Proceedings of the 5th International Symposium on Grape Breeding

12-16 September 1989
St. Martin/Pfalz, FRG

Vitis Special Issue 1990

VITIS

Special Issue
1990

Proceedings of the 5th International Symposium on Grape Breeding

12-16 September 1989
St. Martin/Pfalz, FR of Germany

Edited by: Bundesforschungsanstalt für Rebenzüchtung
Geilweilerhof, Siebeldingen, FR of Germany

Proceedings of the
5th International Symposium on Grape Breeding
12-16 September 1989, St. Martin/Pfalz, FRG

Vitis, Special Issue, 1990
ISSN 0042-7500

„VITIS" Berichte über Rebenforschung mit Dokumentation der Weinbauforschung – Herausgegeben im Verlag der Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D 6741 Siebeldingen, unter Mitwirkung von G. ALLEWELDT, Siebeldingen; H. P. OLMO, Davis; J. V. POSSINGHAM, Adelaide; P. RIBÉREAU-GAYON, Talence. – Redaktion: G. RILLING und M. KLEINERT, D 6741 Siebeldingen. – Druck: Pfälzische Verlagsanstalt GmbH, 6740 Landau/Pfalz. – Printed in Germany.

Preface

This final document brings to an end the 5th International Symposium on Grape Breeding. It comprises, either in full length or as abstracts, the papers and posters presented during the conference and gives convincing evidence of the world-wide interest in the importance of grape breeding with particular focus to the genetic resources of *Vitis*, biotechnology, and to the development of vines resistant or tolerant to pests and diseases.

Recalling the scientific discussions and social events of the symposium from the distance of a year, I believe it was a success. Therefore, I wish to express my gratitude and thanks to

- all who kept the young tradition of this symposium alive,
- the Ministry of Food, Agriculture and Forestry, Bonn, as well as all others who financially supported the symposium and helped to make the meeting a success,
- the Office International de la Vigne et du Vin, Paris, for assuming the patronage of the symposium.

We are now looking forward to future symposia in this series.

Geilweilerhof, July 1990 G. ALLEWELDT

Contents

Section 2: Genetic improvement of grapevine form or function

Section 3: Resistance/tolerance to pests and diseases

Section 4: Resistance/tolerance to abiotic stress factors

Section 5: Tissue and cell culture

Section 6: Clonal selection

Welcoming address

G. ALLEWELDT

Ladies and Gentlemen,
dear friends and colleagues:

On behalf of the Organizing Committee I have the honour to welcome all guests and participants of the Vth International Symposium on Grape Breeding.

In particular I welcome

- Dr. PADBERG, who is representing our Federal Minister of Food, Agriculture and Forestry, Mr. KIECHLE, Bonn
- State Minister ZIEGLER, Ministry of Agriculture, Viticulture and Forestry, Mainz

and our colleague

- Professor FREGONI, Vice-president of the O.I.V., who represents the Office International de la Vigne et du Vin, Paris.

This Symposium is the fifth in the series and is intended to provide an opportunity for exchange of information between workers from all over the world, to discuss and evaluate advances in grape breeding and to share their expertise.

I welcome the members of the International Symposium on Clonal Selection who decided to be part of the body of this Symposium.

Viticulture is and has always been accompanied by a desire to improve the vine and its product for the benefit of both the producer and the consumer. Thus, improvements in viticulture and in new cultivars are still factors which govern the prosperity of viticulture world-wide.

Nowadays, having hardly recovered from the catastrophe caused by mildew and phylloxera which befell Europe's vineyards in the last century, new challenges have to be faced:

1) The environmental stress caused by the use of agrochemicals urges the utilization of genes resistant to pests and diseases in order to underpin our efforts to introduce integrated pest control.
2) The maintenance and evolution of genetic resources is the essential prerequisite for the protection of future requirements of grape breeders.
3) An unavoidable improvement of fruit and wine quality, an overproduction of wine and table grapes in some regions and the unavailability of adapted cultivars in others (particularly in the tropics and subtropics), requires urgent breeding attention.
4) The existing new possibilities offered by gene technology must be introduced into breeding programs.

These problems can only be solved through the comprehensive exchange of research information and by international cooperation.

May this Symposium renew the spirit of challenge in vine breeding, tighten the bonds of friendship and encourage all of us to face and to surmount the forthcoming demands.

I am personally saddened to have to tell you of the recent death of two of our colleagues: Prof. Dr. DARIS of Greece and Dr. FÜRI of Hungary.

We had hoped to welcome them to this Symposium but this was not to be.

In conclusion I want to express my gratitude to our Federal Minister for his generous financial support, to the many sponsors of our Symposium, to the Office International de la Vigne et du Vin, Paris, for taking over patronage, and last but not least, to all members of my staff who have helped in the organization of this conference.

Let us have inspiring discussions and, to speak in terms of breeding, let us sow new seeds to enter and surmount the challenges of the forthcoming 21st century.

I declare the 5th Symposium on Grape breeding open !

Section 1: Genetic resources, evaluation and screening

Research on the meaning of the enzymatic systems (GPI and PGM) as parameters for the definition of varieties (*Vitis* sp.): The Italian case of Cabernet franc

A. Calo, A. Costacurta, R. Di Stefano, G. Calo and G. Paludetti

Istituto Sperimentale per la Viticoltura, Viale XXVII Aprile, 26, I-31015 Conegliano (TV), Italy

Summary: Several studies carried out at Davis and Conegliano showed that isozyme analysis of the GPI and PGM enzymatic systems agrees with the conventional definition of the variety in ampelography.

Differences were reported among varieties but not among biotypes of the same varieties. The only exception recorded was in the population of Cabernet franc in which GPI and PGM reveal two different types (A – the traditional type encountered in France and B – type encountered in the Italian region of Veneto).

Further ampelographic, ampelometric, phenological and chemical studies on the polyphenolic and aromatic substances in fruits have shown considerable differences between the two types. Such differences demonstrate that the type B is a different variety and not a clone of Cabernet franc. Preliminary ampelographic analysis and the equality in GPI and PGM patterns lead to the conclusion that the type B very probably is Carmenère.

Therefore, the hypothesis of variety discrimination based on the analysis of GPI and PGM is valid and this method is useful to help to characterize the varieties.

Key words: variety of vine, biotype, Italy, ampelography, biometry, analysis, morphology, leaf, berry, enzyme, pyrazine, polyphenol, phenol.

Introduction

Generally, ampelography provides the definition of vine varieties according to morphological, phenological and chemical features that may differ in more or less significant ways. Conventionally, when the basic morphological features of a variety's population are the same, clones are distinguished according to the genotypical variations of the physiological and/or phenological and/or chemical features.

Studies carried out at Davis and Conegliano over a 2-year period (1987-1988), at first on 225 varieties and subsequently on 9 variety populations and 63 clones of these (8), showed that isozyme analysis of the GPI and PGM enzymatic systems by starch gel electrophoresis of leaf extracts agrees with the conventional definition of the variety in ampelography.

Differences were reported among varieties but not among biotypes of the same varieties, probably because the genes of which these enzymes are the main expression, did not cause any significant variations of the morphological, physiological and chemical features, practically and conventionally considered in the variety's definition.

So far, the only exception recorded was the population of Cabernet franc, widely employed in Italy, in which the GPI and PGM reveal two different types, i. e. A – the traditional type encountered in France and B – the type mainly encountered in the Italian region of Veneto. The aforementioned types have significantly different features requiring further ampelographic, ampelometric, phenological and chemical studies on the polyphenolic and aromatic substances in fruits in order to provide objective differences that the operative world is also suggesting.

The Cabernet varieties seem to date back to the Biturica mentioned by Plinius and Columella, in relation to the ancient synonyms Viduve and Vidure, according to Petit-Lafitte (who relied on the authority of Vinet, an expert of the 18th century). Although impossible to prove, it is quite evident that the varieties date back to a very early age, a fact of which trustworthy evidence is found in Gironde, where Cabernet franc was well-known at the times of the Cardinal of Richelieu.

During the diffusion of these varieties, started in the 17th century, several populations spread out with different synonymities or distinctions, still existing at the present time. The distinctions involved varieties, i. e. Cabernet franc, Cabernet Sauvignon, Carmenère and then a series of synonyms recalled by the different ampelographies (14, 15, 17, 20). It may be worth underlining the fact that in the 19th century, the ampelographical expert, Count ODART, mistook Cabernet franc and Cabernet Sauvignon, since the latter was still not very well-known in French regions other than Gironde.

In Italy (10, 11, 16), apparently Cabernet franc was imported for the first time by the Count of SAMBUY in Piedmont at the beginning of the 19th century. By the end of the century, the variety was cultivated in approximately 45 provinces, from Piedmont to Southern Italy. Noteworthy cultivations were present in Veneto, where the variety had come directly from France through BORTOLO CLEMENTI, the Count of SCHIO, Count CORINALDI and other vine-growers.

The origins of the vines and thus of the populations were naturally diverse, as still can be noticed in some vineyards propagated with this material.

The Istituto Sperimentale per la Viticoltura has been performing selections since 1980 on the above-mentioned populations that led to the identification and now to the characterization of the types examined in this report.

Materials and methods

Research was conducted in 1987-1988 by the Istituto Sperimentale per la Viticoltura in two fields situated on the Eastern Venetian plain in entirely different pedoclimatic conditions. In each field of comparison the two types of Cabernet franc were represented by 24 vines per biotype with 6 repetitions each.

Examination involved the following features:

1. Biochemical features

The starch gel electrophoretic analysis of leaf extracts was performed for the enzymatic systems glucose phosphate isomerase (GPI) and phosphoglucomutase (PGM). The methods and procedure employed were those previously reported (3, 18).

2. Morphological features

Testing of these features was performed according to the instructions of the international O.I.V. descriptor list. Features are described in Table 1. The following informations were also recorded:

- phenological ages considering the average time of the phenomenon's appearance;
- potential and actual fertility of the buds along the fruit bearing tendrils (7).

3. Ampelometric measurements

These tests analyze the features provided by measurements and the relations that define the leaf, in particular:

- ratios between veins: L_1/L; L_2/L_1
- depth of petiole sinus: S_1/L_1; S_2/L_2
- angles between veins: α between L and L_1; β between L_1 and L_2; γ between L_2 and L_3
- leaf length/width
- petiole/leaf length

Measurements were performed on adult leaves selected in summer from the 5th to the 8th node of the main shoots.

The following grape bunch characteristics were also considered:

- berry weight in g
- berry number per bunch
- bery volume in ml
- petiole length in cm
- berry dimensions (length and width) in cm
- bunch size (width and length) in cm

The data were gathered upon ripening from 10 bunches and 100 grapes.

4. Berry samples were taken weekly from veraison to ripening to establish the following juice components:
   - total acidity (g/l)
   - malic acid (g/l)
   - pH
   - tartaric acid (g/l)
   - sugar (% ml)
   - potassium (g/l)

5. Volatile berry components and berry skin phenolic components

5.1. Berry pyrazines

1 kg of berries were homogenized and steam distilled to establish the amount of pyrazines. The distillate was extracted 3 times with 25 ml of CH_2Cl_2. After solvent distillation, the extract was analyzed by gas chromatography-mass spectrometry by means of the S.I.M. programme with acquisition of ions 123-138, 124-137, 124-151, respectively, related to 2-ethyl-3-methoxy pyrazine, 2-isopropyl-3-methoxy pyrazine, 2-isobutyl-3-methoxy pyrazine.

5.2. Volatile juice components

250 ml of juice was extracted with C_5H_{12} : CH_2Cl_2 in a 60 : 40 ratio for 12 h. After the solvent's evaporation by distillation, the extract was subjected to gas chromatography.

5.3. Phenolic berry skin components

Anthocyanins: These were extracted from 10 berries with ethanol + 0.1 % of concentrated HCl and with mixture ethanol : H_2O : concentrated HCl in the ratio 70 : 30 : 1.

Phenolic acids: The ethanol extract of skins was employed for the analysis of the hydroxy cinnamoyl, tartaric acids.

6. Organoleptic tests were performed on wines produced by the microvinification of the two types of Cabernet franc and tasting the grapes by means of the statistical duo-trio test.

Results and discussion

1. Isoenzymatic analysis

As may be seen in Fig. 1, the two types of Cabernet franc have different GPI and PGM patterns.

2. Morphological features

Of all the features examined (see Table 1), the following proved to be repeatedly different and steady in the two types of Cabernet franc (Table 2).

Type B has higher anthocyanic pigmentation levels in the shoot tip, more blisters on the upper leaf side, a larger amount of hairs between veins on lower leaf side and a looser cluster. These features will be emphasized in further detail by analyzing the ampelometric tests.

Moreover, a particular feature is the special shape of stamen that may be spiral-shaped in type B, as previously illustrated (7) on this population (see Fig. 10).

3. Ampelometric tests

Table 3 illustrates the features that proved to be significantly different among those examined.

The analysis of the results concerning the leaves shows that the dimension of the apex lobe determined by the ratios L_1/L and S_1/L_1 are major in type A than in type B.

With regard to the bunch, type B, as already mentioned, is looser (longer bunch, fewer berries and less compact) and it has larger berries (size and volume).

4. Berry juice components

The course of sugar development in the berries, of the degradation of total tartaric and malic acidity, and of the pH increase does not differ considerably from one type to the other (Fig. 2).

Type B, however, ripens earlier and thus it attains higher sugar in berries earlier and, at the same time, has a significant degradation of total acidity, related to the decrease of both malic and tartaric acid, whereas the pH is higher.

In the juice, there is also a considerable difference in potassium concentrations: higher in type A, an aspect that is probably interesting in enological terms.

Table 1: Morphological features examined

O.I.V. CODE	FEATURE
001	young shoot: form of tip
003	" " : intensity of anthocyanin coloration of tip
004/005	" " : hair density ot tips
007/008	shoot: color of internodes
009/010	" : color of nodes
011/013	" : density of hairs of nodes
068	mature leaf: number of lobes
075	" " : blistering of upper side
076	" " : shape of teeth
079	" " : general shape of petiole sinus
081	" " : particularities of petiole sinus
084/085	" " : density of hairs between the veins (lower side)
088/089	" " : density of hairs on main veins (upper side)
090/091	" " : density of hairs on petiole
151	inflorescence: sex of flower
153	" : number of inflorescence per shoot
204	bunch: density

Table 2: Features proved to be repeatedly different and steady

OIV CODE	FEATURE	EXPRESSION LEVEL	
		TYPE A	TYPE B
003	young shoot: intensity of anthocyanin coloration of tip	3	5
075	mature leaf: blistering of upper side	5	7
084	" " : density of hairs between the veins (lower side)	5	7
204	bunch : density	6,65	5,42

Table 3: Ampelometric measurements proved to be significantly different among those examined

FEATURE	CABERNET FRANC TYPE A	CABERNET FRANC TYPE B
L_1/L (in cm)	0,893	0,842
S_1/L_1 (in cm)	0,544	0,497
S_2/L_2 (in cm)	0,628	0,591
α (in degrees)	48,62	53,5
β (in degrees)	51,52	55,27
γ (in degrees)	48,32	53,25
berry weight (in grammes)	1,54	1,90
berry volume (in ml)	1,39	1,67
berry length (in cm)	1,43	1,52
berry width (in cm)	1,33	1,45
average number of grapes	99,44	85,32
bunch length (in cm)	12,26	13,04

5. Volatile grape components and berry skin phenolic components

5.1. Berry pyrazines

The two types (A and B) differ in the 124 ion chromatogram profile and partly the 151 ion profile, related to 2-isobutyl-3-methoxy pyrazine, the content of which seems to be considerably higher in type B (Fig. 3). The presence of a great number of peaks leads to the conclusion that the preparation system generates adulterations.

5.2. Volatile berry juice components

The profiles of the samples are practically similar (Fig. 4). There are C_6 components originated by the enzymatic attack of linoleic and linolenic acids, besides other unidentified components and a considerable amount of benzylic alcohol.

5.3. Phenolic berry skin components

Anthocyanins: The analysis of the chromatogram HPLC of the anthocyanins (Fig. 5 and 6) revealed that the two types of Cabernet franc differ in the percentage of peonidin monoglucoside (higher in type B), acetates (higher in type B), and p-cumarates (higher in type A). The anthocyanins of both varieties are trisubstituted. The amount of anthocyanins in the skins of type B is approximately double the amount of type A (Table 4).

Table 4: Anthocyanins %

ANTHOCYANINS	CABERNET FRANC TYPE A	CABERNET FRANC TYPE B
Delphinidin monoglucoside	6,57	6,02
Cyanidin monoglucoside	0,67	1,37
Petunidin monoglucoside	6,96	5,96
Peonidin monoglucoside	3,66	10,84
Malvidin monoglucoside	41,45	38,77
Delphinidin monoglucoside acetate	1,50	1,63
Cyanidin monoglucoside acetate	0,11	0,31
Peonidin monoglucoside + Malvidin monoglucoside acetate	12,99	20,58
Delphinidin monoglucoside p.Cumarate	1,48	0,87
Cyanidin monoglucoside p.Cumarate	0,06	0,07
Petunidin monoglucoside p.Cumarate	1,83	0,51
Peonidin monoglucoside + Malvidin monoglucoside p.Cumarate	21,16	11,28

Table 5: Differences between type B of Cabernet franc and type A

Morphological differences

1. Major anthocyanic pigmentation of shoot tip
2. Major blistering of adult leaf upper side
3. Major presence of hairs between veins of adult leaf lower side
4. Spiral shaped flower stamen
5. Minor bunch compactness

Ampelometric

1. Lower ratio L_1/L
   Lower ratio S_1/L_1
   Lower ratio S_2/L_2
2. Major angle α (between L and L_1)
   " " β (between L_1 and L_2)
   " " γ (between L_2 and L_3)
3. Looser berry bunch:
   * longer bunch
   * lower number of berries
4. Major berry dimension:
   * size
   * weight
   * volume

Biochemical and chemical

1. Different isoenzymes for enzyme systems GPI and PGM
2. Grape pyrazines: higher content of 2-isobutyl-3methoxy pyrazine
3. Skin anthocyanins: higher content of peonidin monoglucoside
   higher content of acetates
   lower content of p-cumarate
   double quantity of total anthocyanins
4. Phenolic acids: higher content

Organoleptic

Clear identification of the two types and a more herbaceous taste in type B.

Phenolic acids: The chromatogram at 320 nm shows that type B has a higher incidence of these components. The same test performed at 280 nm (Fig. 7 and 8) shows that type B contains a large amount of an unidentified component with a characteristic spectrum (Fig. 9), of which there are no traces in type A or in wine.

6. Organoleptic tests

Continuous organoleptic research on wines has allowed the preparation of a merit list in which Cabernet franc type B appears to be more appreciated and with a specific individuality. Furthermore, grape tasting performed by means of the duo-trio test indicated that the two types may be distinguished quite clearly (80 %), with a major mark of the herbaceous taste of type B.

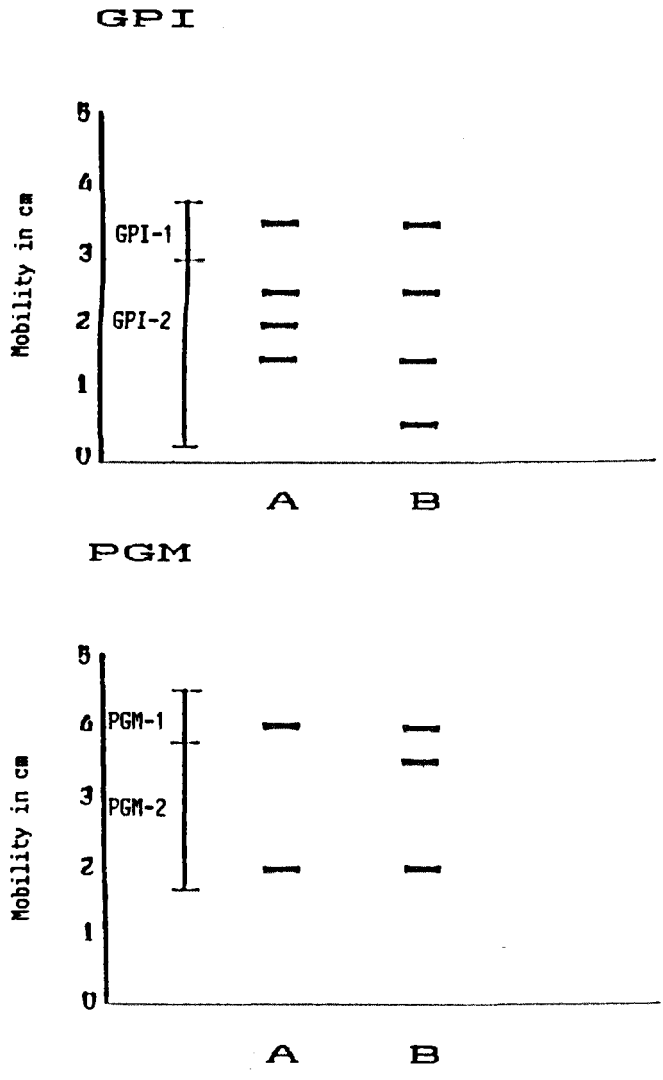


Fig. 1: GPI and PGM patterns in Cabernet franc type A (A) and type B (B).

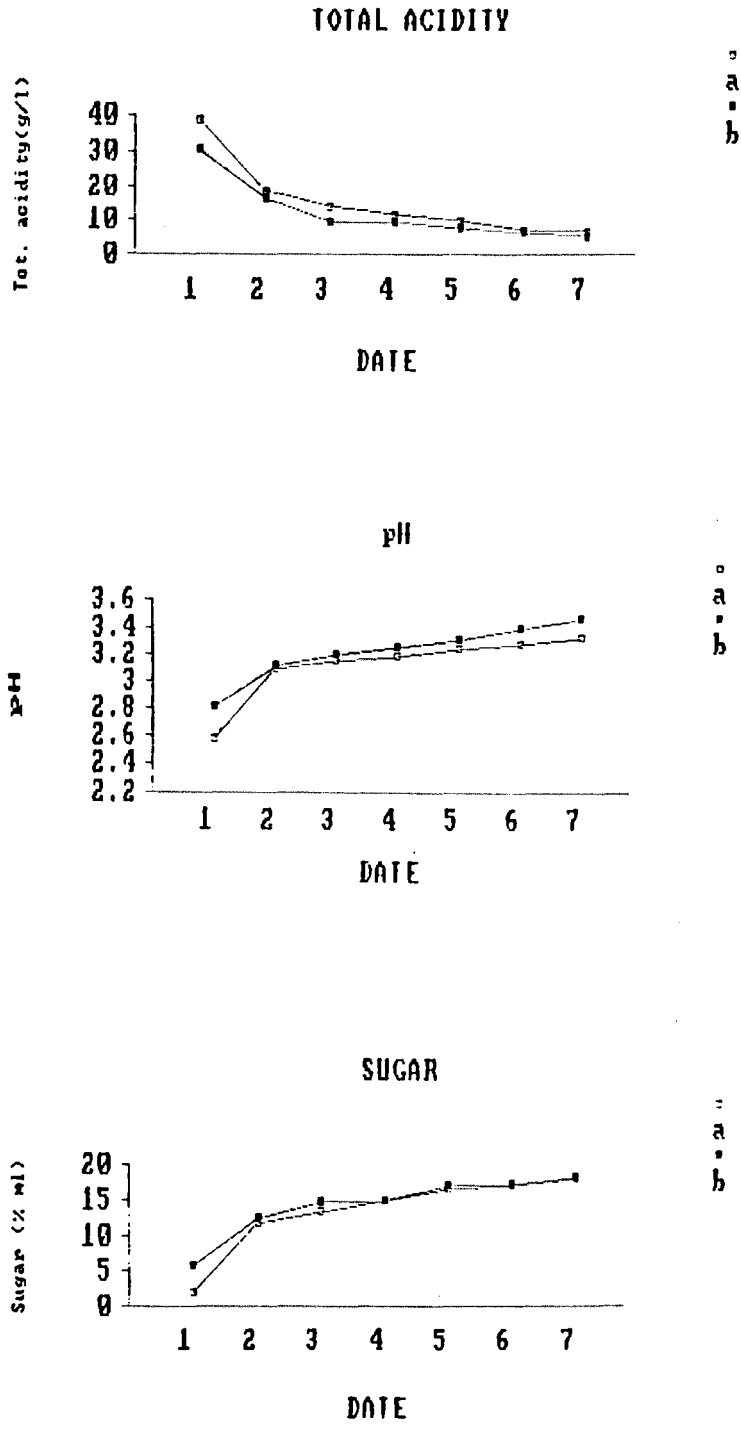
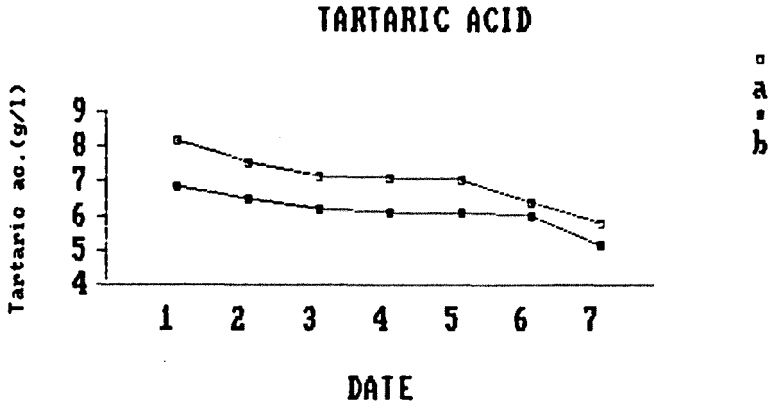
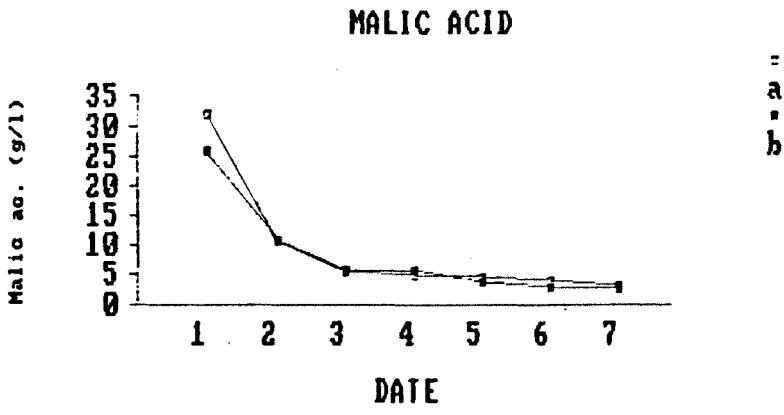


Fig. 2: Berry components of Cabernet franc type A (A) and type B (B). (Continued overleaf.)





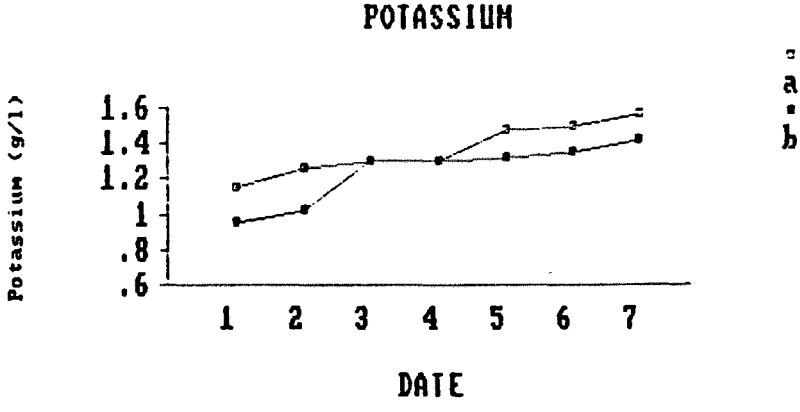


Fig. 2 (continued).

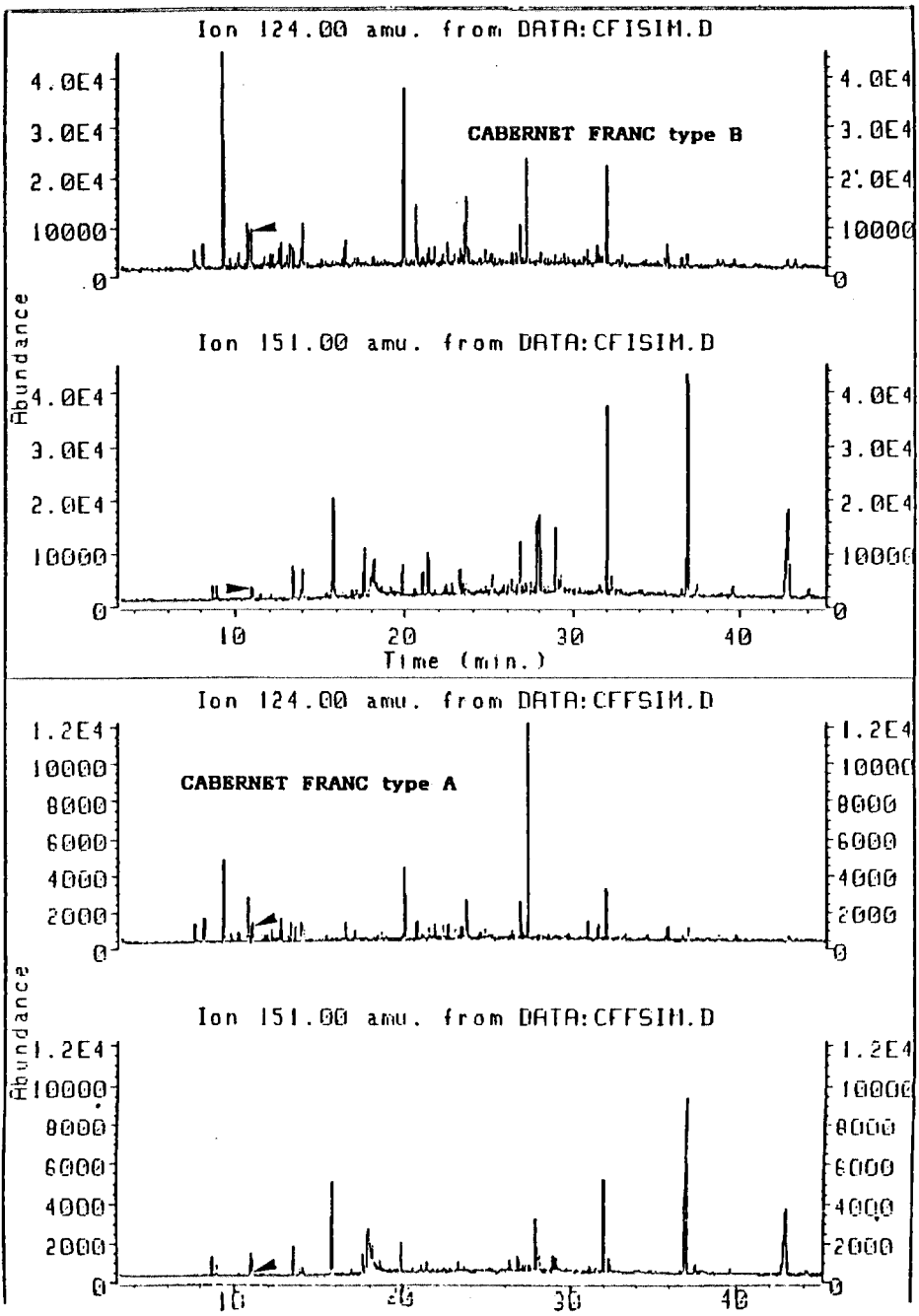


Fig. 3: Ion chromatogram of the CH_2Cl_2 extract of the homogenizated grapes. ◄ = Ions 124 and 151 are the ones related to 2-isobutyl-3-methoxy pyrazine.

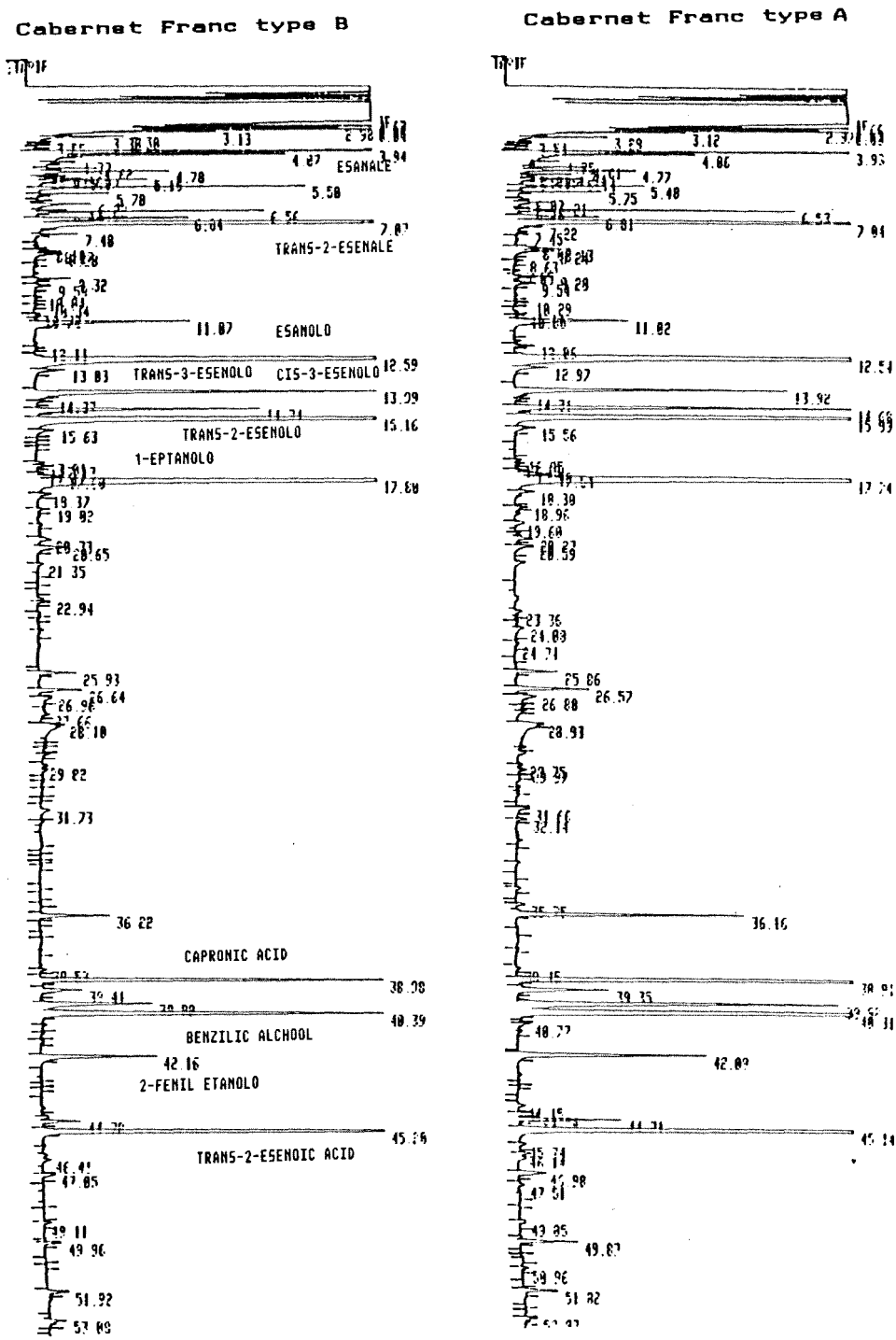


Fig. 4: Chromatogram of grape juice volatile components.

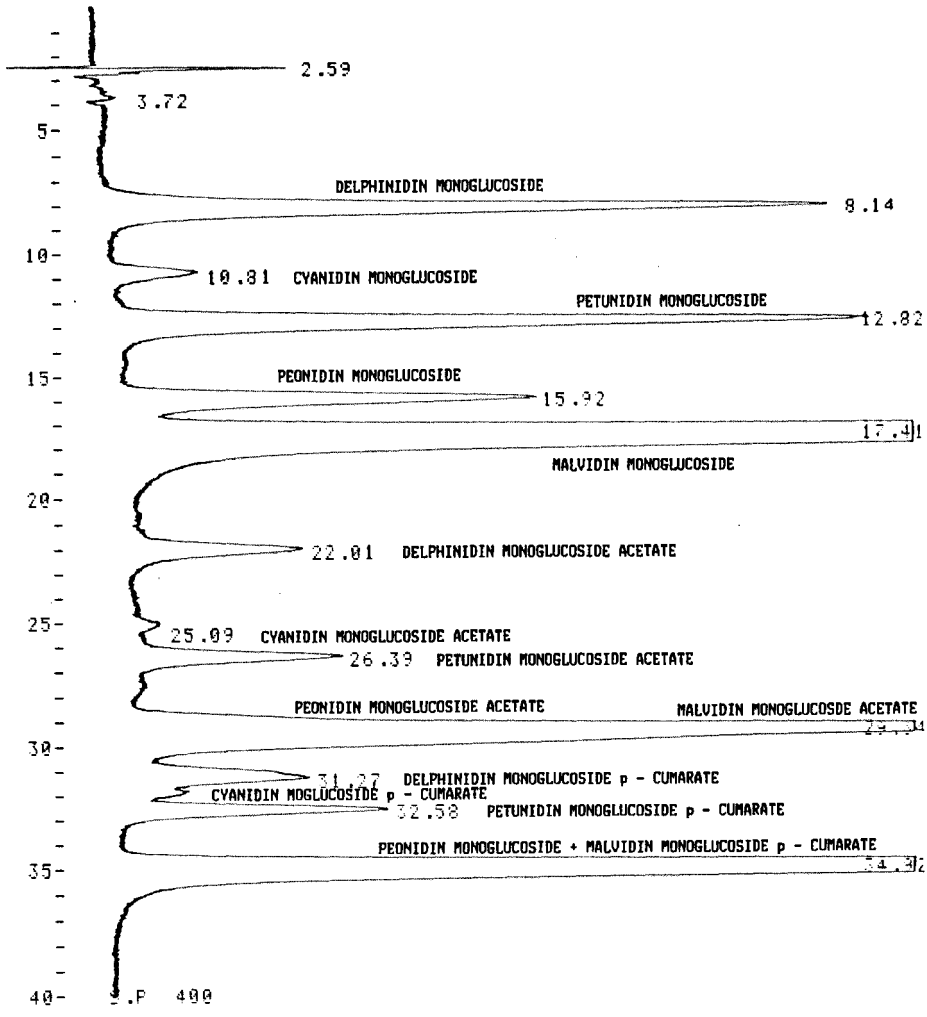


Fig. 5: HPLC chromatogram of the anthocyanins of the skins of Cabernet franc type A. λ = 520 nm.

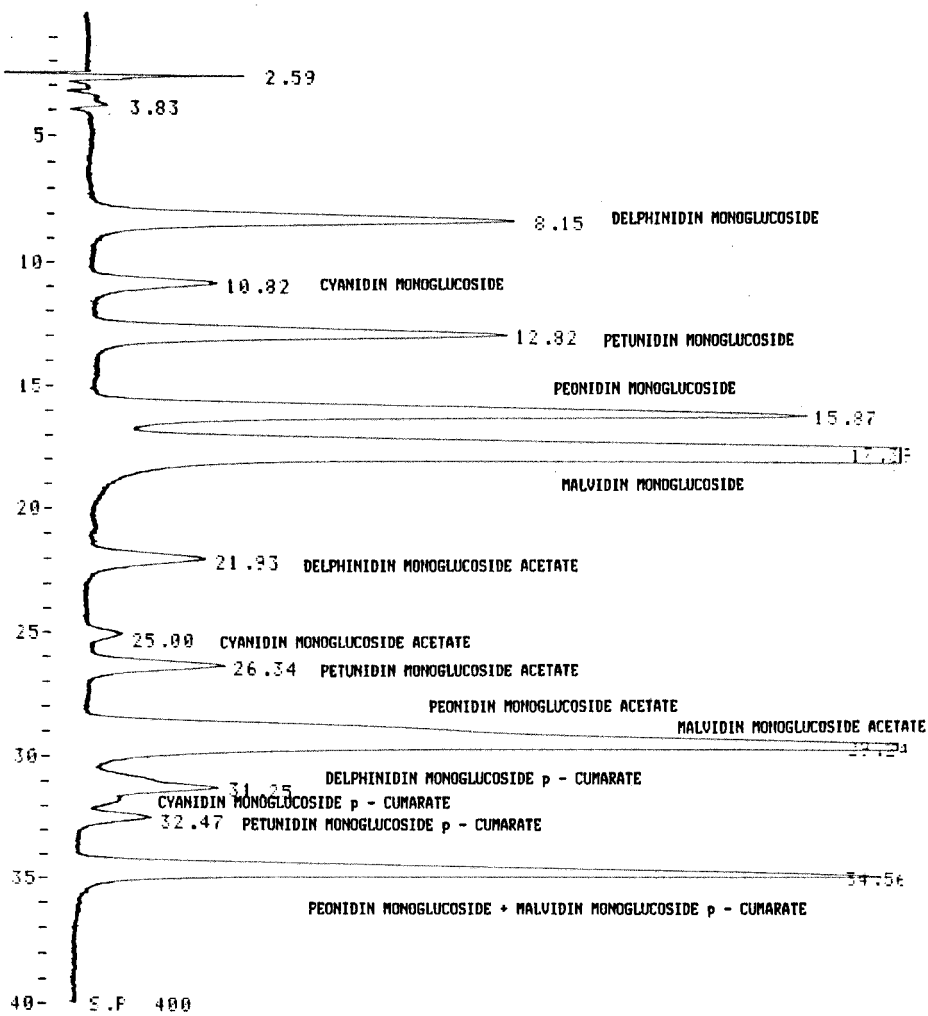


Fig. 6: HPLC chromatogram of the anthocyanins of the skins of Cabernet franc type B. λ = 520 nm.

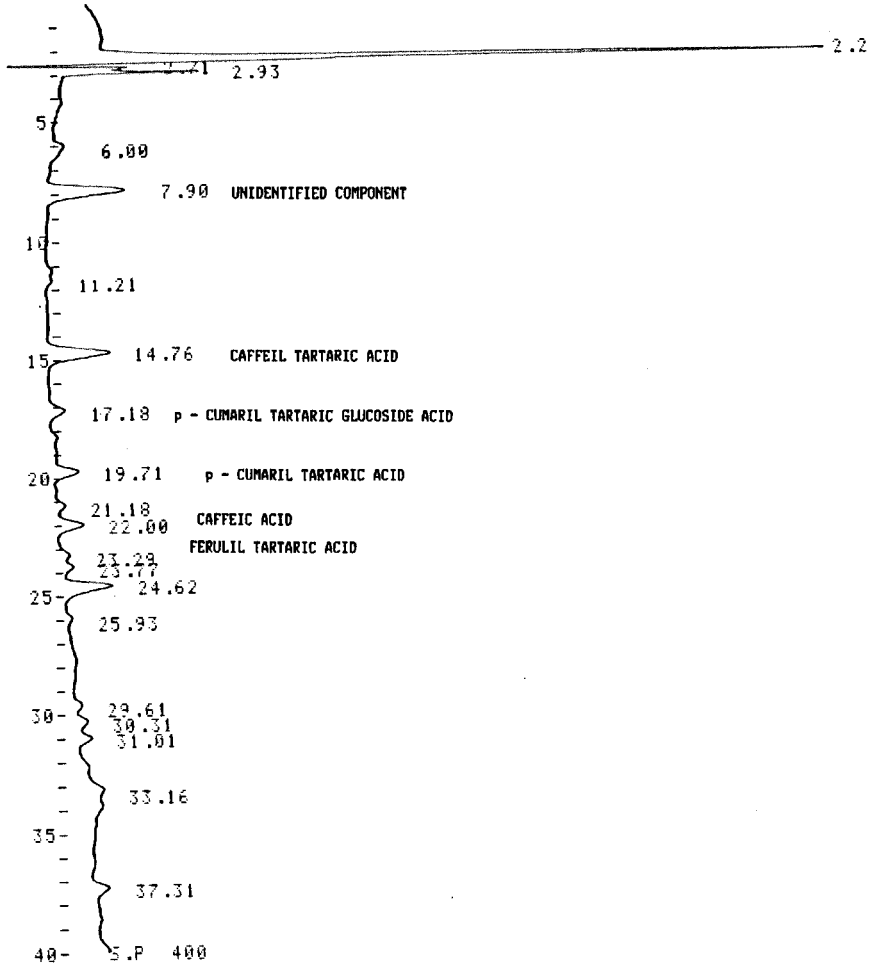


Fig. 7: HPLC Chromatogram of the phenolic acids of the skins of Cabernet franc type A. λ = 280 nm.

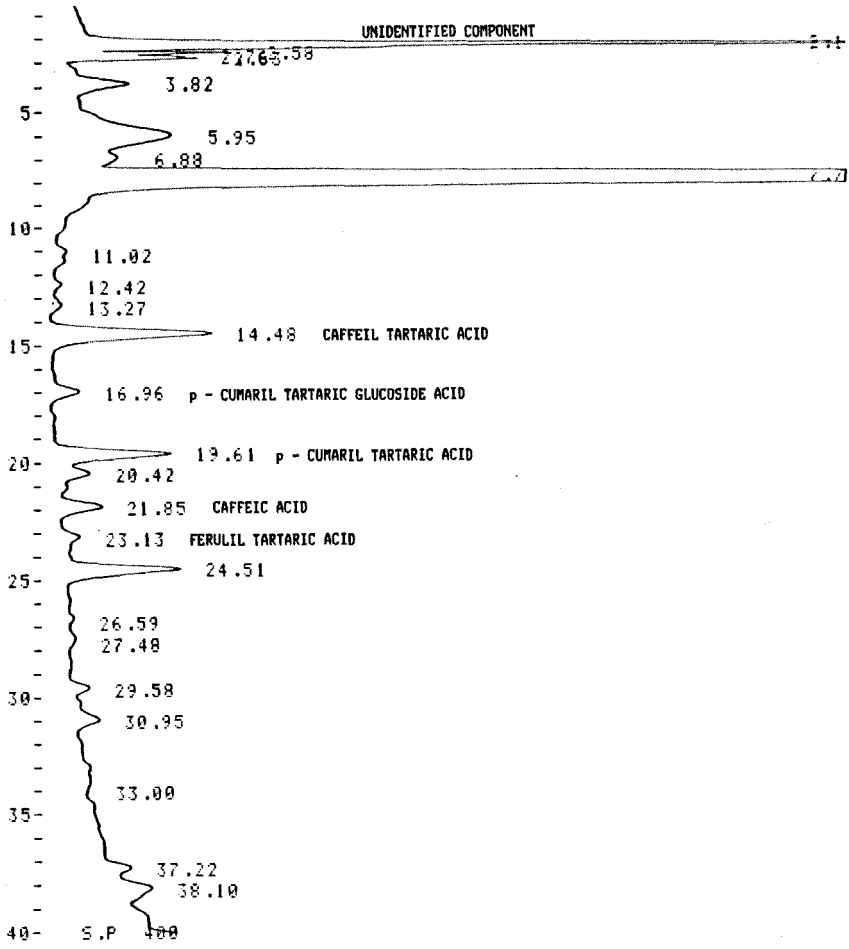


Fig. 8: HPLC chromatogram of the phenolic acids of the skins of Cabernet franc type B. $\lambda = 280$ nm.

Conclusions

A series of studies carried out on different varieties and on clones of the same varieties proved that isozyme analysis of the GPI and PGM enzymatic systems could be used as a discriminating factor when identifying varieties since different patterns may occur between varieties but not between the clones of the variety.

However, the population of Cabernet franc employed in Italy exhibited two different types (A and B) for GPI and PGM.

In this report the aforementioned types were carefully analysed for several morphological, ampelometric and chemical features. Considerable differences were recorded between type B (mainly diffused in Veneto) and type A (the traditional type popular in France). Such differences, illustrated in Table 5, led to the conclusion that it is a different variety and not a clone of Cabernet franc.

Type B is very probably Carmenère, whose population spread in Veneto since last century and has been frequently confused with Cabernet franc. This hypothesis is confirmed by preliminar ampelographic analysis as well as the equality in GPI and PGM patterns and it will be moreover examinated with further checks.

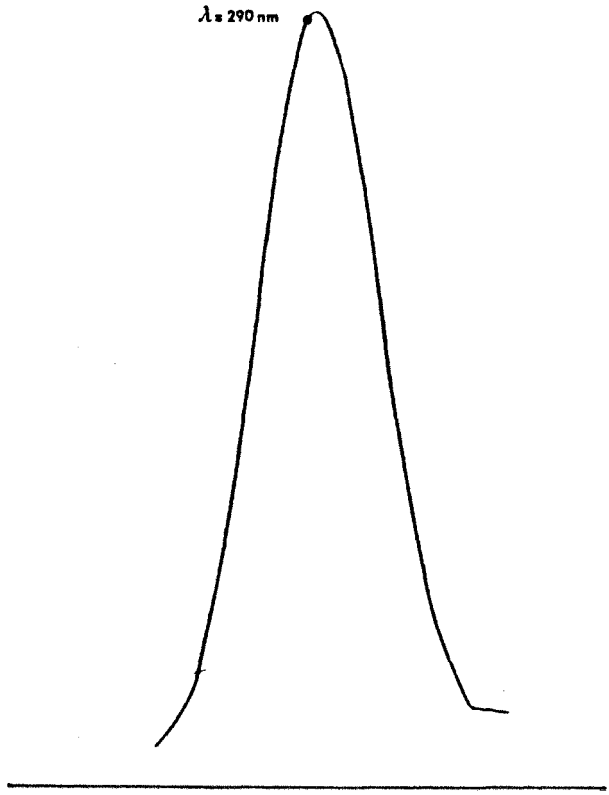


Fig. 9: Spectrum of the unidentified component present in the skins of Cabernet franc type B.

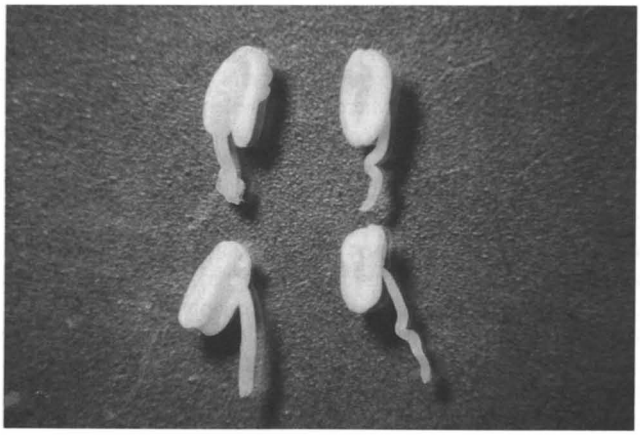


Fig. 10: Shape of the stamen in the two populations of Cabernet franc. Left: stamen of type A; right: spiral-shaped stamen of type B.

Therefore, the hypothesis of a variety discrimination based on the analysis of GPI and PGM is basically valid and thus the method is presently useful to help to characterize the varieties and in future will be suitable for the definition.

References

ALBACH, G.; KEPNER, R.; WEBB, A.; 1959: Comparison of anthocyanic pigments of red Vinifera grapes. Amer. J. Enol. Viticult. **10**, 164.

ALLEWELDT, G.; DETTWEILER, E.; 1986: Ampelographic studies to characterize grapevine varieties. Atti IV Simp. Intern. Genetica della Vite, 55-59.

ARULSEKAR, S.; PARFITT, D.E.; 1986: Isozyme analysis procedures for stonefruits, almond, grape, walnut, pistachio and fig. HortScience **21**, 928-933.

ASTEGIANO, V.; CIOLFI, G.; 1974: Indagine sui costituenti antocianici dei vini rossi piemontesi. Riv. Viticolt. Enol. **27**, 497-507.

BACHMANN, O.; 1989: Isoenzymes as a tool for grape cultivar identification. Riv. Viticolt. Enol. **42**, 27-31.

BOURSIQUOT, J. M.; VIGNAU, L.; BOULET, J. C.; 1989: Ricerche sull'utilizzazione dell'ampelometria. Riv. Viticolt. Enol. **42**, 37-52.

CALO, A.; COSTACURTA, A.; CERSOSIMO, A.; CANCELLIER, S.; MUCIGNAT, D.; 1988: La selezione clonale dei vitigni bordolesi: Cabernet Franc N, Cabernet Sauvignon N, Merlot N. Riv. Viticolt. Enol. **41**, 181-200.

-- -- ; -- -- ; PALUDETTI, G.; CALO, G.; ARULSEKAR, S.; PARFITT, D. E.; 1989: The use of isozyme markers to characterize grape cultivars. Riv. Viticolt. Enol. **42**, 15-22.

-- -- ; LIUNI, C.S.; 1963: Indagini sulla fertilità del Cabernet franc. Atti Accad. Ital. Vite e Vino, Vol. **XV**.

COSMO, I.; 1940: Rilievi ampelografici comparativi su varietà di *Vitis vinifera*. I vitigni bordolesi. Italia Agricola 7, 473.

-- -- ; POLSINELLI, M.; 1960: Principali vitigni da vino coltivati in Italia: Cabernet franc. Vol. I. Ministero Agricoltura e Foreste, Roma.

DI ROVASENDA, G.; 1877: Saggio di una Ampelografia Universale. Torino.

DI STEFANO, R.; CORINO, L.; 1984: Terpeni ed antociani di uve rosse aromatiche. Riv. Viticolt. Enol. **37**, 581--595.

GALET, P.; 1962: Cépages et Vignobles de France. Imprimerie du Paysan du Midi, Montpellier.

MASSE; PULLIAT; 1877: Le Vignoble, Vol. II. G. Masson Editeur, Paris.

MOLON, G.; 1906: Ampelografia. Vol. I, II. Milano.

ODART, A. P.; 1849: Ampélographie Universelle ou Traité des Cépages. Librairie Agricole De Dusaco, Paris.

PALUDETTI, G.; CALO, G.; 1988: Elettroforesi di estratti enzimatici in *Vitis* sp.: nota metodologica. Riv. Viticolt. Enol. **41**, 365-374.

RAPP, A.; HASTRICH, H.; ENGEL, L.; 1978: Analyse par chromatographie capillaire des constituants aromatiques des vins et des raisins; possibilités d'identification des variétés. Génétique et Amélioration de la Vigne, Bordeaux, 417-428.

VIALA, P.; VERMOREL, V.; 1901: Ampélographie, Vol. II, Paris.

Vitis vinifera - a chemotaxonomic approach: Seed storage proteins

A. Scienza [1]), R. Anzani [1]), O. Failla [1]), F. Mattivi [1]), P. L. Villa [1]), G. Tedesco [2]), P. G. Righetti [3]), C. Ettori [3]), S. Magenes [3]) and E. Gianazza [3])

[1]) Istituto di Coltivazioni Arboree, via Celoria 2, I-20133 Milano, Italy

[2]) Dipartimento di Biologia, Sezione di Botanica Sistematica, via Celoria 26, I-20133 Milano, Italy

[3]) Dipartimento di Scienze e Tecnologie Biomediche, Sezione di Biochimica, via Celoria 2, I-20133 Milano, Italy

Summary: The IEF pattern of the constituent peptides for the storage protein from *Vitis vinifera* endosperm is used for the construction of a dendrogram relating 74 seed specimens.

Key words: seed, protein, analysis, Vitis vinifera, variety of vine, clone, ampelography, Italy.

Introduction

In a previous report (Gianazza *et al.* 1989), we investigated the major endosperm proteins of *Vitis vinifera sativa* utilizing seeds from cv. Chardonnay. The storage protein was found to be a globulin, homogeneous by size (M_r > 400 kDa after PAGE) and highly heterogeneous by charge, 23 bands being resolved by IEF, with pIs 4.8-5 for the major components. From SDS-PAGE under reducing and non-reducing conditions, the native structure appeared to be assembled from non-covalently bound subunits, with M_r ca. 65 kDa, which in turn were composed of disulfide-bridged peptides, M_r = 19-21 kDa and 38-44 kDa. The focusing pattern of the denatured protein (8 M urea after -S-S- reduction) included 15 acidic (pIs = 4.25-4.80) and 2 alkaline (M_r = 26 kDa, pIs = 6.8-6.9) components.

We compare here the subunit composition of the storage proteins from a number of cultivars grown across Italy. This data base is then used for the construction of a dendrogram relating the various cultivars to one another.

Materials and methods

The seeds from 54 cvs of *V. vinifera sativa* from the following Italian regions: Val d'Aosta (AO), Piemonte (TO), Liguria (GE), Lombardia (PV), Trentino (TN), Veneto (VE), Friuli (TS), Emilia (BO), Toscana (FI), Sardegna (CG), Puglia (BA), as well as from different clones of cvs Schiava (SC, N = 5), Malvasia (MAL, 6) and Trebbiano (TB, 7) and of two *V. vinifera silvestris* specimens (Reppi2 and Reppi3, SI), were collected at vintage 1988. For each sample, the endosperm dissected from 30-35 seeds was ground and extracted with 10 volumes of 0.2 M glycine (Gianazza *et al.* 1989).

Isoelectric focusing was performed on immobilized pH gradients (IPGs) (Bjellqvist *et al.* 1982) in the pH range 4-5.5 (1-D) or 4-6 (2-D experiments). The IPG plates were polymerized according to standard procedures (Righetti and Gianazza 1987) with Immobiline monomers purchased from LKB; the washed and dried gel slabs were reswollen in 8 M urea - 0.5 % carrier ampholytes (0.25 % 4-6 Ampholine and 0.25 % 4-6.5 Pharmalyte, from Pharmacia-LKB Biotechnology, Uppsala, S). Prior to loading, the protein samples were diluted 1 : 1 with 8 M urea - 2 % 2-mercaptoethanol. Gels were run overnight at 500 V, followed by 1 h at 1,300 V. For 2-D mapping, the gel strips from the 1st d run were equilibrated for 15 min in electrode buffer

(according to LAEMMLI 1970) with 3 % SDS and 2 % 2-mercaptoethanol added. The 2nd d separation was on a 7.5-17.5 % T polyacrylamide gradient, at 50 mA/gel (140 x 140 x 1.5 mm).

A total of 57 bands were analyzed in the 1-D pattern of the various samples. Data reduction was for presence/absence of any given component – disregarding quantitative variations. A dendrogram using average linkage was constructed with the statistical program SPSS-X run on a VAX-VMS computer (Istituto Agrario Provinciale, San Michele all'Adige, TN).

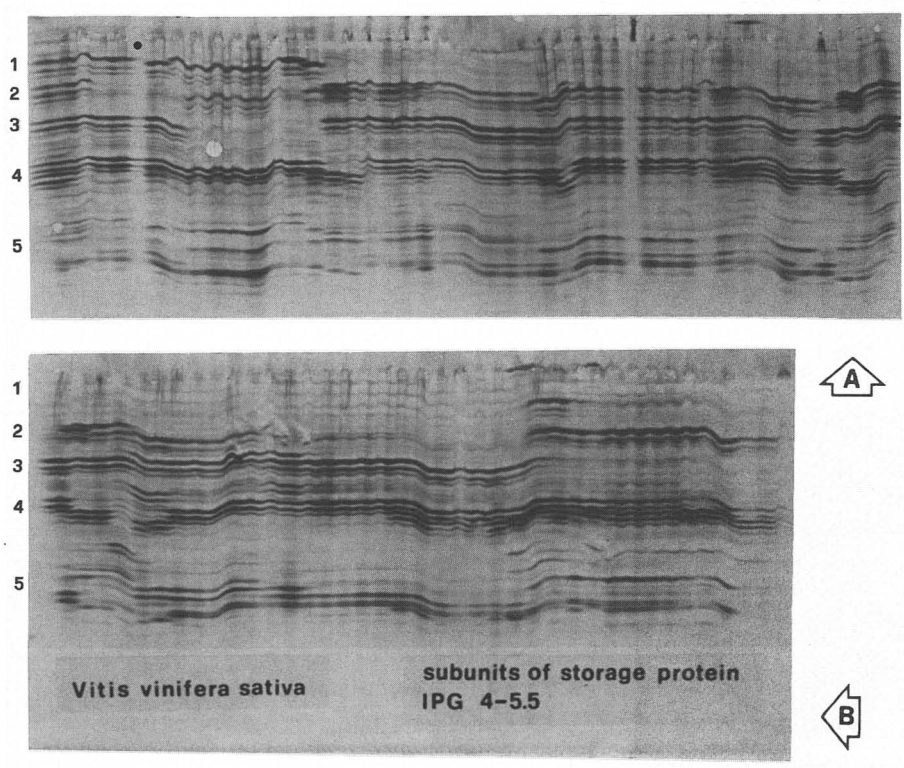


Fig. 1: Isoelectric focusing on immobilized pH gradients (IPG) 4-5.5 of the endosperm proteins from *Vitis vinifera* seeds. 15 μl of a glycine extract diluted 1 : 1 with 8 M urea – 2 % 2-mercaptoethanol were applied per lane. The gel had a polyacrylamide matrix T % = 4, C % = 4 and was made to contain 8 M urea and 0.5 % carrier ampholites in the pH range 4-6.5. Gels were run at 15 °C for 12 h at 500 V, then for 1 h at 1,300 V. Coomassie stain according to RIGHETTI and DRYSDALE (1974). Samples: gel A = Reppi 2 (*V. vinifera silvestris*), Cagnina, Ribolla Nera, Canina, Cannonau, Lambrusca d'Alessandria, Malvasia Brindisi, Verdicchio, Trebbiano Valtenesi, Tb. Soave, Tb. Lugano, Torbiana Soave, Carignano, Malvasia Casorso, Ancellotta, Schiava Grigia, Coda di Volpe di Labico, Lambrusco Salamino, Lambrusco Sorbara, Fumat, Lambrusco Grasparossa, Malvasia Candia, Malv. Candia N. A., Lambrusco Oliva, Lambrusco Maestri, Brunello, Prugnolo, Sangiovese, Aglianico, Grechetto Bianco, Malv. Lecce, Malv. Lunga Chianti, Croatina, Freisa, Bonarda, Pignola, Sc. Lombarda, Uva d'Oro, Tb. Romagnolo, Sc. Gentile, Trollinger, Lambrusco F. F., Neyret, Reppi3 (*V. vinifera silvestris*); gel B = Trollinger, Lambrusco F. F., Neyret, Reppi3 (*V. vinifera silvestris*), Malv. Asolo, Lagrein, Vermentino Spoletino, Pigato, Vermentino Finale, Favorita, Colorino, Marzemino, Teroldego, Mammolo, Vernaccia San Giminiano, Sc.Grossa, Tb. Toscano, Nebbiolo, Barbera Bs., Barbera, Ribolla Spizade, Croà Rosso, Sc. Grossa, Vernaccia, Rossetta di Montagna, Dindarella, Cabrusina, Cividino, Picolit, Corvina Cl. 7, Corvina Cl. 47, Rondinella, Rondinella Cl. 77, Timoraccio, Refoscone, Vien de Nus, Canaiolo, Malvasia.

Results

Fig. 1 A and B shows the isoelectric focusing pattern of the storage protein subunits from the seeds of various *V. vinifera* cvs. The sequence of the samples is based on pattern similarity as evaluated by visual inspection for the presence (from left to right) of cathodal to anodal major components. 10-15 prominent bands in 5 clusters and a number of minor components can be recognized in each lane. None of the major components is present in all samples, i. e. a typical pattern includes only 2-4 band clusters.

The relationships between the different clusters are better evaluated from the 2-D maps of Fig. 2 A-D. The M_r of the polypeptide chains tend to increase with their pI, but clusters 1-2 and 3-4 share essentially the same size.

The dendrogram in Fig. 3 depicts the dissimilarity hierarchy between the banding patterns for the various cultivars. The same results were obtained when specifying either the squared Euclidean or the city block measure as the agglomeration method. Within the widely grown cultivars, 5 out of 7 Trebbiano clones are virtually identical, while the specimens from cvs Schiava and Malvasia are found more scattered across the tree. From the point of view of the geographical origin, the cultivars from Veneto appear the most, and those from Val d'Aosta the least homogeneous.

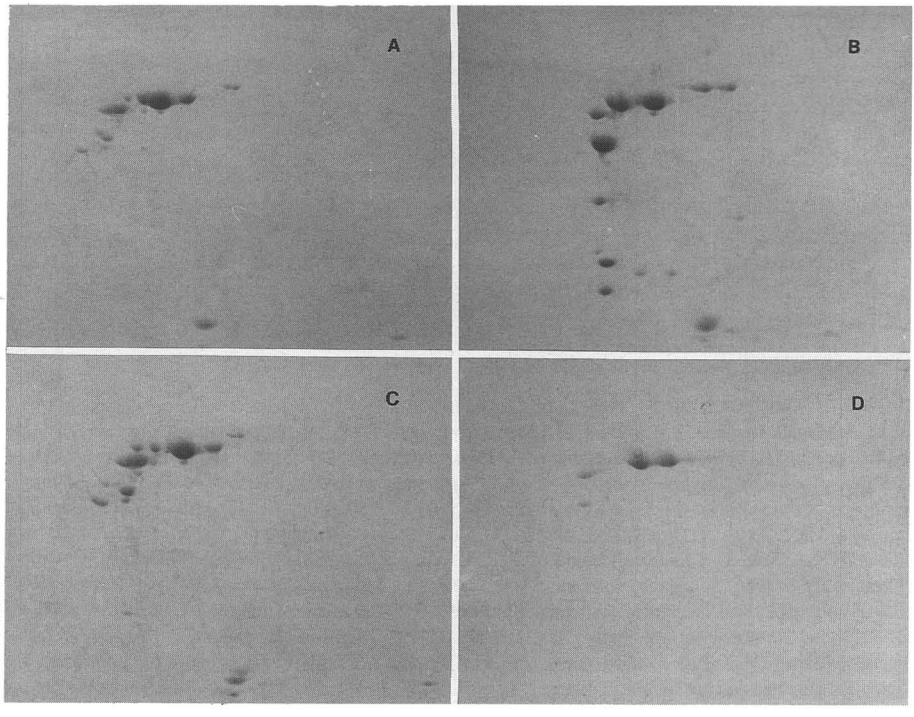


Fig. 2: Two dimensional mapping of the endosperm proteins from *Vitis vinifera* seeds. 1st d: isoelectric focusing in presence of 8 M urea under reducing conditions on a 4-6 IPG; 2nd d: SDS-PAGE according to LAEMMLI(1970) on a polyacrylamide gel T % = 7.5-17.5 %, after reduction and denaturation of the sample. Coomassiestain. A = Canina, B = Trebbiano Lugano, C =Prugnolo, D = Schiava Grossa.

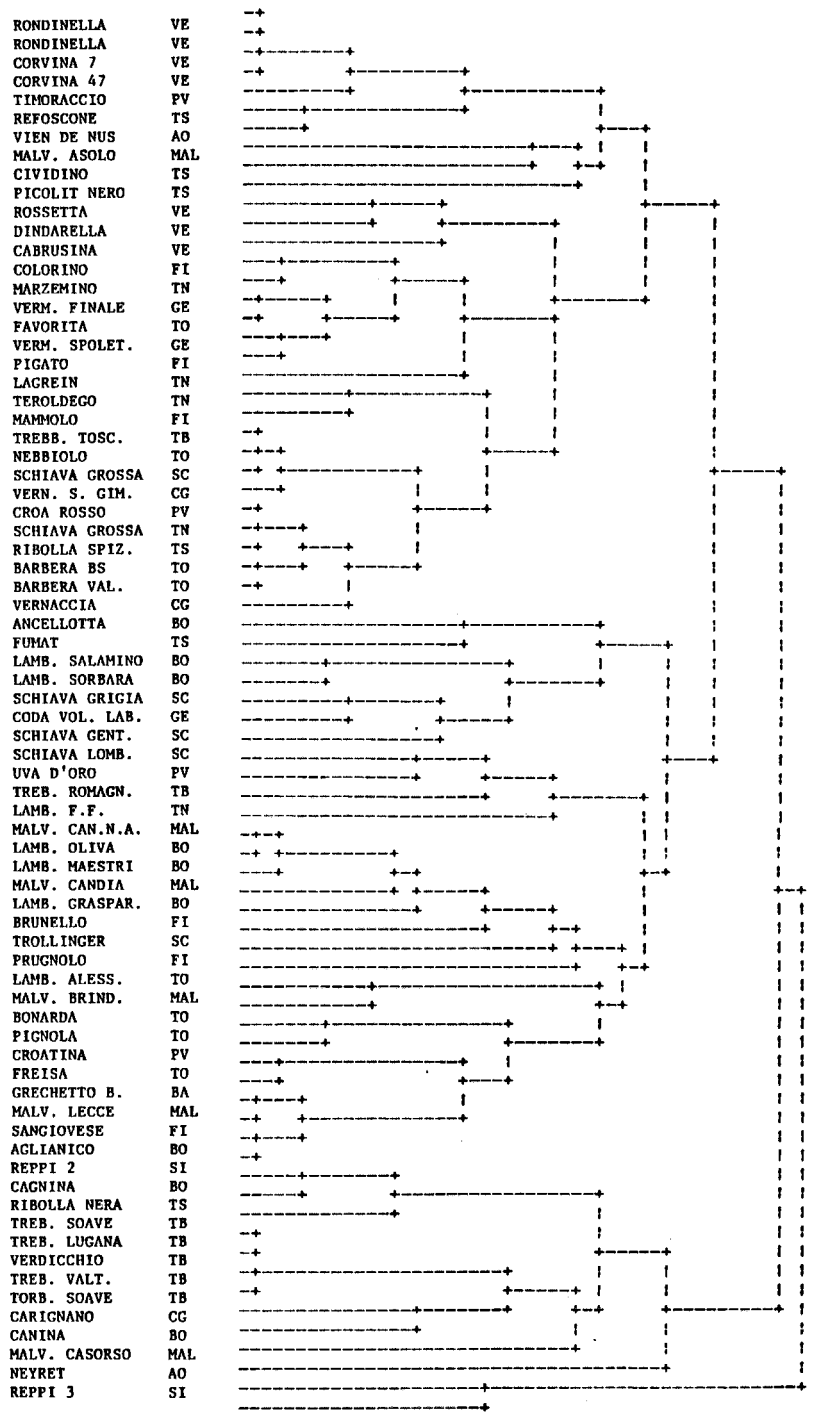


Fig. 3: Dendrogram relating various *Vitis vinifera* sativa cvs.

Discussion

Our current investigation on the seed proteins from *V. vinifera* addresses several problems:

a) the biochemistry of the storage protein (structure and biosynthesis),

b) the ability of various sets of biochemical parameters – i. e. the IEF banding pattern of different endosperm proteins, with or without enzymatic activity – to characterize a given cultivar, or clone, for identification purposes, and

c) their significance in constructing genealogic trees for taxonomy studies.

As for the first point, the present results generalize the findings of GIANAZZA *et al.* (1989) on cv. Chardonnay, and assign pI-M_r relationships between the 5 major protein clusters. Further studies will include: thorough purification of the storage protein from cytosolic components, separation of individual peptides from the different clusters and comparison of their V8-protease fragments while searching for sequence homologies. The hypothesis of a proteolytic processing for the protein subunits from a 65 kDa polypeptide chain (GIANAZZA *et al.* 1989) will be tested by analyzing the set-up of the storage protein across seed development as well as by quantitating in a panel of samples the low- and high-M_r species resolved by SDS-PAGE under reducing vs. non-reducing conditions.

We have defined the size of our samples (N = 30-35) from the evidence of a variability (for presence/absence as well as for relative concentrations) for the various bands of the native storage protein in cv. Chardonnay (GIANAZZA *et al.* 1989). We could then show that this sampling was indeed representative of the average genetic make-up of a given cultivar (to be published). In order to get an impression of the data dispersion around the median, we are currently analyzing the extracts from single seeds in a number of clones. The banding pattern for storage protein subunits is usually fairly stable across a given clone and thus represents a useful parameter in systematic studies.

The dendrogram based on the Coomassie-stained pattern of the proteins focusing (under denaturing conditions) in the pH range 4-5.5 is often close to the ones in which more data, from the analysis of a number of enzymes, were also included (to be published). Thus, the simplest analytical approach – requiring no special chemical nor further processing of the gels – may provide sufficient information for correct cultivar clustering.

References

BJELLQVIST, B.; EK, K.; RIGHETTI, P. G.; GIANAZZA, E.; GOERG, A.; WESTERMEIER, R.; POSTEL, W.; 1982: Isoelectric focusing on immobilized pH gradients: Principles, methodology and some applications. J. Biochem. Biophys. Methods **6**, 317-339.

GIANAZZA, E.; TEDESCO, G.; VILLA, P.; SCIENZA, A.; CARGNELLO, G.; RIGHETTI, P. G.; OSNAGHI, A.; 1989: Proteins from *Vitis vinifera* seeds. Plant Sci. **62**, 73-81.

LAEMMLI, U. K.; 1970: Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature **227**, 680-685.

RIGHETTI, P. G.; DRYSDALE, J. W.; 1974: Isoelectric focusing in polyacrylamide gels. J. Chromatogr. **98**, 271-321.

– – ; GIANAZZA, E.; 1987: Isoelectric focusing in immobilized pH gradients: Theory and newer methodology. In: GLYCK, D. (Ed.): Methods of Biochemical Analysis, 215-278. Wiley-Interscience, New York.

A model of discriminant analysis on the basis of descriptor variables for the ampelography of *Vitis* sp.

A. Camussi [1]), A. Calo [2]), A. Costacurta [2]), C. Lorenzoni [3]) and E. Ottaviano [1])

[1]) Dipartimento di Genetica e di Biologia dei Microorganismi, Università di Milano, Italy

[2]) Istituto Sperimentale per la Viticoltura, Conegliano, Italy

[3]) Istituto di Agronomia, Botanica e Genetica Vegetale, Università di Piacenza, Italy

Summary: Use of descriptor variables in ampelography is recommended to simplify recording of data and to enable useful comparisons. Parametric assumptions are, however, poorly satisfied especially with regard to statistical interference. In the paper some statistical procedures to improve the discriminant ability of descriptor variables are considered. The use of variances and covariances of variety by year interactions is suggested for the error matrix within a multiple discriminant analysis procedure. The adequacy of this model is verified in a 3-year experiment with Italian wine varieties. The discriminant power, as evaluated on the basis of the estimated distances among varieties, is satisfactory.

Key words: ampelography, shoot, leaf, berry, biometry, analysis, descriptor variables, multiple discriminant analysis, genotype by environment interaction, normality assumptions.

The basic aim of ampelography is the complete description of vine varieties in order to provide the most precise definition of their features. The identification of highly discriminating factors is important in order to derive an efficient and reproducible classification of varieties. Use of descriptor variables, as suggested by the O.I.V. protocols, is recommended to simplify data recording and to favor useful comparison. These variables, expressed as rating scales, are difficult to analyse statistically especially with regards to statistical inference.

In the present paper, some statistical procedures are considered to improve the discriminant ability of descriptor variables, particularly as regards data collected in different years of experimentation. As an example, discriminant analysis is applied to data from a set of 31 vine varieties evaluated over 3 years at the Istituto Sperimentale per la Viticoltura (Italy).

Multivariate approach: the canonical analysis

Discriminant analysis is a well established statistical procedure (see, for example, Srivastava and Carter 1983, 231-252), nevertheless a short summary of the main characteristics is given below.

Multiple discriminant analysis (often referred to as canonical analysis) is a very powerful tool used to reduce the complexity of a multivariate system of observations by means of linear functions (discriminant functions) of original variables: they are estimated so that the divergency among groups (here varieties) will be maximized on the basis of the variability existing within groups. The coefficients (a) defining the canonical variates (y) are found by the maximization of

$$\Sigma_i^k N_i (a' \bar{x}_i - a' \bar{x})^2 (a' S a)^{-1}$$

where $\bar{x}_i$ is the mean vector of the i-th group (variety) and $\bar{x}$ is the overall mean vector. N_i is the number of observations of the i-th group and S is the variance-covariance matrix of errors. The solution is found by solving

$$(B - \lambda S) a = 0$$

where λ is an eigenvalue of $S^{-1} B$, and B is the between groups variance-covariance matrix:

$$B = \Sigma_i^k N_i (x_i - \bar{x}) (x_i - \bar{x})' / (k - 1)$$

If we achieve a dimensional reduction taking into account only the canonical variates related to the largest eigenvalues of $S^{-1} B$, the following results can be obtained:
(1) an elimination of most of the redundancy of the original multivariate system, where the traits are correlated to a different extent;
(2)a statistical tool to assign a new object (plant), whose origin is unknown, to one of the groups incluced in the analysis;
(3) a projection of the mean values (centroids) of the groups in the orthogonal space defined by canonical variates, with possible taxonomic derivations on the basis of a reduced but significant number of axes. As the last point is concerned, use of standardized discriminant functions is generally suggested:

$$z_i = a'_i (x - \bar{x})$$

An application of discriminant analysis to the classification of vine varieties

As matter of exemplification, the procedure was applied to describe and discriminate 31 vine varieties. Data were recorded at the Istituto Sperimentale per la Viticoltura in Susegana (Eastern Venetia) in 3 different years considered as repetitions with 3 stocks per variety within each year (Calo et al. 1989).

The traits used in the analysis are reported in Table 1. Numerous traits were recorded but only those showing in the 3 years a variability among groups (varieties) greater than the variability between plants within groups are considered here. Following this simple criteria, a minimal level, as regards the discriminant power, is assured. Most of the traits are expression of underlying quantitative continuous variables. Others describe qualitative characteristics.

Normality assumptions and the matrix of errors

Our data are expressed according to different r a t i n g s c a l e s . Since the canonical analysis is an application of, or better is based on, the multivariate analysis of variance (MANOVA), it is assumed that the variables used in the analysis are continuous and jointly follow a multivariate normal distribution. When the variables are discrete, as in the present case, the assumption of normality is generally not obvious.

We have undertaken the discriminant analysis on the basis of the following considerations:
(1) The generalization of the c e n t r a l l i m i t t h e o r e m to the multivariate case says that if $X_1 \ldots X_p$ have variances $\sigma_1^2 \ldots \sigma_p^2$ and correlations ρ_{ij} ($i = 1 \ldots p, j = 1 + 1 \ldots p$), then the means $\bar{x}_1 \ldots \bar{x}_p$ of a sample of size N have a joint distribution that as N increases approaches to a multivariate normal distribution with variances $^1/_N \sigma_1^2 .. ^1/_N \sigma_p^2$ and with correlations ρ_{ij} the same as those of the X's. The robustness of most multivariate statistical tests is based on this theorem, provided that variances are independent from means (see Mariott 1974, 15). For this reason our sampling design includes replications in different years and within year.
(2) Even if the distribution of single variable is not normal, the distribution of a linear function of numerous variables is approximately normal and the normality increases with the number of variables entering the linear function (Seal 1964, 139). A 'caveat' is the fact that the coefficients applied to 1 or 2 of the non-normal variables allow to dominate the results (this point will be raised later).
(3) Incidental deviations from normality of single component variables will not cause distortion of the point estimates, which are of main interest in discriminant applications (for a discussion on the consequences of deviations from normality, see Scheffé 1959, chap. 10).

Table 1: Descriptor variables from O.I.V. protocol

Variable	Code	Description	Scale
003	I2	Young shoot: intensity of anthocyanin coloration of tip	F
004/005	I3	Young shoot: density of hairs of tip	F
007/008	I4	Shoot: color of internodes	A
009/010	I5	Shoot: color of nodes	A
012/014	I7	Shoot: density of hairs on internodes	F
068	I10	Mature leaf: number of lobes	C
068/B	I11	Mature leaf: angle between weins (L and L1)	
068/C	I12	Mature leaf: ratio L1/L	
075	I13	Mature leaf: blistering of the upper side	F
079	I16	Mature leaf: general shape of petiole sinus	E
081	I17	Mature leaf: particularieties of petiole sinus	A
084/085	I18	Mature leaf: density of hairs between the veins	F
090/091	I21	Mature leaf: density of hairs on petiole	F
093	I22	Mature leaf: lenght of petiole as compared to middle vein	F
202	I28	Bunch: size	F
206	I29	Bunch: length of peduncole	F
220	I31	Berry: size	F
225	I33	Berry: color of skin	D
236	I37	Berrry: particular flavor	B
238	I38	Berry: length of pedicel	F
301	I41	Time of bud burst	F

(scale values: A = 1 2 3, B = 1 2 3 4, C = 1 2 3 4 5,
D = 1 2 3 4 5 6 7, E = 1 2 3 4 5 6 7 8 , F = 1 3 5 7 9)

Choice of the matrix of error variances and covariances

The choice of an adequate model in MANOVA is also of importance for the arguments in the previous section. As the replication within years refers to single plants (stocks), we have considered as variance-covariance S matrix, the matrix from the effects of interaction varieties by years (see table below). In this way we achieve two important points. First, the unit of observation becomes the mean value over three stocks within each year assuring a better fit to normality than individual plants. Second, the weighting of the variability among varieties is performed on the basis of the joint performance over 3 years, which increases the discriminating power of more stable traits. This aspect is in agreement with the simple coefficient of discrimination of LUBISCHEW (1962) as regards single traits.

MANOVA model

Items	D.F.	Matrices
Between years	2	-
Between varieties	30	B
Int. year x varieties	60	S
Residual from the model	186	-

It is possible to asses the adequacy of this model with the MANOVA assumptions by the examination of the frequency distribution of the errors (variety x years effects) as shown in Fig. 1 for some traits included in the analysis. The fit to normal distribution is generally good and particularly with the traits that are more relevant to the discriminant process: this aspect is in agreement with the expectations of point (2), above.

The use of interaction effects in the contest of discriminant analysis is original and we think that it can meet the requirements of a multivariate analysis based on rating scores.

Table 2: Eigenvalues and percentages of variance explained

Root No.	Eigenvalue	Pct.	Cum. Pct.
1	210.27516	61.62073	61.62073
2	44.70333	13.10022	74.72096
3	27.76431	8.13628	82.85723
4	16.22094	4.75351	87.61075
5	12.59509	3.69097	91.30171
6	7.89587	2.31387	93.61558
7	4.74879	1.39162	95.00720
8	3.19846	.93730	95.94451
9	3.17576	.93065	96.87516
10	2.50119	.73297	97.60813
11	1.93070	.56579	98.17391
12	1.45800	.42726	98.60118
13	1.13728	.33328	98.93445

Evaluation of the discriminant power

The eigenvalues (λ_k) whose value is greater than 1 and the relative percent of variance explained are reported in Table 2. The first 7 eigenvalues account for 95 % of the total variation among varieties and we achieve a dimensional reduction by ignoring the smallest 14 eigenvalues. Canonical loadings (correlations between original traits and the canonical variates) allow a biological interpretation of the results of linear transformation. They are reported in Table 3.

The null hypothesis of no differences between varieties was rejected by a Hotelling-Lowley test following MANOVA, which, when approximated by an F statistics, received the value 20.09.

Mean values of varieties according to the first 7 canonical variates were used to derive taxonomic aspects. Since canonical variates are orthogonal, the simple square distances between

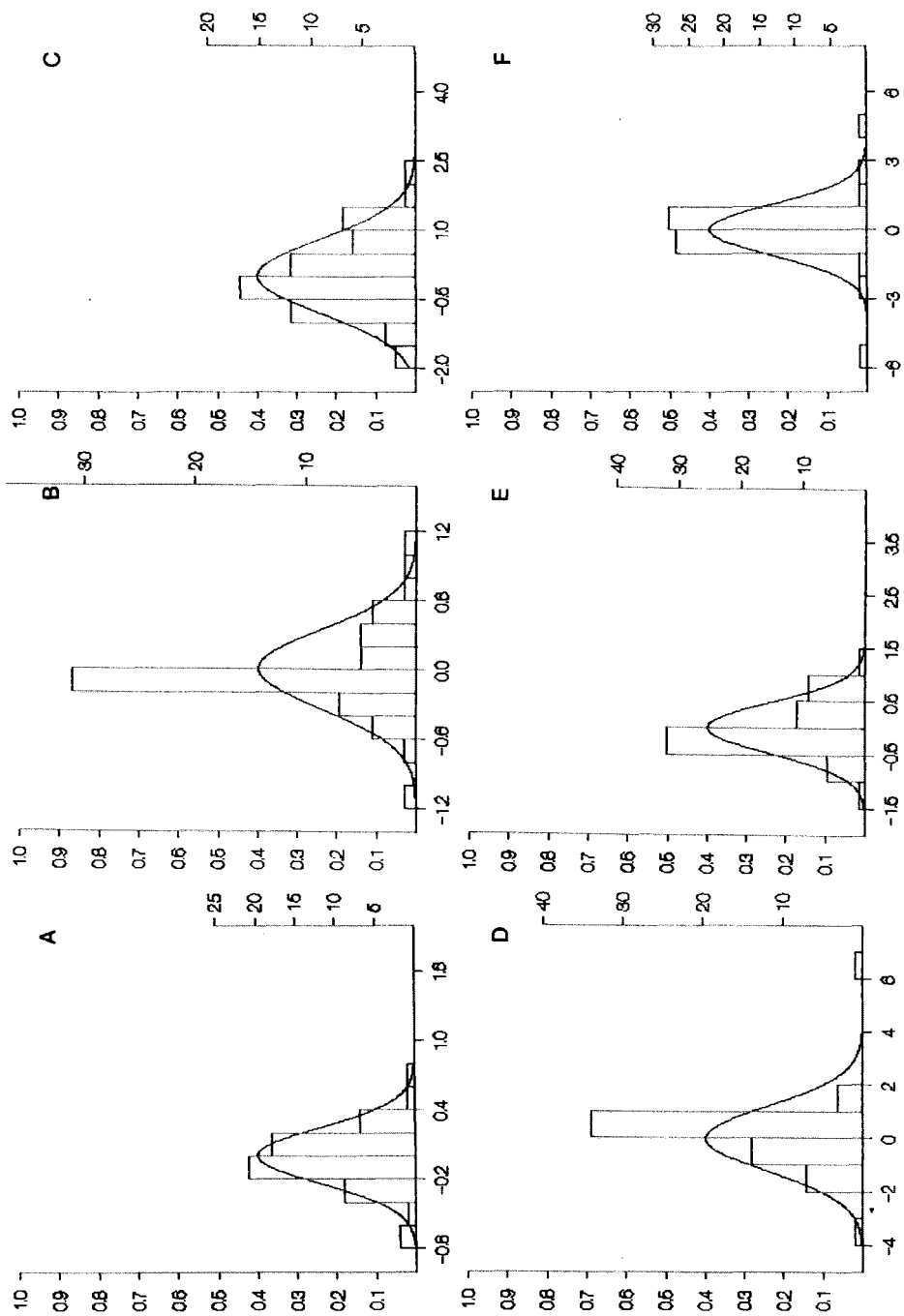


Fig. 1: Frequency distribution of errors (variety x years interaction effects) as regards some traits included in the analysis: A) shoot, color of internodes; B) shoot, color of nodes; C) shoot, density of hairs on internodes; D) mature leaf, density of hairs between the veins; E) berry size; F) color of berry skin. In the ordinates: count of cases (on the right); proportion of case per standard unit (on the left).

varieties correspond to Mahalanobis' generalized distances:

$$D^2_{i\,i'} = (\bar{z}_i - \bar{z}_{i'})' (\bar{z}_j - \bar{z}_{i'}) = (\bar{x}_i - \bar{x}_{j'})' S^{-1} (\bar{x}_i - \bar{x}_{i'})$$

The significance of a distance can be obtained referring to the critical values at different levels of α. These critical values can be obtained by an approximation of the T^2 statistic to F:

$$T^2 = [(\nu - p) / (\nu - p + 1)] F_{\alpha;\, p,\, \nu-p+1}$$

and noting that $T^2_{ii'} = [(N_i N_{i'}) / (N_i + N_{i'})] D^2_{ii'}$. ν are the degrees of freedom of S and p the number of variates used in computing the Distance (here p = 7).

The critical values of distances received the following values:

$$\alpha = 0.05 \;\; D^2 = 3.22, \;\; \alpha = 0.01 \;\; D^2 = 4.05$$

Table 3: Canonical loadings

	Canonical variates						
	1	2	3	4	5	6	7
I2	-.031	-.005	-.075	-.148	-.212	.028	.240
I3	-.034	-.202	-.046	.163	-.107	.035	.173
I4	-.037	-.070	*-.347*	-.210	-.007	-.265	*.466*
I5	-.020	-.030	*-.330*	-.225	-.010	-.257	.244
I7	-.017	-.238	-.013	.168	-.064	-.189	-.115
I10	-.038	-.158	.034	.137	-.255	*.394*	.135
I11	-.005	.001	.040	.082	-.101	-.220	-.036
I12	.003	.017	-.026	.044	-.016	-.052	.069
I13	-.052	-.003	-.062	.147	-.164	-.103	.188
I16	-.018	-.108	.170	.211	*-.528*	*-.388*	-.163
I17	.008	-.006	-.015	.078	-.055	-.121	-.049
I18	-.132	*-.495*	-.068	.188	.060	-.091	.086
I21	-.020	-.094	.052	.010	.012	-.264	-.062
I22	-.059	-.032	-.119	.083	.059	-.187	-.121
I28	-.006	-.265	.063	*-.342*	-.192	*.310*	*-.465*
I29	.011	-.085	-.065	-.099	-.084	.027	-.247
I31	-.020	-.027	-.006	-.246	-.170	.234	-.272
I33	*-.774*	.273	.187	-.078	.134	-.170	-.290
I37	-.002	.110	.027	.181	-.060	.060	.244
I38	-.013	-.034	.024	-.119	-.198	.059	-.209
I41	.033	-.095	*.614*	*-.433*	-.032	-.243	*.319*

A graphical representation of the discriminant results is the projection of varieties in the plot of the first 2 canonical variates. Even though two-dimensional diagrams may be somewhat misleading, these plots can reveal the divergencies among groups (clusters) of varieties. Varieties whose relative distances are not significant at the $\alpha = 0.01$ level were included in the same cluster, following a UPGMA (unweighted pair group with arithmetic mean) procedure (SNEATH and SOKAL 1973, 230-234). The plot shows some varieties not included in clusters and clusters with a reduced number of members (Fig. 2). This pattern can be regarded as a good result for a discriminant process.

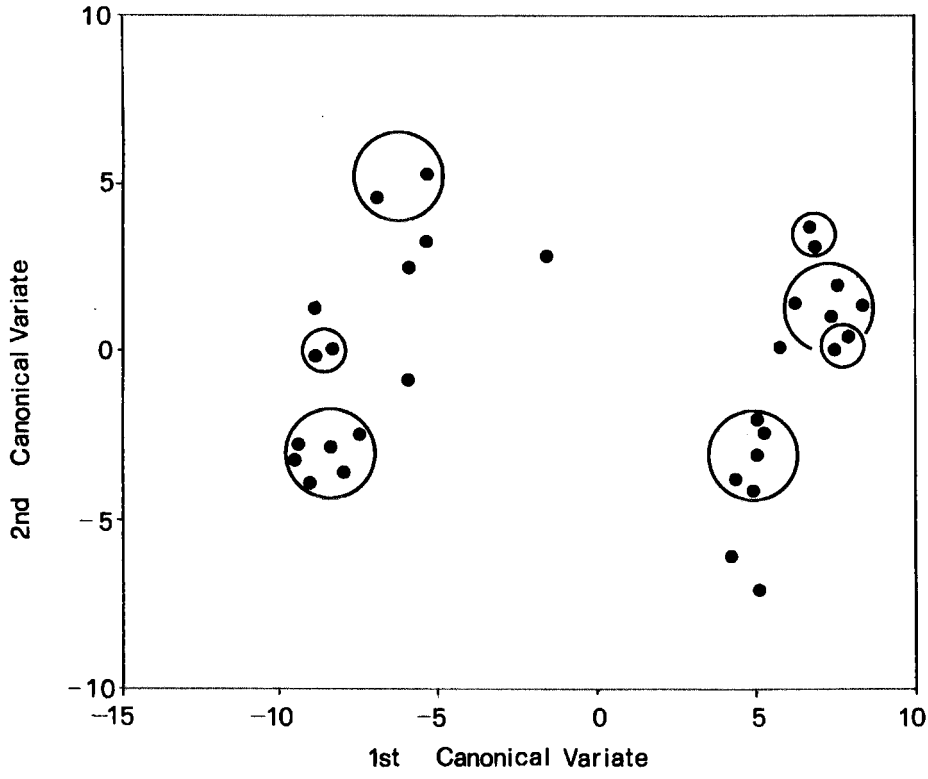


Fig. 2: Projection of variety centroids in the plot of the first 2 canonical variates. Circles include variates with relative distances not different from zero at $\alpha = 00.1$ level of significance.

Regarding the discriminating power of single original descriptor variables, the matrix of loading reveals that the first canonical variate is mainly dominated by the color of the berry skin, while in general the large weight is assumed by traits linked to vegetative characteristics of the plant. Though these results are preliminary and presented merely as an example of a statistical procedure, the standardized discriminant coefficients of the first 3 canonical variates are reported in Table 4. The inspection of figures can be of great interest when choosing the variables with a higher discriminant power in the context of the examined covariation structure.

The use of interaction effects in the context of discriminant analysis is original and we think that it can meet the requirements of a multivariate analysis based on rating scores. More particularly, assuring a good fit to normality, it allows the use of usual statistical procedures, as stepwise selection and related statistical tests to choose the most discriminant descriptors.

Acknowledgements

C. A. is grateful to Prof. T. CALINSKI for useful discussions on the topic.

Table 4: Standardized discriminant function coefficients

	Function No.		
Variable	1	2	3
I2	-.19235	.36414	-.13183
I3	-.15294	-.13837	-.05140
I4	-.22991	-.13317	-.56496
I5	.00150	-.05174	-.45131
I7	-.05234	-.34306	.04656
I10	-.31959	-.19086	.02672
I11	.40305	.31301	.03755
I12	-.23082	-.30224	-.11383
I13	-.34818	.15428	-.10186
I16	.00050	-.09670	.18503
I17	.18351	.26616	-.15830
I18	-.47610	-1.29967	.04751
I21	.40265	.35096	.07206
I22	-.25843	-.39268	-.46230
I28	-.00298	-.28686	.08117
I29	.10030	-.19566	-.25988
I31	-.18423	-.29449	-.38828
I33	-1.12760	.23569	.11122
I37	-.04240	.26009	.16466
I38	-.06007	.23255	-.07179
I41	.00432	-.38915	.80115

Literature cited

CALO, A.; COSTACURTA, A.; GIUST, M.; OTTAVIANO, E.; CAMUSSI, A.; LORENZONI, C.; 1989: Preliminar contribution to the identification of ampelographic and ampelometric features for the characterization of vine varieties (*Vitis* sp.). Riv. Viticolt. Enol. **42**, 71-76.

LUBISCHEW, A. A.; 1962: On the use of discriminant functions in taxonomy. Biometrics **18**, 455-477.

MARIOTT, F. H. C.; 1974: The Interpretation of Multiple Observations. Academic Press, London, New York.

SCHEFFÉ, H.; 1959: The Analysis of Variance. J. Wiley and Sons, New York.

SEAL, H.; 1964: Multivariate Statistical Analysis for Biologists. Meuthen and Co. Ldt., London.

SNEATH, P. H. A.; SOKAL, R. R.; 1973: Numerical Taxonomy. The Principles and Practice of Numerical Classification. W. H. Freeman and Co., San Francisco.

SRIVASTAVA, M. S.; CARTER, E. M.; 1983: An Introduction to Applied Multivariate Statistics. North-Holland, New York.

Improvement of *Vitis vinifera sativa* D. C. taxonomy

L. P. Troshin, P. N. Nedov, A. I. Litvak and N. I. Guzun

National Institute for Grape and Products of Grape Processing 'Magarach', 31, Kirov Street, Yalta (Crimea), USSR

Summary: A method of mathematical statistics for evaluation of the taxonomic usefulness of characters included in the *Vitis vinifera sativa* D. C. cultivar clustering has been developed. The method works as follows: The irregularity of the distribution of characters in taxons is calculated, and the probability of achieving such estimates is calculated when all taxons are assigned to the same general population after a certain character. The method does not depend on the type of the distribution of characters and is computer-programmed (a CM-4 computer). Using the method, it is possible to conclude that the higher the taxon hierarchy, the smaller is the amount of taxonomically useful characters the taxons differ in, i.e. in order to differentiate taxons of lower hierarchy a smaller amount of descriptive characters is necessary. Var. *transcaucasica* and var. *mediiasica* have been isolated in the taxon convar. *orientalis* subconvar. *caspica* Negr. and in the taxon subconvar. *antasiatica* Negr., while the taxon convar. *pontica* Negr. has been supplemented with subconvar. *meridionali-balcanica* and *georgica-caspica* along with subconvar. *balcanica* and *georgica* Negr.

Using discriminant analysis, assignment of many cultivars to proper taxons has been verified. The phenogenetic proximity of taxons has been established. Some problems of the theory of evolution and those of practical breeding are discussed.

Key words: ampelography, biometry, systematics, taxonomy, variety of vine, gene resources, Asia, Europe, Africa.

Introduction

Progress in the theory and practice of grape breeding is due to achievements of ampelography and genetics used by originators in creating genotypes after the models of ideal cultivars (Golodryga and Troshin 1978). Understanding of the combining ability of gene complexes of the initial forms depends on their assignment to proper taxons.

Studies of wild grape in Bulgaria (Negroul *et al.* 1965), Moldavia (Yanushevich and Peliakh 1971), Daguestan (Pirmagomedov 1980), Georgia (Ramishvili 1988) and in the Crimea (Troshin and Sviridenko 1980) have shown a relationship between the local cultured and wild grape and have made it possible to establish its origin.

The origins of some large-fruited table grape cultivars still need elucidation. Vidal (1948) suggested that large-fruited wild grape growing in the Atlas Mountains may have been used as initial material for popular selection of large-fruited grape cultivars in North Africa.

Taking into account the vast area of wild grape distribution and its isolated location in certain regions with different natural and climatic conditions, it is impossible to consider the species *Vitis silvestris* Gmel. homogeneous (Vavilov 1931; Vasilchenko 1955). In every ancient centre of culture, wild grape has its own morphological and biological peculiarities, which makes it necessary to name it after the places of growth(the Daguestan grape, the Crimean grape, the East Georgean grape, the West Georgean grape, the Transcarpathian grape, etc.).

Local grape cultivars are also classified by authors after their places of origin.

According to the classification of Negroul (1946, 1968), grape cultivars are arranged into three ecological-geographical groups. Several hierarchic taxons have been isolated in two of these groups, but Negroul himself thought that the problem needed further elucidation. Studies on grape classification were also done by Ivanova (1947), Aliyev (1956), Nemeth (1967), Gramotenko (1975, 1978), Tsertsvadze (1986).

Materials and methods

Grapevines on their own roots (studied since 1970) and grafted plants (studied since 1983) were described and measured according to standard ampelographic methods. In the matrices of initial data, 30 quantitative characters were determined using a natural scale (scale of relations), and 74 qualitative characters were established using a numerical scale (scores 1-5) (Methodical Instructions on Grape Breeding, 1974). More recently, the O.I.V. numerical scale (1984) (scores 1-10) was used.

The quantitative characters included 11 morphometrical, 6 phenological, 9 biological-agricultural, 2 chemical-technological and 2 adaptive characters. Botanical, uvological, physiological and other characters were considered alternative (Troshin and Fedorov 1988).

Grape cultivars were classified after complexes of characters according to their origin (Gramotenko and Troshin 1988). The validity of the classification was checked using methods of cluster and discriminant analysis (Kendall and Stuart 1976).

In establishing patterns of a classification it is necessary to determine the taxonomic usefulness of characters. For this purpose, the criterion of Kruskal and Wallis can be useful (Zaitsev 1984), but we concluded that taxonomically useful characters were those minimizing transgression of clusters of the 'teaching' sampling. Of non-dimensional methods, the criterion of Mann-Whitney can be used as index of the transgression value (Sachs 1972), but its disadvantage is that, with more than two clusters and a considerable transgression among some of them, the value of the total criterion may become so large that even under condition of complex isolation of several clusters the character will be considered insignificant.

We suggest a criterion of our own which is based, for all pairs of clusters, on how many times cultivars of the first cluster are included into the variation range of the second cluster. Thus, the criterion is defined as follows:

$$\text{Criterion} = \sum_{j=1}^{N} \sum_{i=1}^{M_j} \sum_{k=1}^{N} A_{ijk} \; ; \text{ where:}$$

N – amount of groups
M_j – amount of cultivars in the jth group
A_{ijk} = 1 if the value of a character of the ith cultivar belonging to the jth group is included into the variation range of the kth group; otherwise,
A_{ijk} = 0.

In determining the significance of the criterion, we tried to make theoretical calculations of its critical values considering the values of the criterion between pairs of taxons to be an independent sampling from a corresponding distribution. But the check using the method of Monte Carlo (Afifi and Azen 1982) showed substantial systematic deviations from the values obtained. It is due to the fact that with 3 taxons, A, B and C, for instance, the value of the criterion for taxons A and B depends to a great extent on its values for A - B and B - C. Thus, the sampling cannot be considered independent. We failed to take into account the relationships and chose the method of Monte Carlo.

We made a special program for a CM-4 computer to calculate numerical values of the criterion to sort out characters according to the value of the criterion and to estimate the probability of error, when the assumption that all the taxons belong to the same general population after this or that character was omitted. In doing so, there is no need to produce the numerical value of the criterion as it is not the values themselves that count but the importance of every character for the classification.

The estimates of the taxonomic usefulness of characters do not depend on the type of distribution of large samplings and can be calculated with as few as two cultivars in a taxon;

naturally, the larger the amount of cultivars, the more reliable are the estimates (Troshin *et al.* 1988).

Results and discussion

The results obatined of the classification of grape cultivars made it possible to isolate some new taxons in addition to those already known (Gramotenko and Troshin 1988).

Cultivars of the taxon convar. *boreali-africana* Gram. are characterized by a long vegetation period, late budbreak, very late leaf fall and weak resistance to frosts and fungal diseases. The taxon contains local table and wine cultivars of Morocco, Algeria and Tunisia, e. g. Ahmar bou Ahmar, Farrana, Khadari, Ribier, etc. Their adaptivity in the European part of the USSR is poor, but they can be of interest there as initial material in breeding for large-fruitedness.

Cultivars of the taxon *orientali-mediterranea* Gram. are characterized by a long vegetation period, weak frost resistance, firm berry flesh and tough berry skin; in many cultivars, muscat-flavoured fruit is common. As far as their morphological and biological characters are concerned, these cultivars have a certain phenotypic similarity to those of the previous group, but the East Mediterranean group is older and differs genetically from the other ones. Cultivar types of muscadine grape and Chasselas are examples of this group.

Grape cultivars of Spain, Portugal and the southern part of France differ substantially from those of Germany and the central part of France. The former cultivars are characterized by a long vegetation period, late leaf fall and weak frost resistance. As far as their biological characters are concerned, cultivars of the south-western part of Europe are closer to the North African ecological-geographical group though with regard to their geographical position they enter the West European group. That is why the subgroup subconvar. *pyrenaica* Gram. was established within this group. In table cultivars, Bican, Vermentino, Gros Vert, Catalon d'Hiver are representatives of this subgroup, while Mourvedre, Morastel, Sersial are its representatives in wine cultivars.

The South Balkan subgroup subconvar. *meridionali-balcanica* Trosch. can be considered analogous to the previous subgroup as far as its origin is concerned. The South Balkan subgroup contains cultivars Limberger, Kabassia, Kadarka, Maisky tcheurny, etc. characterized under conditions of the Crimea, the Don and the Kuban regions by low sugar content and larger size of leaves, berries and bunches. Greek and Albanian cultivars are also included into this subgroup.

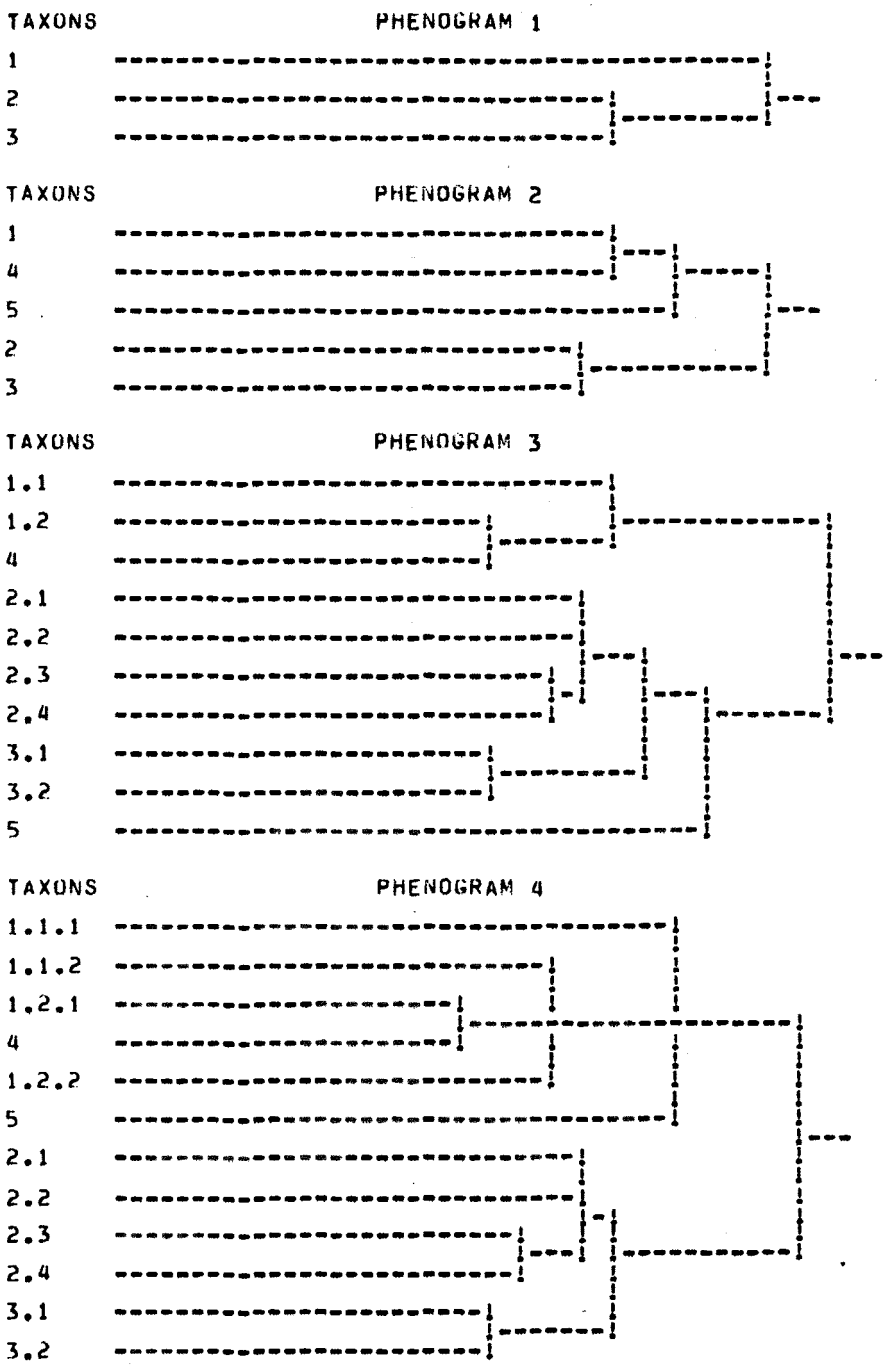
Some Georgean grape cultivars of this subgroup subconvar. *georgica* Negr. which is included into the Black Sea Basin ecological-geographical group have characters of the group of eastern wine grape *orientalis caspica* Negr. Their intermediate position between these ecological-geographical subgroups made it necessary to arrange them into the subgroup subconvar. *georgica-caspica* Gram. contained by the Black Sea Basin ecological-geographical group. This separate subgroup contains cultivars Rkatziteli, Tchinouri, Boudechouri tetri, Sirgoula, Goroula, etc.

We developed the taxonomic scheme of Negroul having classified Transcaucasian and Middle Asiatic cultivars with regard to their uses, too.

While establishing phenogenetic relationships among taxons, we evaluated the taxonomic usefulness of characters. In doing so, values of quantitative characters were transformed into scores of the numerical scale as required by the use of methods of multidimensional analysis.

Having analyzed the taxonomic usefulness, estimates of 104 characters of 120 grape cultivars arranged into 12 taxons revealed the following correlation: the higher the taxon hierarchy and the larger the amount of taxons of the same level, the smaller the amount of taxonomically useful characters the taxons differ in. Thus, in order to classify taxons of a lower level (cultivar types, cultivar groups), a smaller amount of descriptive characters is necessary (Troshin and Fedorov 1988).

Taxonomically useful characters ($P < 0.05$) for 12 taxons had the following probability estimates (%):



Phenograms of cluster analysis: Phenogram 1 - 3 taxons; Phenogram 2 - 5 taxons; Phenogram 3 - 10 taxons; Phenogram 4 - 12 taxons. The names of taxons are given in the text.

- cobwebby pubescence among veins of young leaf (O.I.V. descriptive character code 053): 100
- cobwebby pubescence among veins of mature leaf (084): 100
- berry size (220): 100
- berry shape (223): 100
- texture of berry flesh (235): 100
- cobwebby pubescence of shoot tip (004): 99
- duration of interphase period 'beginning of budbreak - beginning of bloom': 99
- coefficient of shoot fruiting (total of bunches/total of shoots ratio): 96
- field resistance of leaves to mildew: 95
- degree of lignification of terminal shoots: 89
- length of shoot internodes (353): 78
- bunch size (202): 75
- berry length (221): 68
- use of fruit: 66
- average bunch weight: 56
- coefficient of shoot fruitfulness: 44
- blistered aspect of upper surface of leaf blade (075): 39
- degree of shoot fruitfulness: 33
- titratable acidity of juice: 33
- density of fruit in bunch (204): 19
- color of berry skin (225): 14
- degree of lignification of shoots: 12

Taxons were arranged into clusters after taxonomically useful characters and congruence of cultivars with corresponding taxons was estimated using discriminant analysis. The results obtained are shown in phenograms (see Fig.).

Phenogram 1 shows that the taxons convar. *pontica* NEGR. (2) and convar. *occidentalis* NEGR. (3) reveal a greater degree of the phenogenetic similarity to each other after a complex of characters than, if compared separately, to the taxon convar. *orientalis* NEGR. (1). This disagrees with NEMETH's (1967) belief that the ecological-geographical group convar. *pontica* NEGR. is the oldest and the group convar. *occidentalis* NEGR. is the youngest. Cultivars of these two taxons could have been formed during thousands of years, both on the basis of local wild grape under artificial selection and as a result of introgression of genes of cultured forms coming from warmer Mediterranean countries.

The phenograms obtained show that the taxon convar. *orientalis* NEGR. differs from the taxons convar. *pontica* NEGR. and convar. *occidentalis* NEGR., longer lines in the phenogram of the former taxon indicating later evolutionary changes. Earlier, NEGROUL (1968) and SMIRNOV (1974) reported the origin of Middle Asiatic cultivars to be secondary.

Using ampelographic data of the two ecological-geographical groups established by GRAMOTENKO (1978) in doing cluster analysis made it possible to affirm the phenogenetic relationship between the taxons convar. *pontica* NEGR. and convar. *occidentalis* NEGR. and to reveal that the taxons convar. *orientalis* NEGR. and convar. *boreali-africana* GRAM. (4) formed another phenone showing in its turn a greater degree of similarity to convar. *orientali-mediterranea* GRAM. (5) than to each of the former 2 taxons (Phenogram 2). The intermediate position of the taxon convar. *orientali-mediterranea* GRAM. shows that genes of its cultivars are found in cultivars of the remaining 4 taxons. It agrees with literature data concerning the earlier origin of East Mediterranean cultivars, which reflects the development of human civilization since the Mycenaean times.

Phenogram 3 also shows the intermediate geographical position of the taxon convar. *orientali-mediterranea* GRAM. The phenone convar. *occidentalis* NEGR. is made by the subgroups subconvar. *gallica* NEM. (3.1) and subconvar. *pyrenaica* GRAM. (3.2), and the phenone convar. *pontica* NEGR. contains the subgroups subconvar. *georgica* NEGR. (2.1), *balcanica* NEGR. (2.2), *meridionali-balcanica* TROSCH. (2.3) and *georgica-caspica* GRAM. (2.4), the subgroups within each

phenone being closely related. It can be seen from the phenogram that the subgroup subconvar. *caspica* NEGR. (1.1) contained by the eastern ecological-geographical group of cultivars is older than the subgroup subconvar. *antasiatica* NEGR. (1.2), which agrees with the data of SMIRNOV *et al.* (1987).

Further arrangement of data into clusters made it possible to obtain Phenogram 4 showing that the taxons convar. *occidentalis* NEGR. and convar. *pontica* NEGR. preserved their hierarchy in regard to the other taxons. The taxons convar. *orientalis* NEGR., *boreali-africana* GRAM. and *orientali-mediterranea* GRAM. formed a separate phenone. Within this phenone, the taxons convar. *boreali-africana* GRAM. and convar. *orientalis* subconvar. *antasiatica* NEGR. var. *transcaucasica* GRAM. *et* TROSCH. (1.2.1), i. e. North African and Transcaucasian table cultivars, revealed the highest degree of relatedness. Transcaucasian wine cultivars (1.1.1) along with East Mediterranean cultivars are the oldest. Thus, it is possible to conclude that Middle Asiatic wine (1.1.2) and table (1.2.2) cultivars were formed later.

Conclusions

Based on ampelographic material including representatives of various ecological-geographical groups of cultured grape, we made an attempt to improve the natural system of classification.

As the results obtained show, the principal centre of cultured grape origin is the East Mediterranean region from where grapevine moved to the eastern, northern and western parts of Eurasia and to the north of Africa following great trade ways of ancient civilisations and with migrations of ancient tribes. This conclusion agrees with the opinion of DE CANDOLLE (1885).

Using methods of modern taxonomy in order to improve the natural system of *Vitis vinifera sativa* D. C. made it possible to find grounds for the relative independence of the 5 ecological-geographical groups and to establish the divergence of taxons within these groups.

The improved taxonomy of cultured grape with isolated hierarchic groups of cultivars of the Euro-Asiatic species makes it possible to provide a more rational introduction of planting material into new regions of culture, to develop agrotechnical methods aimed at increasing productivity and to more effectively use cultivars of various taxons in creating new adaptive genotypes.

Literature cited

AFIFI, A. A.; AZEN, S. P.; 1979: Statistical Analysis. A Computer Oriented Approach. Academic Press, New York, San Francisco, London.

ALIYEV, A. M.; 1956: On grape assortment of the Daguestan ASSR [Russ.]. Bulletin of Scientific and Technical Information of the Research Institute for Viticulture and Enology, 1, 32-34, Novocherkassk.

DE CANDOLLE, A.; 1885: Origin of Cultured Plants [Russ.]. St. Petersburg.

GOLODRYGA, P. YA.; TROSHIN, L. P.; 1978: Biological and technical program of breeding new highly productive grape varieties with complex resistance [Russ.]. In: Grape Genetics and Breeding for Immunity, 259-264. Naukova Dumka, Kiev.

GRAMOTENKO, P. M.; 1975: Natural cultivar types of the eastern ecological-geographical group of grape cultivars (convar. *orientalis* NEGR.) [Russ.]. In: Works on Applied Botany, Genetics and Breeding 54 (2), 156-166. VIR, Leningrad.

-- --; 1978: Improvement of the classification of the European-Asiatic group of grape cultivars [Russ.]. In: Main Directions of the Development of Enology and Viticulture in the USSR. Theses of the Reports for the National Symposium on the Occasion of the 150th Anniversary of the National Institute for Enology and Viticulture 'Magarach', 88-90. Moscow.

-- --; TROSHIN, L. P.; 1988: Improvement of the classification of *Vitis vinifera* L. [Russ.]. In: Prospects of Grape Genetics and Breeding for Immunity, 45-52. Naukova Dumka, Kiev.

IVANOVA, E. B.; 1947: Ampelographic Study on Grape Cultivars of the Khalili Group [Russ.]. Tashkent.

KENDALL, M. G.; STUART, A.; 1976: The Advanced Theory of Statistics. Design and Analysis, and Time-Series [Russ.]. Nauka, Moscow.

NEGROUL, A. M.; 1946: Origin and classification of cultured grape [Russ.]. In: The Ampelography of the USSR, Vol. 1, 159. Pischepromizdat, Moscow.

---; 1968: Problems of grape origin and breeding on the genetic basis [Russ.]. Genetika **3**, 84-97.

--- *et al.*; 1965: Wild Grape of Bulgaria [Russ.]. Kolos, Moscow.

NEMETH, M.; 1967: Ampelografiai Album. Budapest.

O.I.V.; 1984: Code des caractères descriptifs des variétés et espèces de *Vitis*. Office International de la Vigne et du Vin, Paris.

PIRMAGOMEDOV, P. M.; 1980: Wild grape *Vitis silvestris* in Daguestan [Russ.]. Bot. Zh. **68**.(8), 1177-1184.

RAMISHVILI, P. M.; 1988: Wild Grape of the Transcaucasus [Russ.]. Ganatleba, Tbilisi.

SACHS, L.; 1972: Statistische Auswertungsmethoden. Springer-Verlag, Berlin, Heidelberg, New York.

SMIRNOV, K. V.; 1974: Origin and formation of grape assortment in Uzbekistan [Russ.]. In: Grape Varieties of Uzbekistan, 14-28. Tashkent.

--- *et al.*; 1987: Viticulture [Russ.]. Moscow.

TROSHIN, L. P.; ADIBEKOV, O. V.; FEDOROV, YU. K.; 1988: Computers in grape ampelography and breeding [Russ). In: Prospects of Grape Genetics and Breeding for Immunity, 53-61. Naukova Dumka, Kiev.

---; FEDOROV, YU. K.; 1988: Biometrical Analysis of Grape Genofond [Russ.]. National Institute for Grape and Products of Grape Processing 'Magarach', Yalta.

---; SVIRIDENKO, N. A.; 1988: Resistant Grape Cultivars [Russ.]. Tavriya, Simferopol.

TSERTSVADZE, N. V.; 1986: Classification of cultured grape *Vitis vinifera* L. in Georgia [Russ.]. In: Scientific and Technical Progress in the Viticulture of Georgia, 229-240. Tbilisi.

VAVILOV, N. I.; 1931: Wild relatives of fruit trees of the Asiatic part of the USSR and the Caucasus and the problem of origin of fruit trees [Russ.]. In: Works of Applied Botany, Genetics and Breeding, **26** (3), 93. Leningrad.

---; 1987: The Five Continents [Russ.]. Nauka, Leningrad.

VASILCHENKO, I. T.; 1955: New Grape Species for Culture [Russ.]. Acad. Sci. USSR, Moscow, Leningrad.

VIDAL, J. P.; 1948; La Vigne au Maroc.

YANUSHEVICH, Z. V.; PELIAKH, M. A.; 1981: Wild Grape of Moldavia [Russ.]. Acad. Sci. MSSR, Kishinev.

ZAITSEV, G. N.; 1984: Mathematical Statistics in Experimental Botany [Russ.]. Nauka, Moscow.

Activities concerning conservation of *Vitis* germplasm

ERIKA DETTWEILER-MÜNCH

Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, F. R. Germany

Summary: Activities in the field of *Vitis* germplasm conservation in the Federal Republic of Germany are reported. The results of the worldwide inventory of *Vitis* species, cultivars and genotypes are given.

The questionable identity of grapevine cultivars requires appropriate identification tools. Efforts to improve the currently performed methods in this field are described.

The necessity of national and international collaboration is stressed.

Key words: Vitis, germplasm, gene resources, gene bank, ampelography, ampelometry.

Introduction

The loss of plant species is a worldwide stateable phenomenon. Likewise all the grape growing nations are concerned by the loss of genetic resources of *Vitis* species and cultivars, due to expanding cultivation causing the loss of land races and due to progressing civilization and natural disasters.

For example the population of *V. silvestris* was decimated from some 1,000 plants recorded in the last century by BRONNER (1857) (quoted at SCHUMANN 1974) to about 100 specimens recorded by SCHUMANN (1976) today. In the USA similar phenomenons have been observed. For excample, COMEAUX (1984) did not find *V. rupestris* at those sites in Texas described by MUNSON (1909) (quoted at COMEAUX 1984) at the beginning of this century. With the invasion of phylloxera the decline of land races started and nowadays through restrictive legislation old cultivars are omitted anyhow.

Breeding makes use of genetic resources to provide viticulture with cultivars which are adapted to cultivation requirements and more disease resistant. In addition to the search for resistance genes, genetic resources will always be needed as a reservoir for future, still unknown requirements. That is why a maximum of genetic diversity is indispensable.

This attempt is supported by the OIV and the IBPGR. At the beginning in 1984 in close collaboration with both organizations, the Federal Centre of Grapevine Breeding (BFAR) started an inventory of the worldwide existing *Vitis* species, cultivars and genotypes.

Results and discussion

Some of the results are shown on the following tables.

Table 1 gives a general view of the results:

Information was received from 34 countries, 106 lists of grapevine collections were analysed, further information was obtained through 300 ampelographies and varietal descriptions.

The number of recorded prime names is 15,382. The number of recorded synonyms is 11,430 and the number of standing places for all genotypes is 25,742.

Table 2 shows the number of genotypes from collection lists and/or from literature:

The total number of accessions - 15,382 - is divided in genotypes we know from collections and from literature, which are 10,511 and 9,822 respectively.

Table 1: Information obtained from

Countries	34
Grapevine collections	106
Ampelographic publications	300

Results

n° prime names	15 382
n° synonyms	11 430
n° standing places in collections	25 742

Table 2: Number of cultivars known from collection lists and/or from literature

n° prime names	15 382
– in collections	10 511
– in literature	9 822
n° prime names	
– in collections and literature	5 276
– in collections only	5 235
– in literature only*	4 546
– without indications	325

* there seem to be no living examples any more

Table 3: Repartition of prime names on *Vitis* species

	Total	in collections pure	in collections hybrid	in literature only* pure	in literature only* hybrid	no coll.* no lit.
V. vinifera	9345	4627	1722	2320	475	201
I.C.	4225	2909		1189		127
V. species	677	203	83	272	110	9
without specification	1155	950	10	150	17	28

* the loss of these genotypes is probable

Genotypes found in collections and also described in literature are 5,276, found in collections only are 5,235 and described in literature only are 4546 genotypes; without any specifications are 325 individuals.

Most of the genotypes mentioned only in literature have probably been lost.

Table 3 shows the number of the recorded prime names alotted to *V.* spp.:

9,345 recorded prime names belong to *V. vinifera*, 4,225 are interspecific hybrids, 677 belong to *V.* spp. and 1,155 are of unknown membership. The most important column is where genotypes mentioned only in literature are listed. They may no longer exist.

About 2,320 pure *V. vinifera*, probably old land races, have disappeared as have 475 *V. vinifera* crossings, 1,189 interspecific hybrids, 382 *V.* spp. and descendants and 167 of unknown *V.* spp. membership.

The questionable identity of many grapevines complicates the conservation of the grapevine genetic resources. If a cultivar is held under different names, it may be conserved twice or more. On the other hand, if one name is utilized for several different genotypes, some genotypes may be unintentionally lost.

Therefore, identification of grapevines is urgent. Considering the large number of different grapevines in the world (12-15,000), it is important that identification can be accomplished with a minimum number of descriptors. The descriptors should ensure an objective evaluation. Measurable characteristics are prefered because they are reproducible. Furthermore, descriptors should be little influenced by environmental conditions and they should be quick and easy to record and should not depend on a high technical equipment.

My doctoral thesis (DETTWEILER 1987) was carried out with these requirements in mind. 39 grapevines were described through measured leaf characters. Recording of data was made from leaves of three different climatic areas: arid – Jerez de la Frontera/Spain, humid – Lausanne/Switzerland, and an intermediate climate – Montpellier/France. Data processing was performed using discriminant analysis. A mathematically reduced number of 21 descriptors and the berry color were sufficient to obtain a 90 % identification accuracy. An identification analysis

was calculated with data obtained from leaves of 12 cultivars collected at sites other than those mentioned above. The average recognition accuracy was 87 %.

Based upon these results, the following research is currently being performed:

(1) The number of grapevine cultivars treated by discriminant analysis will be increased to test the performance of this identification tool

From more than 500 genotypes leaves have been collected and will be added to the system. But indispensable for varietal recognition is the establishment of the basic model with specimens coming from different climatic areas. Therefore, the collaboration of collection holders from arid regions would be very much appreciated. Interesting collections would be for example:

Greece: Thessaloniki
Italy: Palermo, Bari, Tormancina, Rome
Portugal: Regua, Oeiras
Soviet Union: Tibilissi
Tunesia: Ariana
Turkey: Izmir
Yugoslavia: Novi Sad, Split

(2) The usage of additional berry and seed characteristics

Initial results suggest that measured berry and seed characters are suitable for varietal description and could increase the identification accuracy.

(3) The computerized evaluation of leaf, berry and seed descriptors

Leaf characters will be recorded by means of a digitizer tablet. A computer program has been developed for this purpose. Likewise recording of berry and seed characters will be done with the computerized evaluation of their pictures.

(4) The quest for the application of a minimal descriptor list composed of measurable and notable leaf, berry and seed descriptors

This evening in the workshop 'Ampelographic Methods', we will discuss the principal descriptors for identification and we intend a resolution recommending a preliminary minimal descriptor list and its comprehensive application.

(5) The additional application of isoenzyme analysis and RFLP

At BFAR, Prof. BLAICH and Dr. BACHMANN are currently investigating RFLP and isoenzyme analysis respectively as tools for varietal description. These methods are to be applied if the identification by morphological features is not possible.

Documentation data obtained from the inventory of the worldwide existing grapevines, including their descriptions will be transferred to a national data base if it is established. Information on grapevine species and cultivars would be available from there.

In Germany the establishment of a national germplasm repository is planned. In 1987 the project group 'Plant Genetic Resources in the Federal Republic of Germany' foresaw the installation of specific 'Advisory Committees' for different plant species. Anticipating the implementation of such an 'Advisory Committee for Grapevines', a working group for 'Grapevine Genetic Resources' was initiated. Members are the collection holders from 8 viticulture institutions in Germany. Future goals are the double conservation of genotypes at two different sites, their description and evaluation, the control of virus-free status, the exchange of genetic material and the announcement of any intended changes.

Table 4 is listing the actual number of *V. vinifera*, interspecific hybrids and *V.* spp. existing in German collections:

Table 4: Distribution of grapevine genotypes in the German collections

	occurrence in collections 1 x	2 x	3 x	total number of genotypes	
Total number of genotypes	1272	294	294	1860	
V. vinifera	479	164	211	844	
pure genotypes (- old cultivars)	286	103	110	490	land races
hybrids	193	61	101	355	
I.C.	686	101	60	847	interest in disease resistant genotypes
V. species cultivars	23	8	6	37	
V. species	62	19	17	98	V. species and clones (V. silvestris)
V. species membership unknown	32	2		34	

The total number of distinct genotypes in German collections is 1,861. 1,272 occur in only 1 collection, 294 exist in 2 collections and the same number can be found in at least 3 collections. 844 are *V. vinifera* cultivars, of those no parentage is indicated for 479, so they could be considered as land races. 847 I.C. are maintained in German collections, which reveal the great interest in Germany for genetic resources providing disease resistant genes. 98 *V.* spp. and clones are registered, of that 48 are *V. silvestris* clones.

As I mentioned before, the aim of the German germplasm repositories is the maintenance of genotypes at two different sites to avoid their loss. This means for the more than 1,277 genotypes, currently found in only 1 collection, a 2nd location must be found.

But, at the moment, no adequate evaluation systems for screening the genetic material exist. Therefore, all of them should be retained.

It should be discussed soon how many genotypes should be kept for specific biotic and abiotic stresses. Is it sufficient to keep for example about 50 genotypes resistant to plasmopara or phylloxera or about 20 genotypes for seedlessness if the material assembled comes from the different centers of diversity, America, Eastern Asia and 'Eurasia'?

On an international level since 1984 a bilateral cooperation exists between the Research Institute of Jerez de la Frontera/Spain and the BFAR. The complementary conservation of grapevines is planned. Frost resistant and disease resistant cultivars could be maintained at the Geilweilerhof station, whereas frost susceptible or mediterrean cultivars could be maintained in the Jerez collection.

Besides the conservation *in vivo* at the BFAR, the long-term storage of 675 genotypes *in vitro* and seeds of *V.* spp. is intended to maintain their genetic diversity.

As I mentioned before, varietal identification is dependent on the description of individual cultivars in at least two different climatic areas. Therefore, the Jerez grapevine collection, consisting of about 1,400 genotypes, will also be described.

It would be very desirable if collection holders could contribute and assist in the efforts to identify and to maintain the grapevine genetic resources.

References

COMEAUX, B. L.; 1984: Taxonomic Studies on Certain Native Grapes of the Eastern United States. Thesis, North Carolina State Univ., Raleigh (USA).

DETTWEILER, E.; 1987: Ein Modell zur Unterscheidbarkeit von Rebsorten mit Hilfe blattmorphologischer Merkmale. Diss., Univ. Hohenheim, BR Deutschland.

SCHUMANN, F.; 1974: Untersuchungen an Wildreben in Deutschland. Vitis **13**, 198-205.

Studies on germplasm resources of wild grape species (*Vitis* spp.) in China

ZHANG FENGQIN, LUO FANGMEI and GU DABIN

Beijing Botanical Garden, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Summary: China is the country rich in germplasm resources of the genus *Vitis*, with nearly 40 native species. This paper introduces into their geographical distribution and chief characteristics, utilization and research on 11 wild species.

Key words: gene resources, China, geography, Vitis, variety of vine, new breeding, ampelography, enzyme, selection, must quality, frost resistance, disease resistance.

Introduction

China has a vast territory with complex geographical environments and greatly different climate, soil and topography in various areas, possessing various species of plants. Among these there are abundant species of Vitaceae and *Vitis* germplasm resources. The collection of *Vitis* species and study on them made by the present authors over many years show that nearly half of the 80 or so species of *Vitis* in the world are native to China (and 10 more new species yet to be published), some of which have been directly used in winemaking industry or used for breeding or as rootstock, and *V. amurensis* is the most valuable one. By means of updated biological techniques an overall study of wild grape germplasm resources in China may offer a glorious prospect for breeding.

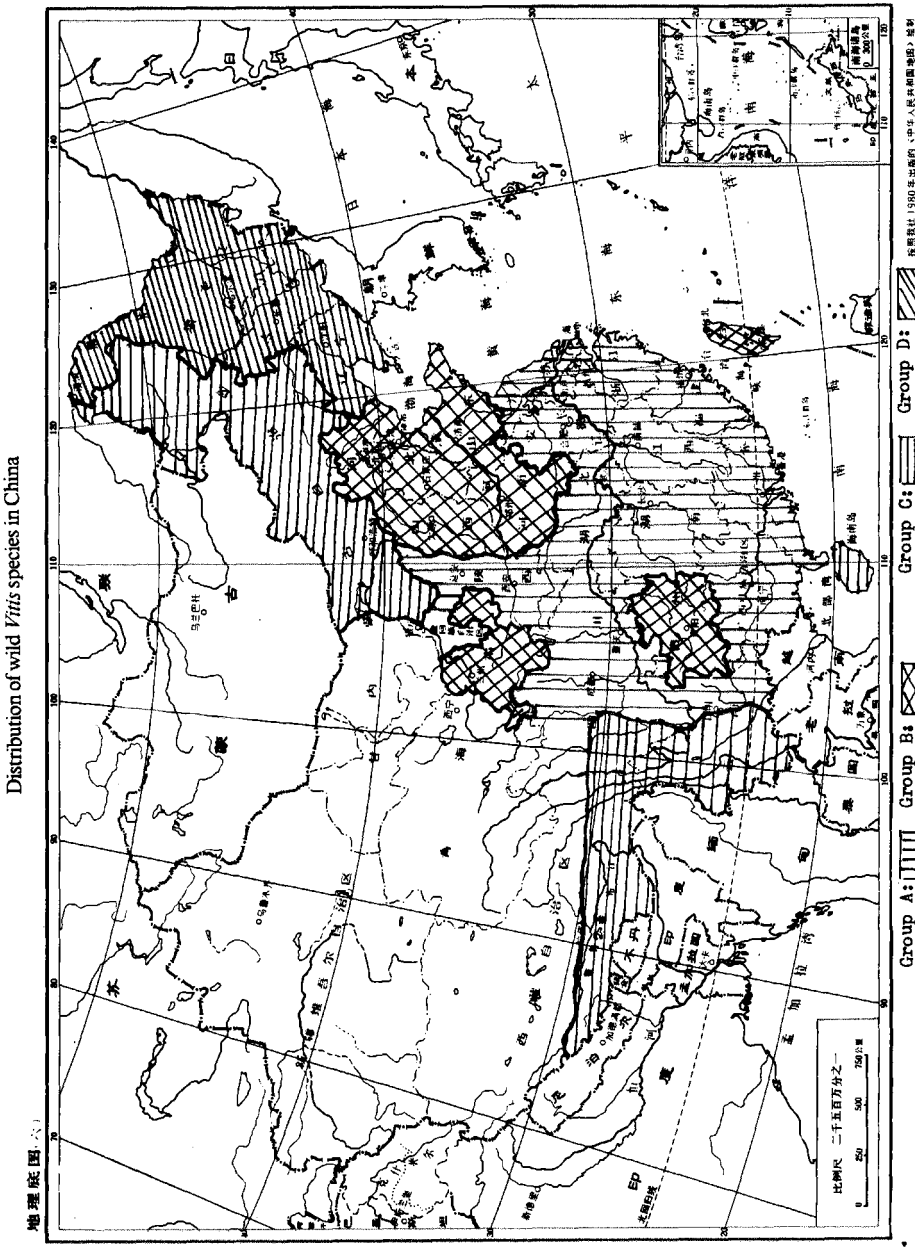
I. Distribution of wild grape germplasm resources in China

There are over 30 species of the genus, distributed almost throughout China. According to their geographical distributions, one may distinguish 4 groups: Group A of 20 species are in Jiangxi, Guangdong, Hubei, Hunan, Henan, Zhejiang, Shaanxi, Fujian, Sichuan, Jiangsu, Anhui, Guangxi, Yunnan, with 12-20 species in each province. Group B of 10 species are in Guizhou, Gansu, Hebei, Shanxi, Shandong, Taiwan, with 6-10 species in each. Group C of 3 species are in Hainan, Tibet and Inner Mongolia, with 2-3 species in each. Group D has only one species in Heilongjiang, Jilin and Liaoning. The distribution map shows that *Vitis* species are most numerous in the middle and lower reaches of the Yangtze River and the region of the Nanling range, whereas in the vast areas of Northwest and Northeast China the number of wild species is very small, and it is noteworthy that in North-East China there is only one species, *V. amurensis*. This is due to their different places of origin, ecological conditions in their areas of distribution and the result of evolution and development of the species.

1. From the map of distribution it can be seen that Jiangxi, Guangdong, Hubei, Hunan, Henan, Zhejiang Provinces have the most species, with over 17 species in each. The further away from this central area of distribution, the fewer species there are. For example, only 1 species is found in North-East China, in Inner Mongolia 2, in Tibet 3, and in Hainan Province 3.

2. Among these species *V. flexuosa* is most widely distributed, followed by *V. ficifolia, V. quinquangularis* REHD., *V. davidii* FOEX, *V. bryoniaefolia* BGE.; *V. amurensis* and *V. yenshanensis* are not found in this area.

3. The central area of distribution is not only abundant in the number of species, but also has species which are regarded as primitive, such as *V. quinquangularis, V. davidii, V. davidii* var.



cyanocarpa, *V. pseudoreticulata* and *V. romanetii*; *V. piasezkii* and *V. wenchowensis*, *V. adstricta* var. *ternata*, *V. hui* are endemic species in the area.

4. Based on the study of the morphology, origin, distribution and eco-geography of each of the species, as well as on their quantitative classification and isoenzyme analysis, we regard Central China as the center of distribution of the *Vitis* species in China, and probably also their center of origin.

II. Several important wild species of grape

1. *Vitis amurensis* Rupr.

The growing tip is yellow-green with a purplish red tone and covered with thick, long, canescent tomentum. The young leaf is yellowish green with a light purplish red tone. The lower surface of the young leaf is covered with extremely thick, light yellow tomentum. The leaf is thick and rough. The leaf is 3-5 lobed or entire, with shallow and obtuse serrature and with setae on the vein of the lower surface. The petiole is often shorter than the mid vein. In autumn, the leaf turns bright purplish red.

It is dioecious, but some have perfect flowers. The cluster is conical or cylindric. The berries are uniform in size, loosely born. The mean length of the clusters measures 12.0 cm, the width 6.7 cm, each weighs 38 g. The berry is small, weighing 0.56 g, near round, diameter near 1 cm, dark purple, with thick skin, less flesh and juice, tastes very sour; on average there are 4 seeds in each berry.

Trained on espalier, the vine is vigorous. In Beijing, it breaks bud in early April, 10-15 d earlier than other common cultivars of *V. vinifera*, and blooms in mid May. In mid and late July shoots begin to mature; the berries ripen in mid August. The growing period is 137 d on average. Active temperatures sum up to 2985 °C. The fertility of bearing canes is best between the 4th and the 9th nodes, in general a shoot bears 1-2 (3) clusters. The juice is bright reddish purple. The juice extraction rate is 51.3 %, the °Brix content 10.5 %, the acid content 2.53 %.

The distribution of species is the most north of China. This species is very hardy. It tolerates severe below zero temperature down to −40-52 °C. It is resistant to powdery mildew, scab and white rot, but not to downy mildew. It is mainly used locally for red wine, but also as pigment for food. It is also a valuable hardy and disease resistant parent for breeding.

2. *Vitis ficifolia* Bunge

The young shoots, petioles and rachis are covered with thick, white, arachnoid tomentum which is later lost. The mature shoot presents dark brown color, covered with thicP, white pubescence. The tendril is long, up to 20 cm but few in number. The growing tips are yellowish-green with a light yellow tone, and covered with thick, milky, arachnoid tomentum. The young leaf is yellowish-green with a dark red tone. The lower surface of the leaf is covered with thick, milky and arachnoid tomentum. Sometimes the lower leaves have a ferrugineous tone. The leaves are thick, scabrous, dark green, finely serrated, 11-25 cm long, most with 3 shallow lobes or entire, 5 deep lobes at lower part of vines. The leaf stalk is often shorter than the mid vein. In autumn, the leaf turns brownish yellow.

The cluster is nearly conical, small and loose; the berries are uniform; the length and width of clusters are 14.5 cm and 9.35 cm respectively, each cluster weighing 29.5 g on average. The berry is small and round, weighing 0.81 g, black, with thick skin and little pulp, tastes sour and astringent; most berries contain 1-2 seeds.

Under espalier training, the vine growth is moderate. Its phenological periods are later than those of common cultivars of *V. vinifera* and a month later than that of *V. amurensis*. In Beijing, it breaks bud in mid April and blooms in early June. The shoot begins to mature in late August and has reached maturity in early October. The growing period lasts 158 d. The accumulated active temperature requires for ripening 3518 °C. Its fruiting ability is the best of all wild species. Over 80 % of all buds form fruiting shoots, often the canes bear 5-7 fruiting shoots, some up to 10, each with 3-4 (5) clusters. The juice is dark red; the juice content is 57.5-63.8 %, the °Brix content 12.2-14.6 %, the acid content 1.35-1.73 %.

This species tolerates below zero temperature down to -20 °C. It can survive winter safely without being buried in North China. It is resistant to scab, powdery mildew, downy mildew. As the berry is small and of poor quality it is not used as table grape. Since the lower surface of the leaf

is covered with very beautiful thick, silvery tomentum, it is used for ornamental purpose. It is also a good high-yield, hardy and disease resistant parent for breeding.

3. *Vitis davidii* Foex

The vine is sturdy and covered densely with spines. The spine is straight or slightly bending on top. The leaf is large with undulated teeth; the upper surface is dark green and the lower surface is gray; only the vein axils are covered with glandular hair; the leaf stalk is usually covered sparsely with small spines.

It is dioecious, but in recent years hermaphrodite individuals were also found with panicles, which are often longer than the leaf. 21.5 cm in length. The berry is large, round, dark purple, 1-1.5 cm in diameter, 3 g in weight; the skin of berry is thick; the berry contains little juice, tastes lightly sweet and matures late; it endures long storage and transport; most berries contain 2 (1-4) seeds. The sugar content is 11.5-15.0 %, the acid content is 0.62-0.91 %.

It tolerates very humid, shaded conditions and hot, dry climates. It is long-lived, high yielding, resistant to scab, anthracnose and other diseases and insect pests. In Nanjing Sun Yat-sen Botanical Garden, it has been used as a pollen parent in breeding for disease resistance.

There is a variety of this species, with nodule vines, *V. davidii* var. *cyanocarpa* Sarg., which is slender, with fewer spines and smaller leaves than *V. davidii*. The cluster is 20-35 cm in length, the berry is larger than that of *V. davidii*, blue in color, contains much juice and tastes sweet. This variety is more tolerant to humid and shaded conditions than *V. davidii* and is a better parent for breeding new cultivars adaptable to the wet and hot climate in South China.

4. *Vitis flexuosa* Thunb.

The vine is slender, the young shoot is canescent, the tendril is thin and long; the leaf is small, wide cordiform or near truncate at the base; the leaf margin is undulate with uneven teeth; the leaf is thin and tough; the vein axils on the lower surface of the leaf are puberulent; the leaf stalk is 3-7 cm long, covered with canescent, arachnoid tomentum.

The panicle is slender, 6-14 cm in length; the berry is round, 6-8 mm in diameter, dark purple, very acid. It contains 2-3 seeds. The °Brix content is 12 %.

This species has strong sprouting ability and adaptability, it tolerates humid and hot climate, lives long, is lightly less resistant to scab than *V. davidii* and *V. adstricta*. There is a variety *V. flexuosa* var. *parvifolia* (Roxb.) Gagnep. within this species.

5. *Vitis pentagona* Diels et Gilg (synonym *V. quinquangularis* Rehd.)

Growing tip and young leaves are covered with white or brownish red, thick, arachnoid tomentum, mature shoots purplish brown. The leaf is narrow ovate or pentagonal, with or without three obscure lobes, 10-15 cm in length; the margin teeth are thin, shallow and obtuse; the base is near truncate or shallow cordiform; the lower surface is covered with thick, brownish red tomentum; leaf stalk is covered with white pubescence.

The vine is dioecious with panicle 8-16 cm long. It produces many clusters with sparsely borne berries; the berry is dark purple and round; 6-8 mm in diameter; the juice extraction rate is 63.7 %; the °Brix content is 17 %; most berries contain 2-3 seeds.

It is resistant to scab and can be used as breeding material for disease resistance.

V. pentagona var. *honanensis* Rehd. has leaves with marked incisions with narrower sinuses. It is distributed over the north slope of Qinling range in Shaanxi Province.

6. *Vitis piasezkii* Maxim.

The young shoot and leaf stalks are covered with brown, puberulent and glandular hairs. Almost all shoots sprouted from the canes bear fruit. Leaves are very variable in shape, either simple or compound on the same shoot. The simple leaf is ovate, 4-9 cm in length; the leaf tip is

acute, the base is wide cordiform; the leaf is lobed, partite or sected with 3-5 leaflets, most have 5 leaflets. The mid leaflet is rhombic, 9-12 cm long. The leaf base is cuneate, with short stalk; the leaflets on both sides have no stalk, the base is oblique; some leaves have all the leaflets with stalks. The leaf margin has coarse teeth. The upper surface is glabrous, dark green; the lower surface light green with yellowish-brown tomentum.

The panicle is 5-15 cm in length; the cluster is cylindrical; the berries are borne evenly. The diameter of the dark purple berry measures 1 cm. The berry is covered with thick bloom, is sweet to eat; the berry brush is scarlet.

This species yields bountifully, with well-adapted character. It is resistant to fungus disease; besides for eating and winemaking it is valuable for breeding.

V. piasezkii var. *pagnuccii* (ROMAN) REHD. has young shoots and leaf stalks nearly glabrous; the lower surface of leaves has little hair.

7. *Vitis adstricta* HANCE

The vine is slender. The young shoots are covered with ferrugineous (in South China) or pale (in North China) pubescence; leaf is small, tripartite with the central part rhombic, or trilobobate, or entire and with few obtuse teeth; the lobes on both sides are unequally bilobous or entire; the lower surface is covered with rusty or canescent pubescence. The leaves turn red in autumn.

The panicle is short, covered with ferrugineous pubescence; the berry is round and dark purple, 8-10 mm in diameter, with °Brix content up to 10-16 %; sweet to eat; the juice proportion is over 60 %.

The plant is resistant to scab, it can be used as a parent for breeding new cultivars which are of good production and resistant to humid, hot climates and also to diseases.

V. adstricta var. *ternata* W. T. WANG is only native to Zhejiang Province. The leaf is completely trisected (ternately compound) and the middle leaflet with or without stalk. *V. bryoniifolia* BGE. is similar to this one. The leaf measures up to 15 cm; it has larger leaves compared with others in North China; the lower surface is covered with thin, white or brown pubescence. Inflorescence measures 12 cm in length; the blue-black berries are borne loosely. The fruit bearing character is good, one fruiting shoot can produce up to 7 clusters.

8. *Vitis yenshanensis*

This is a woody climber with slender vines. Old wood is light brown; the surfaces of young leaves and shoots are mauve, smooth and glabrous. The tendrils are slender. The leaf is small and thin, 7-14.5 cm in length, with 3-5 partitions, the lobe at center is often with 2-3 partitions again; the leaf base truncate or wide cordiform; the margin has coarse and large teeth; the upper surface is smooth and the lower surface is covered with setae. The petiole is thin, purple.

It is dioecious, but some have perfect flowers. The panicle is small, the cluster also small, cylindrical, 6-8 cm in length, subcluster large, weighing 25.2 g. The round berry is small, dark purple; the extraction of the bright-coloured juice is over 60 %; the °Brix content is 21.5 %; acid content 2.3 %; each berry contains 2-3 seeds.

This species tolerates drought and below zero temperature down to −25 °C and is resistant to diseases, especially scab, white rot, downy mildew and anthracnose. Considering the high sugar content, it is a valuable breeding material for resistance.

9. *Vitis romanetii* ROM. or *V. rutilans* GARR.

This species is a sturdy woody climber which grows vigorously. The purple young shoots and leaf stalks are thickly covered with ferrugineous, pubescence and glandular setae (gland spines). The leaf is large, thick, obscurely shallow trilobed or entire; leaf margin finely teethed; the tip spiny; the upper surface is dark green, the lower surface is covered with light ferrugineous, dense

pubescence; the veins are covered with glandular hair; the leaf stalk is 4-9 cm in length and is also covered with dense pubescence and glandular setose hair.

The panicle is loose and as long as or longer than the leaf blade; the rachlets are short. The berry is round, 1 cm in diameter, dark purple. The °Brix content is 12-16 %; the berry is edible but with only weak taste; juice rate is 67 %.

The species tolerates humid and hot climate and resists anthracnose.

10. *Vitis pseudoreticulata* W. T. WANG

The young shoots are at first puberulent and later on glabrous. The leathery leaf is large, cordiform, cordate-pentagonal or reniform; margin entire with fine teeth; the lower surface of the leaf along the vein is covered with short, light brown pubescence or is pruinose.

The panicle is large with many branches. The berry is round, dark purple. Yield is high.

This species is well adapted and tolerant to humid and hot climate; it is useful for breeding new cultivars adapted to the climatic conditions of South China.

11. *Vitis wilsonae* VEITCH or *V. reticulata* PAMP.

The young leaf is sometimes red; the young shoot is covered with white tomentum, later it becomes glabrous. The leaf is thick, leathery, usually entire, with fine teeth; the lower surface along the veins is covered with ferrugineous tomentum; the veins on both surfaces are prominent, forming a clear net, they are often pruinose.

The panicle is slender, 8-15 cm in length; the berry is large, round, 7-12 mm in diameter, dark blue and covered with bloom.

V. wilsonae is a sun-loving vine which does not tolerate shade and wet condition, but is resistant to fungus diseases.

III. Study on and use of grape germplasm resources

1. Selection and use

Many achievements have been made since we began to study and utilize wild grape germplasm in the 1950's especially with research on *V. amurensis*. Its berry is not only used for making superior red wine of famous brand but also for breeding new varieties which are adapted in different regions for its cold and disease resistant genes. For example, in the Beijing Botanical Garden we crossed this species with the cultivar Muscat Hamburg and selected the new cultivars Beichun, Beihong and Beimei which are hardy and disease resistant, with high yield and with high sugar content in fruits. In addition, the juice is bright-coloured and suitable for winemaking. The Institute of Pomology of Jiling Province selected several *V. amurensis* seedlings including Gongniang No. 1 and Gongniang No. 2. The Institute of Grape for Winemaking in Shandong Province crossed Sweet Water with *V. amurensis* and selected new variety Baotuhong. All these varieties possess fine characteristics for winemaking together with hardy property of *V. amurensis*. At present, repeated cross and back-cross for breeding F_2 and F_3 hybrids are being carried out in order to screen out the progenies with superior wine quality. In recent years, the Beijing Botanical Garden crossed F_1 hybrid of *V. amurensis* with European varieties and selected the new variety Beiquan, which is hardy, with fine quality, high yield and disease resistance, and is suitable for white wine. In the mean while, we have also selected a F_3 variety 79-3-172 which is disease resistant and good for winemaking. The Institute of Pomology in Liaoning Province also crossed the F_1 hybrid of *V. amurensis* with the native Chinese variety Longyan and selected a hardy new variety Xiongynebai for white wine. A series of new selections, such as Shuangqing, with perfect flowers, and Tonghua No. 1, Tonghua No. 2, Tonghua No. 3, Changbai No. 6, Changbai No. 9, Zuoshan No. 1 and Zuoshan No. 2, etc., with big cluster and berry have been distributed into production.

In North-East China, *V. amurensis* is used as rootstock to increase the cold resistance and disease resistance for local cultivars to reduce the depth of covering in winter, thereby saving large amount of labour and burying material and expanding the area of viticulture further north. Grafting of tender shoots on rootstocks of *V. amurensis* can also speed up the propagation rate.

In Benxi, Liaoning Province, *V. amurensis* is also used instead of *Parthenocissus* spp. PLANCH. in vertical greening of the city, with excellent effects of beautification.

V. ficifolia is also used as a relatively hardy and good parent for breeding. In the Beijing Botanical Garden, we crossed this species with Muscat Hamburg and Muscat of Alexandria and selected the new varieties Beifeng and Beizi, which have the characters of cold and disease resistance and high yield and are suitable for producing juice with beautiful colour and good taste.

V. pseudoreticulata, distributed in the lower reaches of the Yangtze River, is one of the wild species with utilization value. Through selection, the sugar content of the berry of some individual plants is as high as 19%. With downy mildew resistance, it is also a good parent material for breeding. In recent years, the Institute of Horticulture of Shanghai Academy of Agriculture Sciences used this species as a parent in breeding new varieties.

Furthermore, many wide-spreading wild species such as *V. flexuosa*, *V. pentagona*, *V. davidii*, *V. piasezkii*, *V. bryonifolia*, *V. adstricta*, *V. flexuosa* var. *parvifolia* etc. produce berries good for eating fresh and for winemaking, seeds for oil, roots and the whole plants used for medicine which cures rheumatic disease, muscle pains, broken bones, swelling and inflammation. Some of them are also good ornamental plants for gardening or used as rootstocks. It is worth mentioning that a purple-leaved species of *Vitis*, newly discovered in Xishuangbanna, Yunnan Province, and not yet named, is good to be introduced as a new foliage vine.

2. Studies on resistance

In recent years, Chinese scientists conducted research on resistance of wild species of grapes in North China. They tested the cold resistance of 8 species and a variety, using electric conductance method. The results showed that the cold resistance of *V. amurensis* is the best; that of *V. adstricta*, *V. bryoniifolia* and *V. piasezkii* is reasonably good; that of *V. romanetii* and *V. davidii* is slightly better than that of Muscat Hamburg; and that of *V. pentagona* is poor.

Much research work has been done on the resistance to downy mildew *(Plasmopara viticola)*, scab *(Sphaceloma ampelinum)*, white rot *(Coniathyrium diplodiella)* and anthracnose *(Glomerella cingulata)*, the four major diseases of some wild grape species. Through natural determination in the field, inoculation test in the field and on detached leaves in laboratory, it was shown that the following species are almost immune to scab: *V. flexuosa*, *V. yenshanensis*, *V. adstricta* var. *ternata*, *V. adstricta*, *V. wilsonae* and *V. piasezkii*. Different lines of some species show other resistance, for example *V. davidii*, *V. pseudoreticulata*, *V. piasezkii*, *V. pentagona*, *V. amurensis*, *V. davidii* var. *cyanocarpa*, *V. romanetii*, and two species without Latin names.

So far, no species immune to downy mildew has been found. There exist only disease resistant and susceptible species. The disease resistant species and varieties are: *V. flexuosa*, *V. wilsonae*, *V. bryoniifolia*, *V. pseudoreticulata*, *V. romanetii*, *V. davidii* var. *cyanocarpa*, *V. piasezkii*, *V. hancockii*, and *V. yenshanensis*. The disease susceptible species and varieties are: *V. davidii*, *V. pentagona*, *V. amurensis*, *V. wilsonae*, *V. adstricta*, *V. adstricta* var. *ternata*, *V. betulifolia*, *V. flexuosa* var. *parvifolia*, and a species without a Latin name. Though the number of disease susceptible species is over that of disease resistant species, most of the species are less susceptible to the disease than *V. vinifera* cultivated in vineyards.

The resistances of the lines of *V. davidii*, *V. pseudoreticulata* and a species without Latin name do not vary greatly. But the variation of the resistances between the lines of *V. davidii* var. *cyanocarpa*, *V. piasezkii*, *V. romanetii*, *V. amurensis* etc. is very great.

All of the species mentioned above are not immune to white rot. Nevertheless, in the field they are not infected. After artificial inoculation, they are infected to various degrees. The species of

strong disease resistance are a non-denominated *Vitis* sp., *V. davidii*, *V. amurensis* and *V. yenshanensis*. The susceptible species is *V. romanetii*. But all these species are better in resistance than *V. vinifera*.

The disease resistance of different lines within one species is different. The variation of resistance between the lines of *V. adstricta*, *V. bryoniifolia* and *V. davidii* is little. But lines of *V. piasezkii*, *V. pseudoreticulata*, *V. romanetii*, *V. amurensis* and *V. pentagona* include both disease resistant and susceptible types.

Some lines of a non-denominated *Vitis* sp., *V. davidii*, *V. amurensis* and *V. pentagona* are almost immune to anthracnose. Lines of *V. amurensis*, *V. davidii*, *V. pentagona*, *V. romanetii* and *V. pseudoreticulata* have very strong disease resistance.

All the experiments mentioned above showed that the resistance character is not related to the geographical origin of the species. The disease resistances of lines of some species show evident variation. Only lines which passed disease resistance tests should be chosen as the most reliable parents for effective breeding work of disease resistant cultivars.

Through much research effort it has been determined that the disease resistances of some species are not only inherited but also expressed in the structure of their tissues. For example, the thickness of the skin and the cuticle on the fruit are positively correlated to the potentiality of resistances to white rot and anthracnose. The density of stomata on the leaf is negatively correlated to the resistance of lines of a species to downy mildew. In leaf and berry, the less the content of soluble sugars and the higher the free organic acids are, the stronger is the anti-disease potentiality of a line to scab, white rot and anthracnose. The activity of peroxide enzyme is positively correlated to the resistance potentiality against white rot.

The results of research also show that the isozymes of peroxidase in functional leaf of grapevine possess characters which are stable and can be tested repeatedly within a species. This offers the possibility of identification by use of isozyme test. The isozyme spectrum of peroxidase in berries of wild grape species native to China is distinct from and much more complicated than that of European grapevines. This shows that China is one of the major centers of *Vitis* origin.

References

CHAO WU-JI; 1988: The application of isozyme analysis in the taxonomic study of Chinese wild species of *Vitis*. Viticulture and Wine-Making of China, 1-6.

CHEN JING-XIN; 1979: Yanshan grape, a wild grape of strong resistances and with berries high in sugar content. Zhong Guo Guoshu, 11-15.

HE PU-CHAO; CHAO WU-JI; 1982: Studies on the hardiness of eight species of wild *Vitis* L. in China. Acta Horticulturae Sinica, 17-21.

-- --; WANG GUO-YING; 1986: Study on the resistance against downy mildew of wild *Vitis* species native to China. Acta Horticulturae Sinica, 17-24.

-- --; WANG YAO-JIN; 1988; A study on identification of white rot resistance of Chinese wild species of *Vitis*. Zhong Guo Guoshu, 5-8.

Institute of Botany; 1972: Iconographia Cormophytorum Sinicorum, Tomus II, 769-775. Chinese Academy of Sciences Ed.

LI SHENG-CHEN *et al.* (Eds.); 1960: The Breeding for New Cultivars of Grape, 59-64.

WANG WEN-CAI; 1979: The newly discovered species of *Vitis* L. Acta Phytotaxonomica Sinica, 73-98.

Wang Yao-Jin; He Pu-Chao; 1987: A study on identification of scab resistance of Chinese wild species of *Vitis*. Journal of Pomology, 1-8.

YU DE-JUN; 1979: Taxonomy of Chinese Fruit Trees, 176-185.

ZUO DA-XUN; YUAN YI-WEI; 1981: The geographical distribution and utilization of *Vitis* L. in China. Bulletin of the Nanjing Botanical Garden Mem. Sun Yat-Sen, 25-31.

Evaluation and utilization of vine genetic resources in Czechoslovakia

DOROTA POSPISILOVA

Research Institute for Viticulture and Enology, 83311-Bratislava, Matúskova 25, Czechoslovakia

Summary: The organization of research with plant collections in Czechoslovakia, including grapevine, is described. The collection of *Vitis* genotypes maintained at the Research Institute for Viticulture and Enology includes about 1,500 varieties. Research is directed toward collection, preservation, investigation of varietal characters, use for breeding programs and establishment of a computerized information system. The results obtained up to now and new perspectives are discussed.

Key words: gene resources, gene bank, collection, varietal evaluation, breeding, information system, Czechoslovakia.

Czechoslovakia has a rich tradition in the collection, storage and utilization of genetic resources of cultural plants. Since the beginning of this century, collections of species and varieties have been established in several research centres and intensive work with them was started in the 1930's. Since 1954, the Research Institute for Plant Production in Praha-Ruzyne has assumed the coordinative activity of this work and at present there are 29 research and breeding institutes involved in this field (BARES 1987). At the Research Institute for Plant Production the Board for Plant Genetic Resources was established, the members of which are researchers responsible for individual crop collections and those others from cooperating organizations. The Board is a consultative authority for all institutions researching and utilizing genetic resources in Czechoslovakia. For each crop the work in collections is methodically controlled by responsible persons. The Research Institute for Plant Production in Praha-Ruzyne secures in a centralized way the introduction of plant genetic resources, the evidence of the information system of plant genetic resources and since 1988 the long-term storage of collections of seed species.

The international solution programme is linked up to the cooperation of COMECON member countries under the coordination of the Research Institute for Plant Production in Leningrad. Further cooperation has been developed particularly in the framework of the International Board for Plant Genetic Resources (IBPGR) and of its European programme ECP-GR (European Cooperative Programme on Conservation and Exchange of Crop Genetic Resources) in which Czechoslovakia has participated since 1983. Cooperation is also developed in the framework of the EUCARPIA (European Association for Research on Plant Breeding) gene bank (DOTLACIL 1987).

The work with the vine collection is included in this framework.

In the past, the vine collections created an inseparable component of the viticultural research, breeding and educational institutions in Czechoslovakia. Until the 1950's, their function was more or less collectional or they served pedagogical purposes. The first larger vine collection of approximately 350 varieties was established at the Viticultural Research Station in Mutenice in the 1930's. Later, this collection was transferred to the Research Institute for Viticulture and Enology in Bratislava where it has become the basis of today's large collection. It is also worth mentioning the smaller collection of the Agricultural University in Lednice na Morave which contains from the total number of 287 cultivars 173 interspecific hybrids.

The work with the vine collection is divided into the following programmes:

- Collection and conservation of varieties *in situ*
- Study of collection varieties

- Utilization of collection varieties
- Information system creation.

1. Collection and conservation of varieties *in situ*

Since 1962, we have collected about 1,300 cultivars from many countries of Europe, Asia and America in our central collection, the majority of which are *Vitis vinifera* L. varieties.

For today as well for future, we have directed the introduction of varieties towards the extension of the basis of species of the *Vitis* genus. We also try to obtain new hybrid materials from the interspecific resistant breeding. We consider the registration of our own materials from hybridization programmes as inevitable. The conservation of clones of currently grown varieties as well as those of less economic importance in the collection is also one of our tasks. Systematic clonal selection narrows varietal populations genetically. Therefore, genetic variability of these populations can only be conserved in collections.

Intensification tendencies in viticulture have substantially reduced the varietal composition of our producing vineyards which was found in old private vineyards until 1945. One of our tasks is to conserve these autochthonous or acclimatized varieties in the collection for the needs of the future.

During the last few years, even the supply of foreign varieties in the collection seems to be problematic. Increasingly strict phytoquarantine precautions often hinder the release of labouriously obtained material in the collection. We also struggle with practical aspects of the production unrentability of the collection stands.

The situation of decreasing genetic resources in Czechoslovakia is also being managed from the ecological point of view. At the National Academies of Sciences commissions for protection, conservation and rational utilization of gene pools including also vine were established with the goal of maintaining a diversified ecosystem of plants and animals. The situation is urgent and it is the subject of discussions of the highest managing authorities. Production intensification not only reduces the genetic variability of species but it also threatens the weed flora and the fauna.

2. Study of collection varieties

One of the main tasks in the work with the collection was the study of foreign varieties under the ecological conditions of our country. The entire work was aimed at the following two directions:

a) The collection of 60-70 varieties was studied in approximately 80 ampelographic, biological and agrotechnical characters over 3-year periods. The hitherto obtained results of the study of about 500 foreign varieties and 200 selections from our own crossings were concentrated in 8 final reports with detailed results, ampelographic descriptions of varieties and recommendations to their utilization for growing and breeding purposes as well as for their collection value.

b) In Czechoslovakia we have established and evaluated an ecological experiment with four different geographic and ecological groups of varieties in three significantly different localities. We have attained the following generalized conclusions (Pospisilova 1978, 1979):

- The division of varieties in accordance with the territory of their origin into geographic groups (Negrul) appeared to be correct.
- Regional differences influence the varieties of the *occidentalis* group inexpressively but those of the groups *orientalis antasiatica* significantly. The groups *proles pontica* are intermediate.
- Regional conditions do not influence only the physiological characters (fertility, sugar content, acid content etc.) but they also cause morphological convertibility (leaf size and shape; cluster, berry and grapeseed size). The most significant differences are to be found again in the group of *orientalis* varieties and the least ones in the *occidentalis* group.
- Similarly, the individual varietal groups are also influenced in their growth intensity, lignification and other physiological characters.

These results have practical utilization in the introduction and acclimatization of foreign varieties as well as in the application of their genome in breeding.

3. Utilization of collection varieties

The vine collection is also utilized for practical purposes.

a) It serves for the establishment of breeding collections at viticultural breeding and educational institutions in Czechoslovakia.

b) It is the source for varietal exchange at international level.

c) Some of the collection varieties have practical utilization (Pannónia kincse, Guzal kara, Feteasca regala, Alibernet, Zweigeltrebe etc.). However, the collection consists of only a small quantity of such varieties especially in consideration of the geographical conditions in Czechoslovakia.

d) It is one of the sources for breeding of new vine varieties.

During the last 30 years, considerable work has been done on the breeding of new varieties in Czechoslovakia and particularly this collection of varieties has contributed to their creation.

In spite of a considerably strong representation of traditional white wine varieties, new crossings (of traditional varieties with those from the collection) e. g. Devin, Breslava, Mopr etc. have found their place in practice. Also, the limited number of traditional red wine varieties has been enriched with our new breedings. With the use of French, Italian and Soviet varieties (Castets, Abouriou noir, Teinturier, Aleatico nero and Puchljakovski) we have bred a whole range of new types from among which especially the varieties Dunaj, Váh and Hron have come in practice.

The consumption of table grape varieties in Czechoslovakia is relatively low and this fact induced us to breed table grape varieties suitable for our ecological conditions. With the use of Pannónia kincse, Julski biser, Cardinal, Aptish aba, Dunavski misket and many others we have created a whole range of table grape varieties of significant production importance which are grown in our warmest viticultural regions. These are for example Dóra, Diamant, Opál, Topas etc.

Seedless grape types have their specific place. They were created with the help of collection varieties Katta kurgan, Perletta, Delight, Ceaus roz, Chibrid bessemen V-6 and others. We have bred seedless grape varieties, small-fruited as well as large-fruited, which doubtless have their importance at least for amateur gardeners (Muscat Susanna x Delight 11/8, 12/17; Ceaus roz x Delight 5/1, 6/6, 5/9; Ceaus roz x Perletta 17/40; Ceaus roz x Carica na lozjata x Bolgar 5/11; Ceaus roz x Chibrid bessemen V-6 21/8; Katta kurgan x Perletta 14/44, 15/42; Katta kurgan x Chibrid bessemen 25/34).

The latest trend in plant breeding in Czechoslovakia is the breeding of resistant varieties, especially of table grape cultivars which practically cannot be bred without pertinent genetical resources from our own as well as foreign collections.

4. Information system creation

The effective work with genetic resources is dependent on a functional information system. Such a system was founded in Czechoslovakia at the Research Institute for Plant Production in Praha-Ruzyne under the designation EVIGEZ (Rogalevicz *et al.* 1986). It secures information for all institutions in Czechoslovakia on the following issues:

- Import, distribution and export of genetic resources
- Passport data of genetic resources
- Descriptive data of genetic resources
- Other information

The vine collection is included in this system and at present the passport data of each variety have been entered into the computer. For the descriptive data we have developed the Classifier of the *Vitis* Genus (Pospisilova *et al.* 1988) which allows the coding of 110 descriptors and also contains the codes of botanical taxons. This Czechoslovak vine classifier has become the basis for the elaboration of the International Classifier in the framework of the COMECON member states.

After the passport introduction of varieties into the computer system, it will be continued with data processing through coding of individual descriptors in case of about 700 evaluated varieties for the creation of the database and its universal utilization.

In the sense of the international information activity on the state of the vine genetic resources, Czechoslovakia has become involved in the working programme of the O.I.V. (Groupe d'Experts 'Sélection de la Vigne') with providing of foundations from about 1,000 varieties for the elaboration of the world survey of sorts and varieties of the *Vitis* genus (ALLEWELDT 1987).

Perspectives of the work with vine genetic resources

All activities connected with vine genetic resources in the future will be concentrated on the completion of the collection especially with species and varieties relevant to plant breeding, on the study of the main characters of those varieties of the collection which have not yet been evaluated, and on the completion of the database.

Also important is the collection of *Vitis silvestris* GM. resources from meadow soil forests of the Danubian Lowland, of old regional varieties and participation in international expeditions especially in the Caucasian centre of origin of vine varieties.

At present we are founding a biotechnological laboratory at our institute. One of its activities will be the screening of varieties on stress and pathological factors as a portion of our resistant vine breeding programme. The detection and evidence of genes of major effect (major genes) and their alleles play an important role in genetic resources. It is a very complicated task by the use of classical methods in persistent cultures.

Another possibility for the future is the conservation of the collection *in vitro*. These cultures are useful for genetic studies and manipulation. But up to now, *in vitro* culture does not enable the conservation of germplasm in its original condition for unlimited periods. Therefore, it seems that the *in vitro* collections will only complement the contemporary collections in vine plantations. Concerning the clones, especially the virusfree ones, the collections *in vitro* will play their important role.

Even though much work has been done for the conservation and utilization of vine genetic resources, new plant breeding and cultivation methods will require further work with genetic resources. The most important demand of the present time is the conservation of the genetic variation of cultural plants and its utilization for the benefit of mankind in the future.

References

ALLEWELDT, G.; 1987: The Genetic Resources of Vitis. Table 1, 2, 3. Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, Siebeldingen.

BARES, I.; 1987: Historie studia genetickych zdrojú kultúrnich rostlin. In: 100 vyroci narozenin N. I. Vavilova. Sbornik VURV Praha-Ruzyne, 19-22.

DOTLACIL, L.; 1987: Génová banka a dalsi rozvoj studia genetickych zdrojú v CSSR. In: 100 vyroci narozenin N. I. Vavilova. Sbornik VURV Praha-Ruzyne, 28-36.

PØSPISILOVA, D.; 1978, 1979: Ökologisch bedingte Veränderlichkeit der Weinrebensorten I.-IV. Wein-Wiss. 33, 266-276. Wein-Wiss. 34, 1-8, 143-150, 249-263.

– – ; SEHNALOVA, J.; DROZD, J.; BARES, I.; 1988: Descriptor List, Genus *Vitis* L. VURV Praha-Ruzyne.

ROGALEWICZ, V.; BARES, I.; SEHNALOVA, J.; MARTINKOVA, M.; 1986: Ceskoslovensky informacni systém gentickych zdroju (EVIGEZ). The Czechoslovak Genetic Resources Information System. VURV Praha-Ruzyne.

The role of variety as genetic potential in nutrient utilization

J. André and E. Hajdu

Research Institute for Viticulture and Oenology, H-6000 Kecskemét, Hungary

Summary: A container model trial was set up on sandy soil with 5 replications to study the nutrient regime of 16 wine grape varieties in a 10 year program starting in 1982 at Kecskemét-Miklóstelep in the Institute for Viticulture and Oenology.

Leaf, fruit and wood analysis data and production parameters (fruit and wood weight, frost tolerance) were evaluated every year under identical cultural conditions. Interactions between years, varieties and nutrient elements (N, P, K, Ca, Mg, Zn, B, Fe, Mn) were discussed.

Trials so far have proved the decisive role of variety characters fixed genetically on nutrient uptake and nutrient utilization at given nutrient supply.

Key words: variety of vine, nutrition, mineral, leaf, fruit, wood, yield, must quality, shoot yield, cold resistance.

Introduction

A very important step in breeding is the study of values and production characteristics in a given variety or clone. This study also includes the establishment of the nutrient regime in varieties and clones recommended for production under different conditions (soil, climate, cultivation methods, etc.). For this purpose model container trials were installed in our Institute to test the nutrient regime of 16 wine grape varieties. The trial was planned for 10 years. Observation data of 6 years are presented.

In the test program, the nutrient requirement and utilization ability of different wine grape varieties were determined in sandy soil at different levels of stock nutrient supply.

In the test we wanted to determine:

(i) the effect of different nutrient doses on the nutrient regime of the varieties, on growth, on quality and quantity of cluster yield, on wood ripening and on winter tolerance of buds,

(ii) the role of variety as genetical potential in the rate and quantity of nutrient uptake,

(iii) the effect of fertilizer doses as recommended for a variety in a given production area considering aspects of environmental protection.

Material and methods

The trial started in Kecskemét-Miklóstelep in 1982 on a level site. Methods developed by several Hungarian and foreign authors were followed (Polyak 1968, 1973; Papp 1971; Füri *et al.* 1974; Füri and Kozma 1975; Edelbauer 1976; Méreaux *et al.* 1979; Szöke and Füri 1980; Arutjunjan 1981).

The closed containers were placed, one close to the other, in a 80 cm deep trench, in an unheated plastic tent. The containers were plastic barrels, 80 cm high with 50 cm diameter. The bottom of the barrel was filled with sifted river ballast 15 cm thick (40 kg/barrel) to receive possible stagnate water. The gravel was covered by a plastic net in two layers. The barrel was filled with soil enriched with nutrients (144 kg sand/barrel) and slightly pressed. The nutrients used as stock supply were homogenized with the soil prior to filling in. No maintenance fertilization was given. In the trials the nutrient uptake of 16 varieties was studied at two soil nutrient levels. The water supply was regulated to complete the winter precipitation and remove the surplus water accumulated at the bottom. The superfluous water was pumped out through a plastic tube placed into the barrel.

The own-rooted vines planted in the barrels were rooted in hoses filled with perlite. High cordon training was used with 3 short spurs of 2 buds. Stocks were covered with straw to protect them from winter frosts and the plastic tent was also covered by a plastic film at the end of the growing period (ANDRÉ 1986).

The order of the 16 varieties in trial and sand analysis data are presented in Table 1.

Since the beginning of the trial the following values have been measured continuously:

- Foliage mass
- Topped green weight
- Cluster yield, cluster number, mean cluster yield
- Sugar content and acidity of berries
- Water quantity accumulated at the bottom of the barrel
- Weight of pruned woods
- Frost tolerance of wood in heat chambers.

Every quantitative measurement was completed with an analysis of the sample. Changes in the nutrient uptake were followed by leaf analysis 4 times in the growing period. Yield and wood analysis were performed from the 3rd year.

Table 1: Trial characteristics

STUDIED VARIETIES:

K - 9	F. Kadarka
Medina	Rheinriesling
Chardonnay	RF-48
Ezerfürtü	Jubileum 75
Zweigelt	Blaufränkisch Tf.
Steinschiller	Zengő
Zalagyöngye	M 7
Sztyepnyak	Kunleány

SOIL TYPE: calcareous sandy soil of slight humus content of 0 - 1,0 m depth.

SOIL ANALYSIS RESULTS		TRIAL SOIL NUTRIENT LEVELS	
		LOW	HIGH
pH	8,1 (KCL)		
KA	25		
$CaCO_3$ %	4-5		
H %	0,44-0,50		
Total salt	0		
AL P_2O_5 ppm	86	100	200
AL K_2O ppm	70	150	300
Mg KCl ppm	25	80	150

Results

The role of variety as genetical potential in the nutrient uptake

The measurement and analysis data accumulated in the 6 years (1982-1987) can be evaluated from several points of view. In this case the role of variety was studied as genetical potential in nutrient uptake.

An increase of the nutrient supply caused considerable differences in the uptake, in the mean of the 16 varieties (Table 2). Differences varied in varieties, plant parts and nutrient elements as well. Within one variety, however, even extreme differences did not surpass 76 % between the two treatments (low and high). The low nutrient level served as reference. If, however, the analysis data of the plant parts (leaf, fruit, wood), of the 16 varieties were compared within identical treatments of the varieties, almost 300 % difference was found.

Table 2 shows that the variety plays a decisive role in the nutrient uptake of the plant. Considering any of the 3 plant parts (leaf, fruit, wood), it is clear that doubling the nutrient supply resulted in a 20-50 % mean surplus uptake (maximum 76 %), compared to the low nutrient level. At identical nutrient supply, uptake differences varied between 50-70 % in the average, with 300 % maximum among varieties. The difference can be caused by the different nutrient requirement and different nutrient utilization ability of the varieties fixed in the genotype.

Fig. 1 shows in detail the analysis values of magnesium. Considering the nutrient elements, it can be said that uptake differences among varieties (fixed genetically) were twice as high (in certain elements even more) as obtained by doubling the nutrient supply.

Table 2: Treatments and highest nutrient differences among varieties

NUTRIENTS	HIGHEST NUTRIENT CONTENT DIFFERENCES								
	AMONG TREATMENTS WITHIN THE SAME VARIETY			AMONG VARIETIES					
				LOW			HIGH		
	EXTREME VALUES ± %								
	NUTRIENT EFFECT			VARIETY EFFECT					
	1.	2.	3.	1.	2.	3.	1.	2.	3.
N	11	40	13	20	97	25	20	63	20
P	[76]	50	50	62	91	30	117	54	50
K	37	25	36	53	43	49	51	30	60
Ca	-32	-24	-25	93	68	57	79	122	69
Mg	50	35	36	123	54	64	104	38	29
Zn	25	-36	36	48	80	63	41	67	110
B	38	210	17	69	57	33	71	[288]	45
Fe	49	50	46	50	109	110	53	103	138
Mn	14	-20	-29	72	56	73	84	44	61

Legend: 1. Leaf analysis 2. Fruit analysis 3. Wood analysis

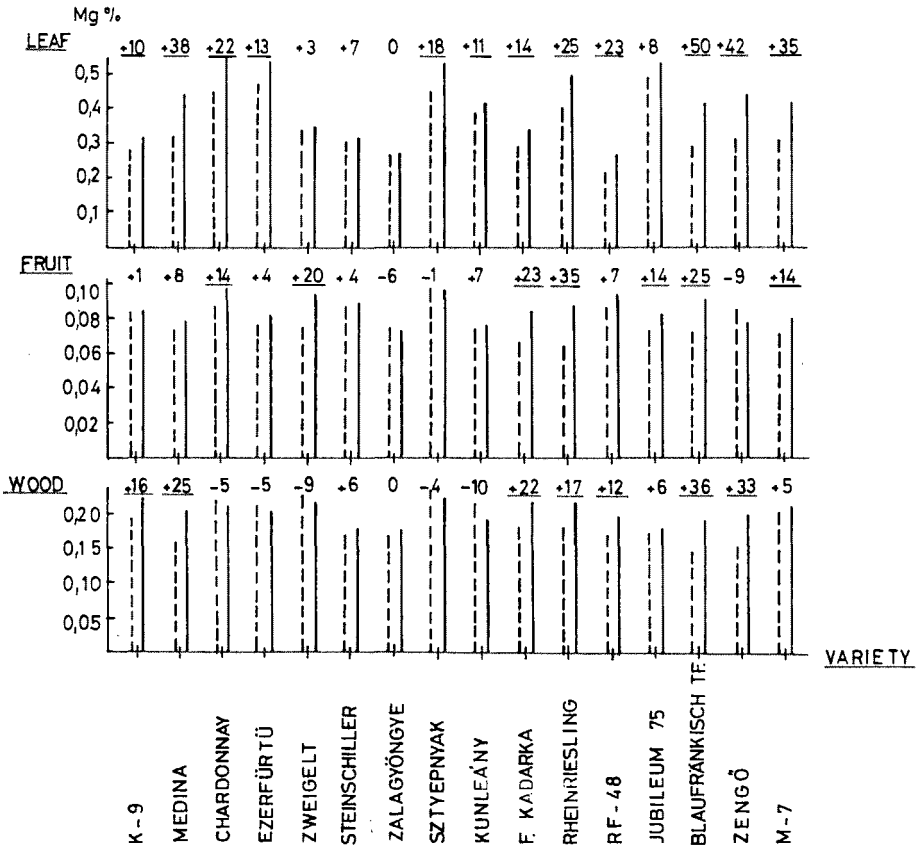


Fig. 1: Average results of magnesium analysis over 6 years (1982-1987).

The role of variety in the nutrient uptake during the growing period

At identical nutrient supply and bud loading, there were significant differences among varieties in the rate of nutrient uptake during the growing period (Fig. 2). The difference among varieties was also expressed in the nutrient elements. In the figure, P, K, Mg uptake rates of varieties with extreme values are shown. When changing the nutrient supply, the difference in uptake among varieties was modified but not eliminated. There was a change in the uptake curve of varieties in the different years as well, probably explained by different climatic factors (precipitation, temperature, light).

These yearly differences are important in a variety at identical nutrient supply with given nutrient elements. In the mean of several years the same variety indicates an uptake trend characteristic of the variety at identical nutrient supply.

It can be concluded that at identical nutrient supply and bud loading the nutrient uptake rate of a variety is regulated by its genetical properties. The rate can be modified by year effects but genotype effects can not be eliminated.

Differences in the nutrient uptake and supply of varieties during the growing period and in certain elements affect the whole life of the plant and its production value. Data obtained so far do not yet allow the determination of these effects precisely.

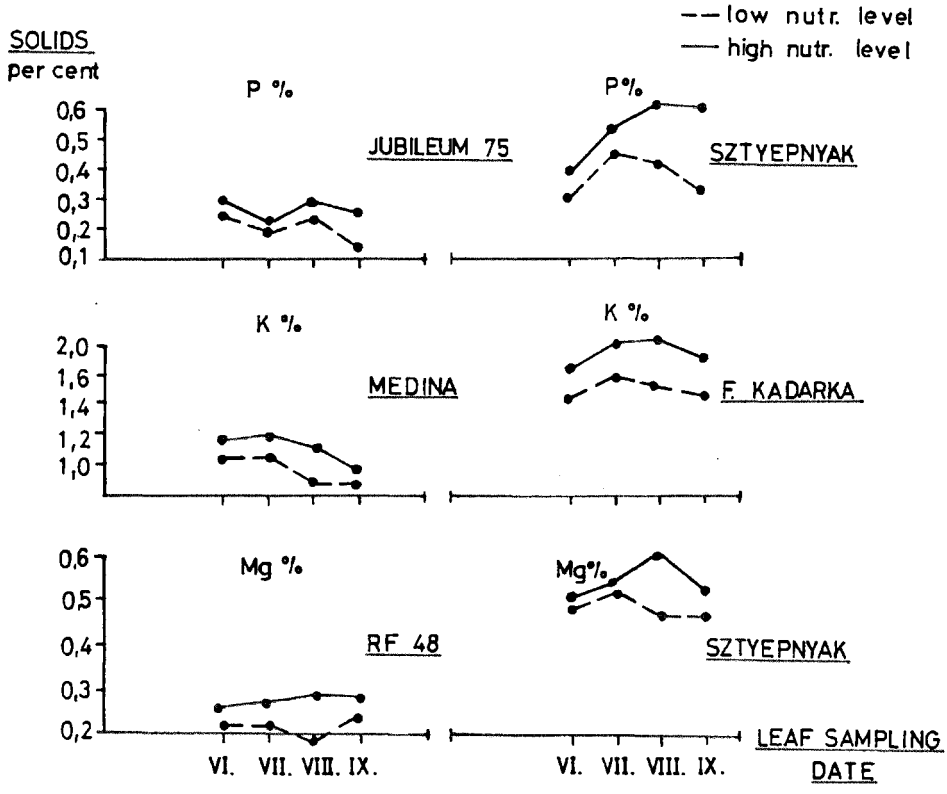


Fig. 2: The effect of variety on nutrient uptake during the growing period based on leaf analysis.

The considerable differences among varieties in the tested parameters despite identical nutrient supply and production conditions can be explained by the different nutrient content - which is specific for the variety - within the growing period.

The role of variety in green, fruit and wood weight, in sugar content and acidity and in frost tolerance of buds

Results show clearly the importance of varieties in the nutrient uptake at identical nutrient supply. This observation is only affirmed by the remarkable variety effect on the studied plant parts (Table 3). While at the double nutrient supply the highest difference produced 3.5 fold surplus (which is very high), the difference due to different genetical properties was much more high, 11 fold.

There is no variety with the same difference at every elements in identical treatments. That is, in the 9 elements tested the nutrient supply can be superior or inferior to the average. So, if we take LIEBIG's rule strict, the notion of general nutrient supply and the variety classing following the rule may seem rather artificial.

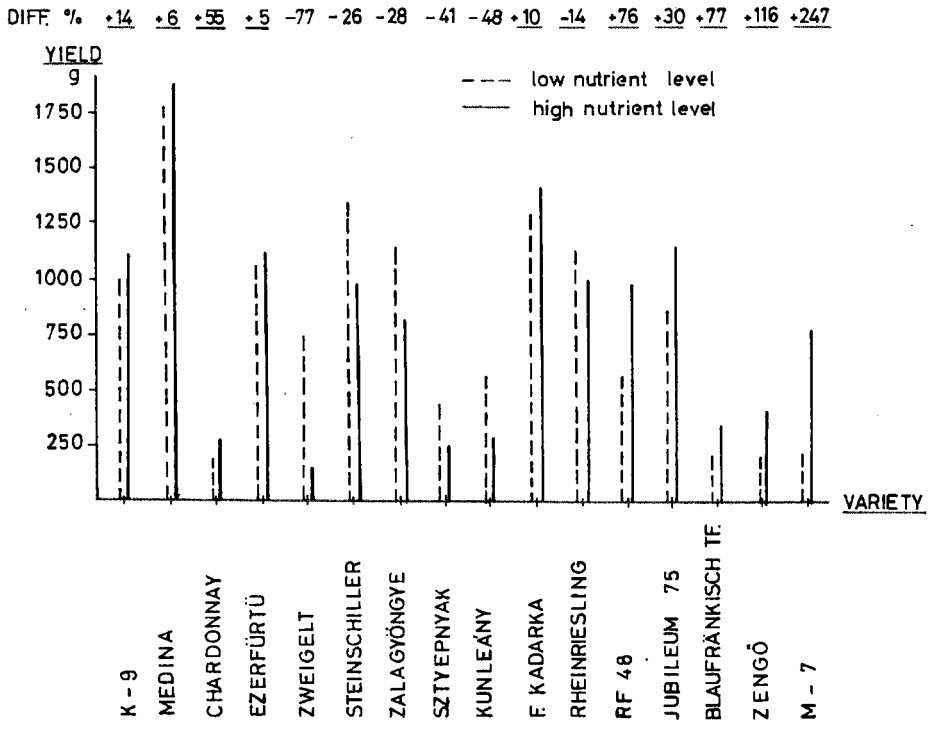


Fig. 3: Mean yield over years 1984-1987 as g/variety (5 vines).

Fig. 3 represents the cluster yield in the 16 varieties at two nutrient levels. If variety reactions to nutrient supply changes are evaluated according to the results obtained in production parameters (see Table 3) and we try to draw conclusions as to the different nutrient requirements and utilization of varieties, we receive quite different results from those of analysis evaluations.

The mean nutrient uptake surplus in a variety proved by analysis does not necessarily coincide with a general increase in the majority of production parameters. It can be stated that in the case of vine the nutrient requirement, nutrient uptake and utilization can only be spoken of as related to a concrete variety or element. Thus, it is very important to know exactly the production value and within it, the nutrient regime of a variety.

Literature

ANDRÉ, J.; 1986: A szölö tápanyagháztartásának vizsgálata homok és lösztalajon, modellkisérletben I. Szölötermesztés és Borászat **8** (3), 22-26.

ARUTJUNJAN, Sz. Sz.; 1981: Szoderzsanie azota, foszfora i kalija v. organah vinograda v. szvjazi sz mineralnüm pitaniem lozü. Biol. Zh. Arm. Erevan **34** (8), 861-866.

EDELBAUER, A.; 1976: 4e Colloque International sur le Contrôle de l'Alimentation des Plantes Cultivées, Gent, 293-301.

FÜRI, J.; KATONA, J.; KOZMA, F.; 1974: A szölö öntözésének és vízháztartásának vizsgálata. Jubileumi Tudományos Napok. Budapest. SZBKI Közleményei, 201-220.

Table 3: The highest differences measured (%) for the studied characters among treatments and varieties

MEASURED PARAMETERS	AMONG TREATMENTS WITHIN THE SAME VARIETY	AMONG VARIETIES	
		AT LOW NUTRIENT LEVEL	AT HIGH NUTRIENT LEVEL
	NUTRIENT EFFECT	VARIETY EFFECT	
GREEN WEIGHT g	230	913	518
WOOD WEIGHT g	127	182	187
FRUIT WEIGHT g	347	1000	1097
CLUSTER NUMBER	262	660	425
MEAN CLUSTER WEIGHT g	186	664	429
MUST SUGAR M/l	-10	128	118
MUST ACIDITY ‰	-16	187	187
FROST TOLERANCE %	240	650	420

-- --; KOZMA, F.; 1975: A szölö evapotranszspirációja. Időjárás. OMSZ. Kiadványa **79** (2), 112-120.

KOZMA, P.; POLYAK, D.; 1968: Tenyészedények a szölészeti kutatásban. Kert. és Szöl. Föiskola Közleményei **32** (1), 123-135.

-- --; -- --; 1973: A szölö ásványi anyagellátottsága, produktivitása és a levélanalizis adatai köz ötti össefüggés. A Kert. Egy. Közleménye **37** (5), 89-103.

MÉREAUX, S.; ROLLIN, H.; RUTTEN, P.; 1979: Effets de la sécheresse sur la vigne. 1. Etudes sur Cabernet Sauvignon. Ann. Agron. **30**, 553-575.

PAPP, J.; 1971: A málna tápanyagigényének vizsgálata. A Kert. Egy. Közleményei **35** (3), 177-187.

SZÖKE, L.; FÜRI, J.; 1980: Tenyészedényes módszer a szölö ásványi táplálkozásának tanulmányozására. Szölötermesztés **3** (2), 1-4.

Creation and study of the Pinot noir variety lineage

A. BRONNER and J. OLIVEIRA

I.N.R.A. Station de Recherches Vigne et Vin, Laboratoire de Viticulture, 8, rue Kléber, F-68000 Colmar, France

Summary: The objective of the study presented here is to obtain pure line genotypes able to transmit to cross progeny the most useful characters to wine grapes, particularly the berry colour of Pinot noir. From the very heterogenous Pinot noir variety we chose a clone INRA-Colmar as the initial parent of a generation series. In the first 3 generations of self pollination we selected only red berries genotypes. After the 4th generation we made 'pedigree' selections with seedling separation, retaining not only the red berries population but also some white ones. This series of generations shows only a weak 'inbreeding' effect. Numerous populations with strong vigour and high fertility were observed. During these crosses, types of bunches and berries have appeared which were very different in shape from the original parent. Types range from the round berries of the Pinot noir to elliptical, ovoid, troncovoid, cylindrical or other kinds of berries. Berry size is also highly variable. We obtained bunches from small to very big with variable compactness. We obtained homogenous populations for such characters as berry colour, sexual type, vigour and fertility. These characters can be considered as homozygous after the 6th generation. This study shows the necessity of making numerous generations of self pollination to obtain homozygous forms of useful characters, which are generally polygenic. These observations must be confirmed by test crosses and in the meantime the self pollination program will be continued to enable us to fix the unstable characters.

Key words: Pinot noir, variety of vine, crossing, self pollination, seedling, selection, pure line, flower, berry, bunch, growth.

Introduction

The research for 'parents' able to transmit to progeny some characters which allow the improvement of red wine grape varieties has been often studied by different authors with the help of complex selection strategies.

At the Laboratoire de Viticulture de la Station de Recherches Vigne et Vin de l'Institut National de Recherches Agronomiques de Colmar, we have obtained numerous progenies by simple or multiple crosses.

In the Pinot noir improvement process, we have found that many of the seedlings from crosses involving Pinot noir as parent have white or little coloured berries and the black berries progeny yield a low quality wine.

These reasons induced us to study the possibilities of obtaining one or several homozygous genotypes for some characters such as sexual type, fertility and black berry colour with a high pigment content, after successive self pollinated generations having as an initial parent a Pinot noir variety clone. Up to now we obtained abundant new plant material whose main characteristics we are going to explain.

Materials and methods

As the initial parent we chose the Pinot noir clone selected by INRA Colmar. It is the most cultivated clone because of its quality.

We realized 6 generations in a greenhouse, one every 2 years, one for seed germination and floral initiation and another year for self pollination, fructification and grape vintage.

In the first 3 self pollinated generations, we did not make a pedigree selection, to avoid too many families. The selection during these 3 generations eliminated all individuals with weak vigour, weak fertility (or sterility), and red, little coloured or white grapes so that only the black wine grape populations of the Pinot noir type were retained. All the female seedlings and some with pollination problems were also eliminated.

After the 4th generation, we made a pedigree selection retaining not only the red berries populations but also some white genotypes with interesting characteristics. The retained genotypes should also generate enough seedlings to permit us to study disjunction.

Table 1 shows our selection diagram and the number of genotypes obtained and selected per generation. Table 2 documents different observations and notations recorded during different generations. In Tables 3, 5 and 6 the notes describing the flower, berry and bunch characteristics are defined.

Table 1: Selection diagram

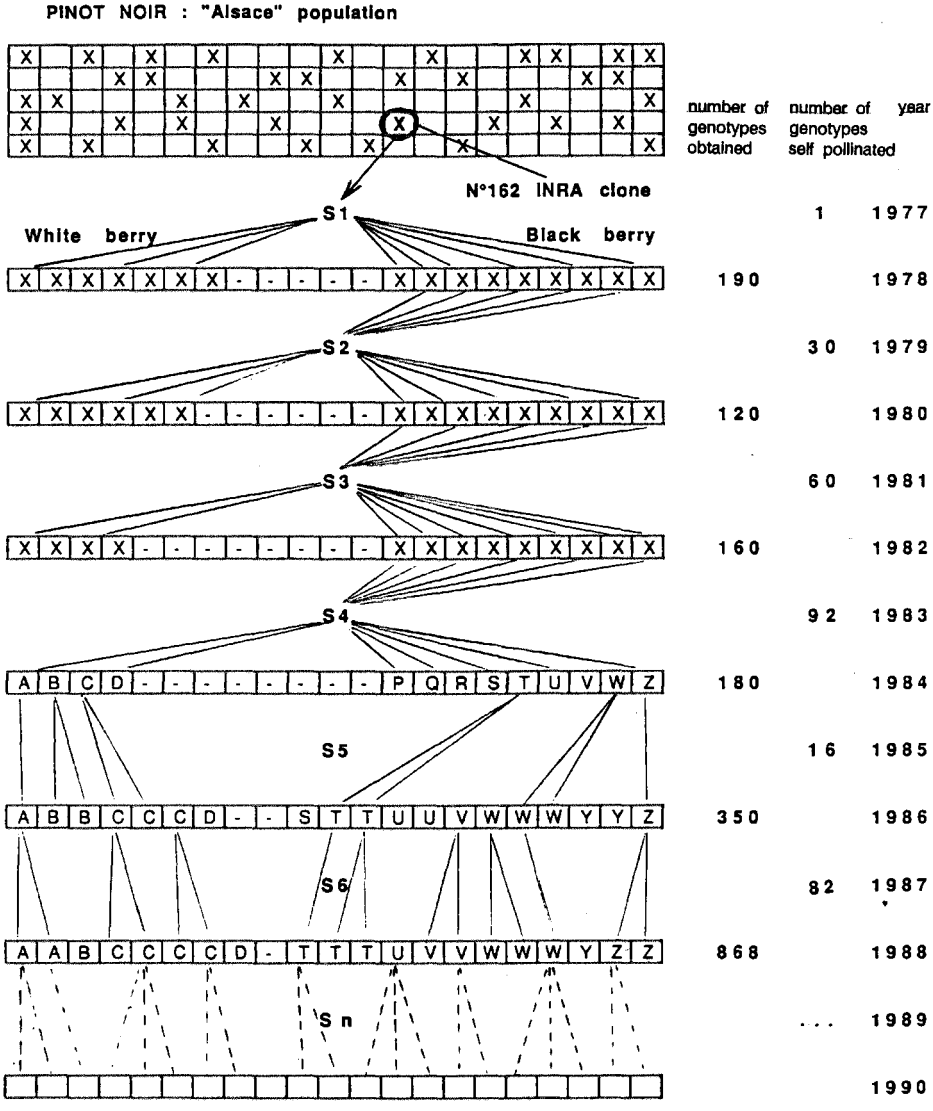


Table 2: Notes taken during the 4th-5th and 6th self pollinated generations

	1st year in greenhouse culture		
	Seedling vigor at the end of the 1st year		
	Base diameter		
	20° internode diameter after the first tendril		
	Length of 20 internodes		
Leaves colouring at the end of vegetation cycle : red colouring or not			
	2nd year in greenhouse culture		
Before	**flowering**		**Code O.I.V.**
	5 buds fertility, number of inflorescences per shoot		-
	Class of inflorescences importance		-
During	**flowering**		
	Early coulure		
	Sexual type		-
	Coulure		-
During	**veraison**		
BUNCH	Size		-
	Length		-
	Density		-
BERRY	Color	+ additional colors	-
	Size		-
	Shape	+ additional shapes	-
	Flavor		-
	Coulure and millerandage		
Ampelographic	**notes**		
	Leaf shapes		-
	Lobing degrees		
	Seedlings vigor by shoots development measures		-

Results

From 1st to 3rd self pollinated generation

During the first 3 self pollinated generations, we only retained the hermaphroditic progenies with black berries of the Pinot noir type.

4th generation (S4)

The 4th generation is composed of 92 genotypes.

Here we observed high vigour with low variation, expressed by length of shoots and by the diameter of the principal axis (Table 4). In the greenhouse, this vigour was the same as that we obtained from a simple cross with *Vitis vinifera* varieties.

Average fertility is 0.7 but with 9 sterile genotypes and 10 weakly fertile: 21 having a fertility level higher than 1.0, i. e. more than one inflorescence per shoot.

The sexual type, which is a very important criterion between generations, could be clearly determined for 49 genotypes: 11 are female and 38 hermaphroditic. The female types were eliminated.

Table 3: Notes code

FLOWER SEX physiological		BERRY COLOUR		BERRY SHAPE	
CODE OIV:	151	225		223	
1	male	1v	white-green	1	flat
3	hermaphrodite	+ 1j	white-yellow	2	slightly flat
5	female	2	rose	3	roundish
		3	red	4	short elliptic
		4	red-grey	5	ovate
		5	dark-red-violet	6	obtuse-ovate
		6	black	7	obovate acuminada
		+ 6a	blue-black	8a	cylindric
		+ 6b	dull-black	+ 8b	wide cylindric
		7	red-black	9	long elliptic
				10	arched

+codes 11 to 31 after GALET P.

BERRY LENGHT		BUNCH SIZE		BUNCH LENGTH	
221		202		203	
1	very short	1	very small	1	<= 10 cm
3	short	3	small	3	15 cm
5	medium	5	medium	5	20 cm
7	long	7	large	7	25 cm
9	very long	9	very large	9	30 cm

(+ = additional classes)

Table 4: Disjunctions in S4

Observations:	number of genotypes	average	minim.	maxim.	coeff. variation
Base diameter	92	6.9	4.8	9	17,80%
Diameter 20°eye	92	6.2	4.5	8.1	12,80%
Length 20 internodes	92	173.7	110.0	245.0	15,70%
Fertility	85	0.7	0	1.7	-

	Notes by class	(class=effectif)						Pinot Noir = class:
Sex	49	1=38	3=11	5=0				3 heterozygous
Bunch size	50	1=18	3=26	5=6				1
Length	50	1=23	3=24	5=3				1
Density	40	3=11	5=12	7=17				3
Colour	49	1=9	3=10	6=30				6
Form	50	3=30	4=15	5=2	6=1	7=1	8b=1	3
Size	42	3=7	5=28	7=7				3
Coulure	49	0=44	5=5					0
	number of plants observed							

Table 5: O.I.V code no. 223

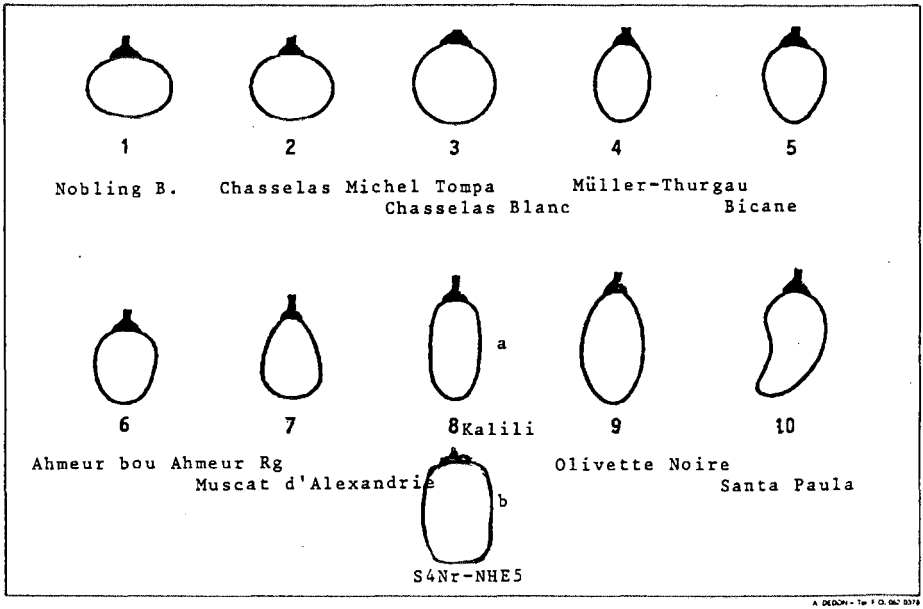


Table 6: Berry shapes according to GALET

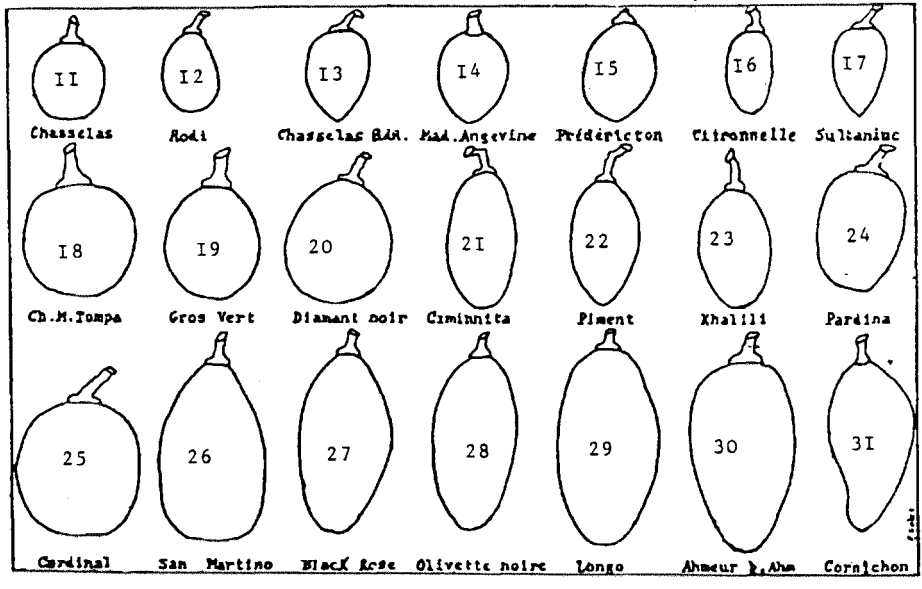


Table 7: Disjunction in S5 of the main S4 genotypes having S6 progenies

S4								S5																								Nb. of S5	
								effect.	inflor. nb				sex		berry colour				berry shape						size bunch				length bunch					giving	effectif
	NI	SX	CB	FB	GO	DM	LG		NI				SX		CB				FB						DM				LG				1 progeny	in
									0	1	2	3	3	5	1	3	5	6	3	4	5	6	7	9	1	3	5	7	1	3	5	7	9	in S6	S6
NHF0	4	3	1	3	5	5	3	70	9	28	19	6	41		39				30			2	5	3	13	16	11		12	17	11	1		19	243
NHN0	1	3	1	4	7	5	3	69	13	31	20	1	44	4	40				14	3	16	2			11	13	11	1	24	12	1			14	150
NHE0	2	3	3	4	5	1	1	5		1			1		1																		1	10
NHE9	1	3	3	3				11		6			5	1				3				2											1	1
NHF5	2	3	3	6	5	3	3	35	4	6	21	2	7		1		8	8							10	1	3		15					2	4
NHE1	2	3	6	3	5	1	1	10		6	3		9					8	5			3			2	6	1		2	6	0	1		3	28
NHF4	2	3	6	3	5	5	3	17	2	9	6		11			3		8	11						5	6			5	6				4	60
NHF6	1	3	6	4	5	1	1	14	3	6	3		5	1				8	1		5	2			3	5			8					6	21
NHI6	2	3	6	3	3	3	3	29	2	11	15		17			1		10	4			5			10	7			10	7				12	84
NHL6	2	3	6	3	5	5	5	16	3	2	10		5	1				6	3			3			1	3	2		2	1	3			3	34
NHN4	1	3	6	4	5	3	3	11	3	3	3		6		2			4		1	4			1	1	2	3		6					4	4
NHN6	1	3	6	3	7	3	3	13	2	6	3		6					7	5			3			2	3	3		2	1	4	1		3	4
NHN7	1	3	6	4	5	1	1	37	13	16	5		13	4				14	4	2	3	5			7	5			14					8	10
								total																									total	total
								337																									80	653

At this stage (S4), the bunch characters are similar to those of Pinot noir but some seedlings differ substantially and we can see in Table 4 how the length and compactness of the bunches increase.

With regard to berries, the original form is class 3, but we see many genotypes of classes 4, 5, 6, 7 and even 8. From size observations it appeared that 83 % of the berries are thicker than those of the initial parent and 25 % of them are very thick.

5th generation (S5)

During the 5th generation obtained by self pollination from the 16 genotypes identified in the 4th generation, 80 genotypes were selected and self pollinated.

When we study the sexual type of the progenies of the 4th generation of self pollination genotypes (Table 7) we notice that 7 genotypes produced only seedlings with flowers of class 3 (hermaphroditic); 6 other genotypes produced only black berries and 4 produced berries of different classes.

Table 8: S5 general characters by S4 family

family identification	vigor	height June-87	buds coulour	leaves size	lobing degrees	lobes number
NHE1	strong	2	red-green	very large	deeply lobed	5
NHE9	medium	1-1.5	red	medium	deeply lobed	5
NHF4	strong	2	red-green	very large	lobed	3 - 5
NHF5	strong	2	red	medium	deeply lobed	5
NHF6	strong	2	very dark-red	small to medium	unlobed	3
NHI6	strong	2	red-green	large	lobed	3 - 5
NHK7	weak to med.	1	red-green	medium	lobed	3 - 5
NHL5	strong	2	red-green	medium to large	deeply lobed	5
NHL6	strong	2	red	large	deeply lobed	5
NHM3	very weak	< 1	red-green	small	lobed	3 - 5
NHM6	very strong	> 2	red	very large	deeply lobed	5
NHN4	strong	2	red	medium	deeply lobed	5
NHN6	medium	1-1.5	red-green	medium	lobed	3 - 5
NHN7	medium	1-1.5	red	medium	unlobed	1 - 2
NHN0	strong	2	red	medium	deeply lobed	3 - 5

Table 9: Germination percentage by S4 family

S4 familly	Total number of seeds	Percentage of germination
NHN6	227	6.6
NHE1	649	7.4
NHE9	57	8.8
NHL6	560	15.3
NHF5	101	21.8
NHE0	268	23.5
NHN7	185	41.1
NHN4	194	43.3
NHI6	486	43.4
NHF4	445	47.8
NHF0	2367	54.1
NHF8	74	59.5
NHN0	1183	61.1

Only 1 genotype of the 4th generation is stable for this character: NHF4. The remaining S4 progenies have berries identical to the original Pinot noir but also progenies with extremely different berry shape such as NHF0: 8a.

The size of the bunches varies greatly but concerning the length notation of the bunches, 4 genotypes yield a stable progeny for this character. Table 8 shows the general characters of the different families. We can see that only 1 family has a weak vigour, 9 show strong vigour and the other 5 are medium. In S4, the coefficient of correlation between vigour and fertility is 0.49: the passage to each following generation induces an automatic selection for vigour. The size of the leaves is not to be considered as an important character because we know that its expression in a greenhouse has no correlation with that in the vineyard.

6th generation (S6)

The 6th generation includes 82 progenies from 16 genotypes of S5 coming from 13 genotypes of S4.

Table 10: Disjunction of the sexual type in S4-S5-S6

S4			S5		S6	
	H	F	H	F	H	F
NHE0	1		1	0	4	0
NHE1	1		9	0	18	0
NHE9	1		5	1	4	0
NHF0	1		41	0	129	0
NHF4	1		11	0	14	0
NHF5	1		7	0	1	1
NHF6	1		5	1	10	1
NHI6	1		17	0	30	4
NHL6	1		5	1	9	1
NHN0	1		44	4	50	5
NHN4	1		6	0	11	0
NHN6	1		6	0	0	0
NHN7	1		13	4	24	2
	H	F	H	F	H	F
Effect.	13	0	170	11	304	14

Table 11: Disjunction of the berry colour in S4-S5-S6

S4 Nr	colour	S5			code colour							S6			code colour						
		1v	1j	2	3	4	5	6	6+	6t	7	1v	1j	2	3	4	5	6	6+	6t	7
NHE0	3 red	1										4									
NHE1	6 black							8				2						12			
NHE9	6 black							3										1			
NHF0	1 white	39										102									
NHF4	6 black				3			8				3						46			
NHF5	3 red	1					8	8												.	
NHF6	6 black							8				3						6			
NHI6	6 black				1			10										30			
NHL6	6 black							6										9			
NHN0	1 white	40										46									
NHN4	6 black	2						4				6			1			3			
NHN6	6 black							7													
NHN7	6 black							14						3				19			
Effect.	13	83			4			76				166		3	1			126			

In Table 9 we can see the seed germination power leading to the seedlings of the 6th generation of self pollination. This power of germination, as per cent, is extremely variable between the S4 families. The vigour in S6 remains strong. Expressed by the measure of the growth of the 1st year primary axis, the vigour differs from 1 to 5.75 m at the end of the development with an average of 2.80 m for 655 plants. The average of shoot growth per family differs in the 2nd year of greenhouse culture from 1.90 to 2.90 m. The fertility level varies from 1 to 3 and was noted for 455 of the 655 plants.

270 plants, i. e. 59.3 %, are fertile. 30.3 % could not be determined because there were only 1 or 2 buds or they had not burst.

In S6 progenies the percentage of fertile plants is very variable but no progeny is stable. As to the sexual type (Table 10) checked in S5, there were 8 progenies out of 13 with no plants with female flowers. However in S6 the plants of these same progenies presented a few female plants. Nevertheless, we obtained some other progenies of sexual type hermaphroditic homozygous. After flowering, we made a notation about coulure and millerandage. We observed large variability on coulure notations of progenies with an average of 3.0 in most of the families and general average of 3.2, which is a high value. 6 progenies had a total coulure on the 82 progenies.

Millerandage notes are as variable as coulure notes, of the same size and with a general average of 3.1.

Table 11 shows the disjunction of berry colour regrouped with regard to S4 families. NHE0, NHF0 and NHN0 families are homogeneous: white berries. NHE9, NHI6 and NHL6 are also homogeneous but with black berries. The other families have heterogeneous progenies. The disjunction of the berry shape is shown in Table 12 where we presented the main shapes. In this table we notice the dispersion of the berry shape within some families and the distance compared to the original shape of the Pinot noir.

6th generation: the progenies

After studying all 82 S6 progenies in relation to generations S5 and S4, we wanted to know if there existed progenies with several homogeneous characters. In many cases we found a homogeneity concerning the colour and the shape of the berry, the vigour of the plant or the shape of the leave. But of the 82 progenies, 1 only was homogeneous for all observed traits. S4.NHN7-S5.REZ2-S6.THP2 to THR1 shown in Table 13.

All the notations on bunches were stable, the values of growth were extremely similar, only fertility was variable. However, the relatively low number of plants does not allow us to conclude with certainty that it is a lineage. More observations will be necessary as well as culture in the vineyard.

Discussion

The aim of the successive self pollinated generations is to obtain pure lines, i. e. individuals that are genetically identical and completely homozygous. However, self pollination induces the well-known inbreeding phenomenons (Levadoux 1950; and other authors): weak percentage of germination, high seedling mortality, low vigour of the survivors and low fertility or even sterility.

In the 6 successive self pollinated generations of the Pinot noir it is obvious that an automatic selection was made with the elimination of seedlings which are weak (appearance of a high percentage of lethal or sublethal genes), sterile, sensitive to coulure and millerandage and/or have female flowers.

Every time it was possible, we applied some technics, which permitted us to preserve a maximum of seedlings in each generation by the amelioration of the percentage of seed germination (Balthazard 1979) and by the use of new breeding techniques.

Table 12: Disjunction of the berry shape in S4-S5-S6

Code	OIV	and	Galet										ovate																	
								flat		roundish		obtuse-ovate and obovate acuminada														cylindric					
S4		S5						S6	2	3	20	5	6	7	12	17	22	24	26	4	8	9	10	15	16	19	21	25	27	29
		3	4	5	6	7	9	1	18	11		13	30								23	28	31							
												14																		
NHE0	4		1							4																				
NHE1	3	5			3				4	11										1										
NHE9	3				2															1										
NHF0	3	30			2	5	3	1	3	14		13	11			1	10	3		19	7	5	1		5		1			
NHF4	3	11						1	6	7																				
NHF5	6																													
NHF6	4	1		5	2							8								3										
NHI6	3	4			5				1	6		13								8										
NHL6	3	3			3					4										4		1								
NHN0	4	14	3	16	2				3	14		17	1							6		1								
NHN4	4		1	4			1			1		6					1					2					1			
NHN7	4	4	2	3	5					3		16	1							2										
NHN6	3	5			3																									
		77	7	28	27	5	4	2	17	64	0	73	13	0	0	1	11	3	0	44	7	9	1	0	5	0	2	0	0	0

Most of the time, we obtained from plants of the 4th, 5th and 6th generation families normal vigour that allowed the plant to initiate flowers.

From a genetical point of view, we chose as an initial parent a clone selected from a variety population including numerous types.

The starting variability was quite narrow. According to GALAIS (1981), the inbreeding effect can increase variability and therefore the progress by generation but also the duration of the

Table 13: NHN7-REZ2 progeny

S4	S5	S6	growth 1st year			Fertility		Colour	Shape	Sex	Growth
			a	b	b-a	oeil 1	oeil 2	shoot	leave		2nd year
NHN7	REZ2	THP0	281	380	99	2	2	1	2	H	120
"	"	THP2	101	189	88	0	0	1	2		98
"	"	THP3	216	345	129	0	0	1	2		131
"	"	THP4	235	342	107	0	0	1	2		158
"	"	THP5	246	367	121	2	1	1	2	H	128
"	"	THP6	265	384	119	1	0	1	2	H	137
"	"	THP7	264	385	121	0	0	1	2		140
"	"	THP8	243	347	104	1	0	1	2	H	152
"	"	THP9	273	374	101	0	1	1	2	H	122
"	"	THR1	247	355	108	1	0	1	2	H	130
Average			237.1	346.8	109.7						131.6
Coeff.of variation %			21.7	16.7	11.4						12.9

		S6	Coulure	Mille-randage	Shape berry	Colour berry	Size bunch	Length bunch	Compact. bunch		
		THP0	3	4	ovate	black	small	very small	compact		
		THP2									
		THP3									
		THP4									
		THP5	4	4	ovate	black	small	very short	compact		
		THP6			ovate	black	small	very short	medium		
		THP7									
		THP8	3	4	ovate	black	small	very short	medium		
		THP9	4	4	ovate	black	small	very short	medium		
		THR1	3	4	ovate	black	small	very short	medium		

Table 14: Summary: sex, colour and shape

	Sex		Colour	berry	Shape	berry
S4	S5	S6	S5	S6	S5	S6
NHE0	*	*	*	*		(*)
NHE1	*	*				
NHE9		*	(*)	(*)		
NHF0	*	*	*	*		
NHF4		*				
NHF5						
NHF6						
NHI6				*		
NHL6			*	*		
NHN0			*	*		
NHN4	*	*				
NHN6	*		*			
NHN7						

*= Character considered as been homozygous

selection cycle. Concerning the sexual type of the flowers, the progress to homozygoty is very slow and corroborates, if necessary, that sexual type is dependent on a complex multiallelic system with effect of relations of epistasie and of occasional dosage (CARBONNEAU 1983). The berry colour, the dimension and length of the bunches become quickly stable.

Concerning the shape of the berries we observed a very large dispersion tat also became stable during the following generations (Table 14). After 6 generations of self pollination and among 82 progenies, only 1 is homogeneous and can be considered as a pure line. The progress towards homozygoty as in the case of the sexual type quoted before is extremely slow: the noticed characters are under the dependence of sexual genes.

Conclusion

Summarizing the reported results leads us to the conclusion that in the case of the clone of Pinot noir chosen as the original parent it is necessary to carry out at least 6 generations of self pollination to obtain 1 homogeneous progeny or lineage among 82. This homogeneity concerns all the phenological characters expressed by the plant issued in the 2nd year of cultivation after sowing in a greenhouse. Many progenies are homogeneous only for one or several characters. This work will be continued with several more self pollinated generations and test crossing of the selections. The advantage of chosing one or the other obtained lineage will then be considered.

Literature cited

BALTHAZARD, J.; 1979: Contribution à l'Amélioration de la Germination des Graines de Vigne. Thèse, Univ. de Dijon.

CARBONNEAU, A.; 1983: Stérilités mâle et femelle dans le genre *Vitis*. Agronomie **3**, 635-649.

GALAIS, A.; 1981: Amélioration des populations en vue de la création de variétés. Le Sélectionneur Français **1981** (29), 5-23.

LEVADOUX, L.; 1950: La sélection et l'hybridation chez la vigne. Ann. Ecole Natl. Agricult. Montpellier **38** (3, 4).

Genetic improvement for crossbreeding in table grape varieties

S. Cancellier, A. Calo and A. Costacurta

Istituto Sperimentale per la Viticoltura, Via XXVIII Aprile, 26, I-31015 Conegliano (TV), Italy

Summary: Genetic improvement by crossbreeding of table grape varieties was realized at the Istituto Sperimentale per la Viticoltura for the achievement of the following main targets: early species, seedless species, species with high content of fructose in grapes and, at the same time, a research concerning the hereditary transmission of these features.

The results are the followings:

- Registration in the National Catalogue of the varieties of 4 new table grape varieties that are interesting for their ripening (I.C. 199, I.C. 218, I.C. 120, I.C. 213).
- Information concerning the heritability of earliness, average weight of grape and bunch for the varieties examined.
- Achievement of varieties that have a ratio between the two monosaccharides considerably tending towards fructose. This feature remains constant throughout the years.

Key words: table grape, variety of vine, Italy, breeding, heritability, berry, bunch, maturation, seedlessness, glucose, fructose.

Introduction

The cultivation of table grapes in Italy has an economically important position in some regions of Southern Italy such as Apulia and Sicily and a reasonably important position in regions of Central Italy such as Abruzzo and Latium.

The most widely cultivated varieties are: Regina and Italia (I.P. 65) which correspond to 80 % of the total production. The remaining 20 % is produced by earlier ripening cultivars such as Cardinal, Regina dei Vigneti, Panse precoce and Primus.

Therefore, offer of table grapes is concentrated in the months of September and October. While some cultivation techniques employed by viticulturists (e. g. covering) allowed an extension of the aforementioned period, market demand in the month of July can only be fulfilled with early ripening grapes. The simultaneous arrival of large amounts of table grapes in September-October sometimes decreases market prices due to an overabundance.

The current spectrum of table grape varieties would thus require improvements mainly for what concerns the time of ripening; so research institutes have the duty to provide all the information concerning current varieties and new ones to viticulturists in order to meet requirements.

Until recently, practically no seedless table grape varieties were grown in Italy for fresh consumption or raisin production. In fact, Italy is a large importer of raisins. Furthermore, we should point out mind that the markets trends in Italy and abroad are directed towards seedless grapes.

The old seedless cultivars, i. e. Sultanina, Perlette, etc., never met with viticulturists' approval, whereas more recent varieties obtained abroad (Sugraone, Red Flame, Pasiga, Perlona, etc.) are currently being considered.

There is also a plan to obtain cultivars containing large quantities of fructose for direct consumption and for the extraction of monosaccharide, considering its dietetic and physiological importance. This would diversify the market for table grapes.

For all these reasons, the Istituto Sperimentale per la Viticoltura started table grape crossbreedings in 1968 in order to examine the mechanisms of hereditary transmission of the characters that were interesting for this purpose and to produce:

A) early ripening varieties with good morphological and production characters;
B) seedless varieties suitable for Italy with a good productivity and early ripening time;
C) table grape varieties with an elevated content of fructose in grapes.

Materials and methods

The crossbreeding systems adopted for the various operating programs were as following:

A) Earliness

Work began in 1968 using as parents the medium ripening varieties: Italia, Alphonse Lavallée, Baresana, and as early ripening varieties: Perla di Csaba, Volta, Primus, Panse precoce, Regina dei Vigneti, Maddalena Bruni.

Self-pollinations were performed with all varieties. The pattern was subsequently varied by introducing some new varieties produced through previous work, i. e. I.C. 120, I.C. 199, I.C. 218, each of them back-crossed with one of the two parents: Italia (I.P. 65).

B) Seedlessness

Work began in 1984 by employing as parents the following varieties with seeds: Italia (I.P. 65), Alphonse Lavallée, Regina, I.C. 199, I.C. 218, and the seedless varieties Perlette, Ruby seedless, Sugraone, and some crossbreedings produced by A. GARGIULO in Argentina, i. e. Perlon, Pasiga, Nerona, etc.

Self-pollinations were performed with all these varieties. Operations were also carried out both with traditional methods and with new *in vitro* techniques (since 1988). *In vitro* culture has been employed to develop both ovules in crossbreedings seedless x seedless and seeds of low germinating varieties such as Regina.

C) Glucose/fructose ratio

In 1976 the analysis of the variability of the glucose/fructose (G/F) ratio and of reducing sugar in the different varieties of the ampelographic collection belonging to the Istituto Sperimentale per la Viticoltura allowed us to select some varieties as follows:

- variety A: low ratio G/F (average of 2 years 0.80)
- " B: high ratio G/F (" " " " 1.21)
- " C: low ratio G/F (" " " " 0.78)

to be introduced into a program of crossbreeding and self-pollination.

Results and discussion

A) Earliness

Tests carried out on the phenological, morphological and productive characters of the descendants from crossbreedings and self-pollination allowed to acquire important knowledge for the following work.

In particular, the analysis of the transmission of phenological times (budding, blooming, colouring, ripening) to descendants, in order to identify the most suitable parents for breeding early ripening varieties, proved high heritability for the period blooming-colouring and for the total cycle (budding-ripening): $h^2 = 0.76$ and $h^2 = 0.69$, respectively.

The analysis of the phenological behaviour of self-pollinated progeny proved that there is considerable variability among the descendants of crossbreedings and that there may exist two mechanisms of transmission for earliness encountered by us in the types:

- Volta (I.P. 105)
- Perla di Csaba

These results also indicate that the cultivar Volta is a parent capable of transmitting its own earliness to descendants (CALO *et al.* 1980). The analysis also shows that the descendants of the cross Italia x Volta have an elevated heritability for the average bunch weight ($h^2 = 0.50$). These dimensions are intermediate and inclining towards the parent with smaller dimensions. Besides these theoretical results, the study led to the identification of some descendants that were not only interesting because of their early ripening, but also for their organoleptic and morphologic characters.

Four of them were registered in the National Catalogue of Varieties:

- Conegliano 199: Italia (I.P. 65) x Volta (I.P. 105)
- Conegliano 218: Italia x Volta
- Conegliano 213: Italia x Panse precoce
- Conegliano 120: selfing of Italia.

Tables 1 and 2 summarize some characters of these varieties, even compared with their parents.

The cultivation prospects are interesting for the varieties Conegliano 199 and Conegliano 218. In fact, they both have an extremely early ripening (they ripen before Cardinal) and interesting market characters (interesting size grapes and bunch, pleasant and even blue-black colour, particular taste).

Conegliano 120 is similar to Italia from which it originates, although grapes are longer and ripen approximately 2 weeks earlier than the parent. This character makes it interesting because it ripens at a time in which there is not a great flow of grapes to the market.

The two early crossbreedings Conegliano have been tested more thoroughly to verify the effect of some cultural operations which improve the average bunch weight and the size of grapes.

Table 1: Phenological times of new varieties and of parents at Conegliano (averages of years 1986-87-88)

PHENOLOGICAL TIME	VOLTA I.P.105	PANSE PRECOCE	ITALIA I.P.65	PERLA CSABA	C.199	C.218	C.213	C.120
BUDDING	17/4	25/4	20/4	15/4	8/4	10/4	16/4	17/4
BLOOMING	2/6	10/6	6/6	3/6	28/5	1/6	5/6	6/6
COLOURING	18/7	31/7	18/8	15/7	15/7	17/7	8/8	15/8
RIPENING	6/8	8/9	26/9	6/8	6/8	7/8	21/8	10/9

Table 2: Some morphological features of the new Conegliano varieties compared to Italia (I.P. 65)

FEATURE	C 199	C 218	C 213	C 120	ITALIA
AVERAGE WEIGHT OF BUNCH (g)	206	328	316	457	575
AVERAGE WEIGHT OF GRAPE (g)	2,30	3,32	2,70	6,7	7,13

Table 3: Effects of grape bunch thinning on new early varieties (averages of 3 years)

	CONEGLIANO 199		CONEGLIANO 218	
	TEST	THINNED OUT	TEST	THINNED OUT
AVERAGE WEIGHT OF BUNCH (g)	206	263	328	405
AVERAGE WEIGHT OF GRAPE (g)	2,30	3,00	3,32	5,00

Table 4: Effects of some agronomic operations on Conegliano 199 in Apulia

	TEST	THINNING OUT	INCISION	GIBBEREL-LINS	THINN.+INCIS.	GIBBEREL.+INCIS.	THINN.+INCIS.+GIBBEREL
AVERAGE WEIGHT OF GRAPE (g)	2,36	3,60	3,20	3,30	3,92	3,90	5,50
AVERAGE WEIGHT OF BUNCH (g)	205	245	266	277	267	323	374

The tests carried out showed how interesting and favourable the response of the crosses considered were (Table 3). In fact, just grape bunch thinning had favourable effects, especially on Conegliano 218.

Researches carried out in Apulia on the variety Conegliano 199 using different techniques (bunch thinning, giberellic acid, etc.) (Table 4), both simple and combined, confirmed the results.

B) Seedlessness

Work started, as already mentioned, in 1984, and the plants produced with traditional methods and by *in vitro* techniques are being trained. We obtained the first production in 1989.

Embryo culture provided different results according to the variety.

There is little knowledge concerning the heritability of seedlessness. Some authors believe that it is controlled by some recessive genes (Spiegel-Roy *et al.* 1986).

The percentage of seedless individuals in the progenies originated by seeded x seedless crossbreedings seems to be rather low. The use of the seedless x seedless crossbreedings allows an increase in the percentage of seedless individuals, although it may aggravate some negative characters in the existing seedless varieties (small size of grapes, low fertility of buds).

C) Glucose/fructose ratio

A program of crossbreeding using as parents the varieties (A), (B) and (C) with a different ratio between monosaccharides at ripening was carried out for some years. These tests allowed the production of a group of plants with a G/F ratio more favourable for fructose. The first results have already been presented in a report to the 4th International Symposium on Grapevine Genetics at Verona (Calo *et al.* 1986). In the following years, the observation of the evolution of the ratio in the parents and in the different progenies continued.

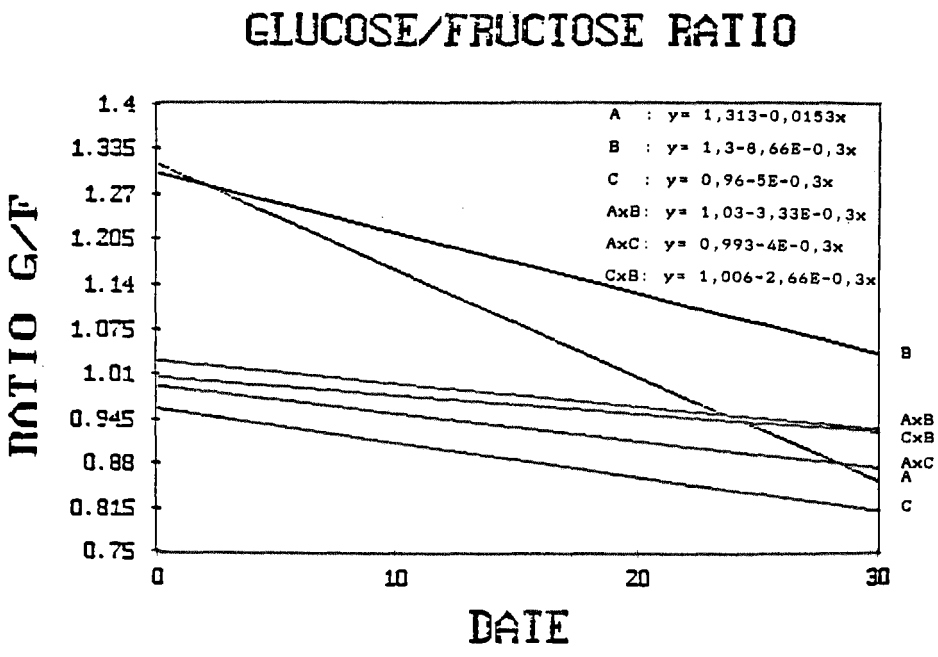


Fig. 1: Course of glucose/fructose ratio in different genotypes during ripening.

The further study emphasized the different behaviour of the two varieties (A) and (C) with a low ratio at ripening, as illustrated in Fig. 1. In fact, type (A) starts with a very high ratio (1.30-1.40) and reaches a low ratio only upon ripening. On the other hand, the low ratio of type (C) starts at the stage of colouring and continues through the entire period.

The analyses of the ratio at ripening confirmed what had already been noticed and illustrated in the previous report. In particular, as can be seen in Fig. 2, there is a movement of the ratio between starting population and population deriving from crossbreeding program, with a manifest increase of fructose as related to glucose.

Fig. 3 shows the marked difference of the G/F ratio in two types of progenies. This difference remains throughout the variability between years, thus confirming the genetic base and polyfactorial origin of the character.

Conclusions

The results concerning the programs described herein are presently encouraging for the earliness character.

For what concerns seedlessness and the production of varieties with a low glucose/fructose ratio in grapes, considerable variability has been observed among progeny which is important for future studies.

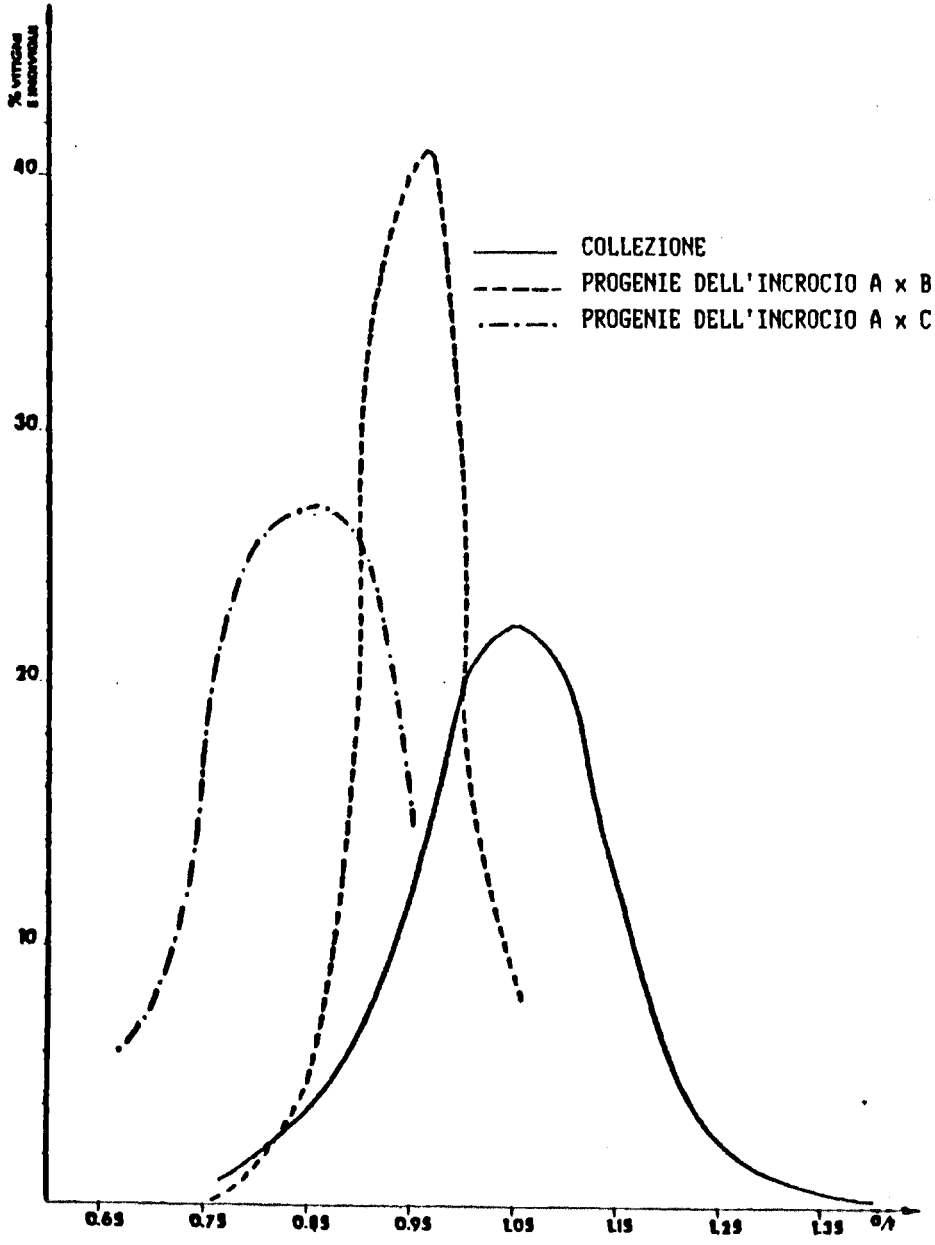


Fig. 2: Distribution of glucose/fructose ratio in the grapevine collection and in crossbreeding progenies.

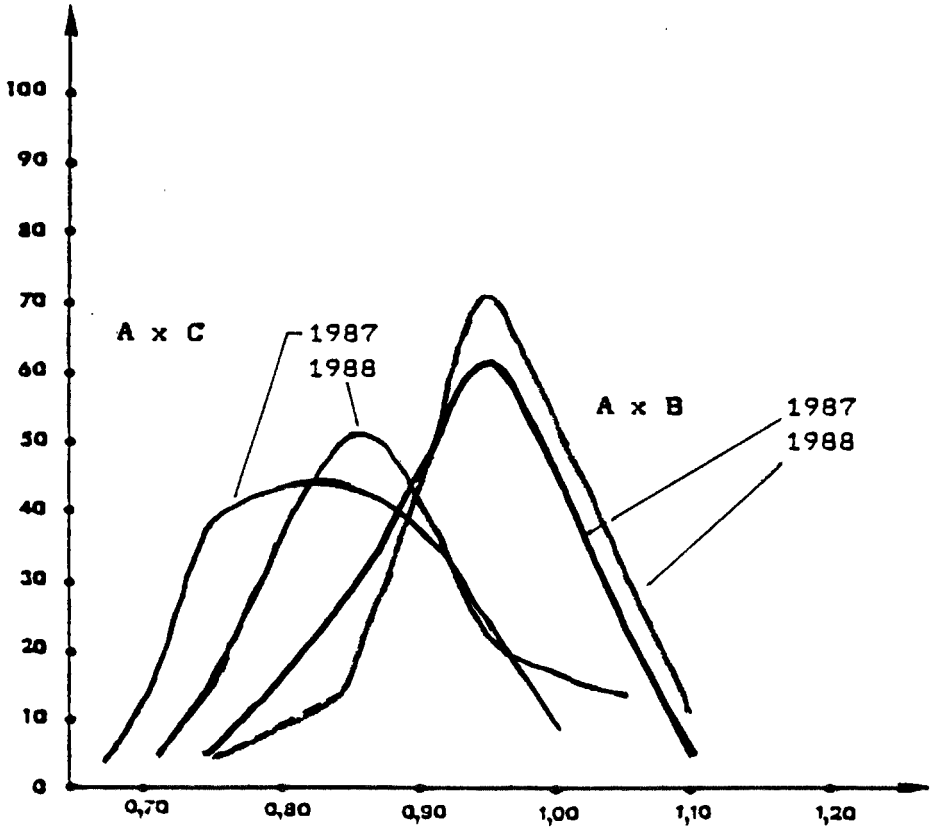


Fig. 3: Distribution of glucose/fructose ratio in two progenies for years 1987 and 1988.

References

CALO, A.; CANCELLIER, S.; COSTACURTA, A.; LORENZONI, C.; 1980: Heredity of earliness of fruit ripening in *Vitis vinifera* L. 3rd Symp. Grape Breeding, Davis, 15-18 June 1980; 227-234.

– – ; COSTACURTA, A.; CANCELLIER, S.; 1986 a: Ereditarietà del carattere precocità in una serie di incroci di *Vitis vinifera*. 4° Symp. Intern. Genetica della Vite, Verona, 13-18 Aprile 1985; 185-188.

– – ; – – ; – – ; 1986 b: Variabilità del rapporto glucosio-fruttosio nelle bacche, in discendenza da incrocio di viti. 4° Symp. Intern. Genetica della Vite, Verona, 13-18 Aprile 1985; 188-191.

COSTACURTA, A.; CANCELLIER, S.; DE LUCA, R.; 1980: Influenze genetiche nel determinismo del peso del grappolo in uve da tavola. Riv. Viticolt. Enol. 33, 481-485.

– – ; CATALANO, V.; 1988: Risultati dell'applicazione di alcune tecniche agronomiche per migliorare le caratteristiche morfologiche e merceologiche dell'incrocio ad uva da tavola precocissimo 'Conegliano 199'. Riv. Viticolt. Enol. 41, 149-157.

SPIEGEL-ROY, P.; SAHAR, N.; BARON, I.; LAVI, U.; 1986: Ovule and seed culture from early ripening seedless and seeded grape cultivars. 4° Symp. Intern. Genetica della Vite, Verona, 13-18 Aprile 1985; 34-36.

Isozyme pattern comparison between tissue-cultured grapevines and mother plants [1])

R. Botta [2]), R. Vallania [2]), M. L. Miaja [3]), G. Vergano [3]), G. Me [3])

[2]) Centro di Studio per il Miglioramento Genetico della Vite, CNR, Via P. Giuria, 15, I-10126 Torino, Italy

[3]) Istituto di Frutticoltura Industriale dell'Università, Via Ormea, 99, I-10126 Torino, Italy

Summary: Isozyme analysis is one of the means suitable to characterize clonally propagated cultivars.

Isoelectric focusing was used to reveal differences in isozyme patterns between tissue-cultured plants and mother plants, for the cultivars Barbera, Queen of the Vineyards, Dolcetto and Delight. In cultivar Barbera both 2n and 4n plants were considered.

Leaf samples were collected from shoots grown on cuttings under controlled environmental conditions and from plants obtained by tissue culture. The buds used for tissue culture were taken from the same shooted cuttings.

Leaf extracts were analyzed by isoelectric focusing considering the following isozymes: AcPH (acid phosphatase), GPI (glucose phosphate isomerase) and PGM (phosphoglucomutase).

The banding patterns of GPI and PGM showed differences among the cultivars, while for AcPH there seemed to be no differences among them in the pH range considered. There were no differences between isozyme patterns of the Barbera 2n and Barbera 4n.

The main difference between *in vitro* plants and mother plants was the amount of isozyme evaluated by densitometric measurements. In all the cultivars, the amount of isozymes for AcPH was higher in mother plants than in *in vitro* ones, while for PGM and GPI it was the opposite.

This can be due to the different environmental conditions affecting cellular metabolism.

Key words: enzyme, protein, analysis, leaf, variety of vine, clone, tissue culture, ampelography.

Introduction

Isozyme analysis of grapevine has been carried out with leaves, berries and canes using either starch gel or polyacrylamide gel electrophoresis (Wolfe 1976; Dal Belin Peruffo *et al.* 1981; Arulsekar and Parfitt 1986; Benin *et al.* 1986; Subden *et al.* 1987; Bachmann and Blaich 1988; Benin *et al.* 1988; Paludetti and Calo 1988). These authors pointed out that isozyme analysis is one of the means suitable to characterize cultivars and clones.

In this investigation isoelectric focusing was used to ascertain possible differences in isozyme patterns between tissue-cultured plants and mother plants for the cultivars Barbera, Queen of the Vineyards, Dolcetto and Delight.

The grapevines used came from a vineyard of irradiated plants (γ-rays) and presented interesting characters: Dolcetto was early ripening; Delight had berries larger than usual; Queen of the Vineyards was parthenocarpic; Barbera was tetraploid. In cv. Barbera a diploid plant was also considered.

Materials and methods

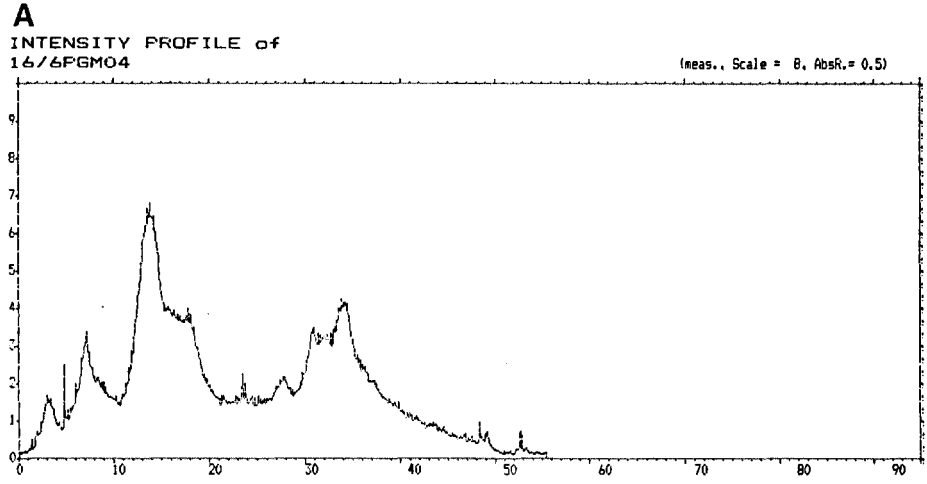
Samples of young, expanding leaves were collected from the 1st up to the 3rd node of shoots grown from cuttings and of tissue-cultured plants. The buds used for tissue culture were taken from the same shooted cuttings.

The cuttings were kept in the Knoop nutritive solution at 22 °C with a 16-h photoperiod.

The tissue-cultured plants were grown on MS (Murashige and Skoog) medium added with 5 μM BAP (6-benzylaminopurine); to stimulate rooting 1 mg/l IBA (3-indolebutyric acid) was

[1]) Contribution no. 208 of the Centro di Studio per il Miglioramento Genetico della Vite, CNR.

A



PEAK AREAS of
16/6PGM04 (Scale = 8, AbsR.= 0.5)

Scaling factor : 1.000
Background : horizontal line
Peak areas : fitted

Peak	Position	Area	Area %	Peak	Position	Area	Area %
1	3.1	162.3	2.3	7	31.2	716.5	10.3
2	7.7	812.6	11.7	8	34.0	512.5	7.4
3	13.6	1379.3	19.9	9	37.3	897.7	12.9
4	17.2	1086.1	15.6	10	43.1	165.6	2.4
5	23.3	874.7	12.6	11	47.4	112.4	1.6
6	27.8	228.1	3.3				

Total Area 6947.8 = 100.0 %

Fig. 1: Cultivar Delight; densitometric measurement of PGM banding pattern. A) *in vitro* cultured plants; B) mother plants. (Continued overleaf.)

added. The environmental conditions were kept at 24 °C during 16 h light and 16 °C during 8 h dark.

Leaf extracts were obtained according to BENIN *et al.* (1986, 1988) with 1 ml of the buffer (pH 8.5) proposed by SCHAEFER (1971) using 0.1 or 0.2 g of leaves.

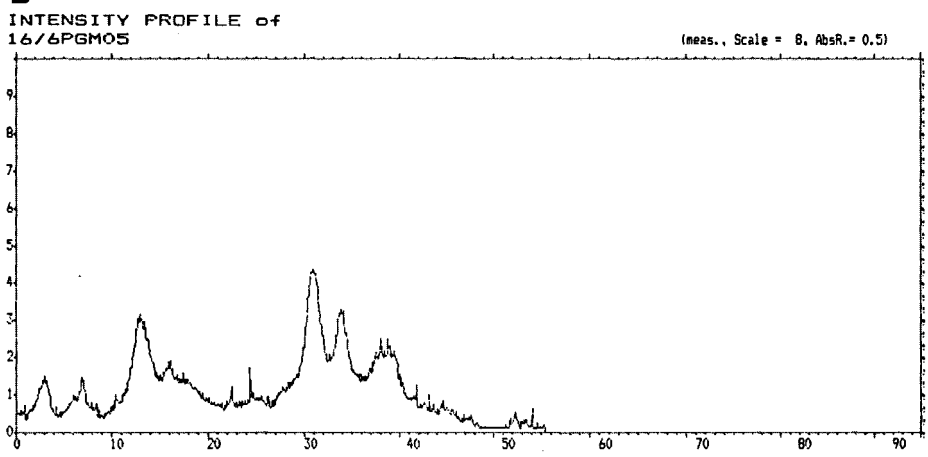
The homogenized samples were centrifuged at 4 °C for 15 min at 10,000 rpm; 20 μl of each sample were put on a polyacrylamide gel plate (LKB Ampholine-PAGplate) for isoelectric focusing.

PAGplate pH range was 4.0-6.5 for AcPH and PGM with running conditions of 2,000 V, 40 mA, 50 W at 4.3 °C for about 2 h. The pH range was 3.5-9.5 for GPI and the running conditions were 1,500 V, 50 mA, 30 W at 5 °C for about 2 h. The isoelectric points were estimated with LKB markers.

Staining solution for AcPH was prepared according to ARULSEKAR and PARFITT (1986); for PGM and GPI a staining solution modified from SHAW and PRASAD (1970) was used.

The densitometric measurements were made with an LKB 2202 ULTROSCAN laser densitometer.

B



PEAK AREAS of
16/6PGM05

(Scale = 8, AbsR.= 0.5)

Scaling factor : 1.000
Background : horizontal line
Peak areas : fitted

Peak	Position	Area	Area %	Peak	Position	Area	Area %
1	2.9	163.7	4.0	7	31.0	487.2	12.0
2	6.7	212.8	5.2	8	33.8	432.6	10.6
3	12.9	426.5	10.5	9	38.2	776.8	19.1
4	16.5	630.1	15.5	10	43.9	203.8	5.0
5	23.7	317.7	7.8	11	45.6	2.7	0.1
6	29.2	411.6	10.1				

Total Area 4065.5 = 100.0 %

Fig. 1 (continued)

Results and discussion

The isozymes studied catalyze reactions in the glucide cycle leading to the formation of sugars and starch that can be accumulated in the leaves.

The leaf amount used for the extraction (0.1 g/ml) of PGM and GPI gave scarcely evident bands for the mother plants. For this reason, the extraction was performed doubling the leaf amount of the mother plants (0.2 g/ml), thus obtaining more evident bands and revealing the different isozyme content of the plants. This difference was confirmed by densitometric measurements (Fig. 1).

The banding patterns of PGM showed no differences between *in vitro* cultured and mother plants and between Barbera 2n and 4n (Fig. 2) and the differences among the cultivars that are depicted in Fig. 3. The isoelectric range of PGM was 4.85-6.45.

Considering GPI, the cultivars showed similar anodal and cathodal bands; in the isoelectric range 5.65-5.92 the cultivar Barbera showed two bands more than the cultivars Delight and Queen of the Vineyards, while the cultivar Dolcetto had only one of them. No differences between *in vitro* cultured and mother plants or between Barbera 2n and 4n were observed. The isoelectric range of GPI was 5.65-7.3.

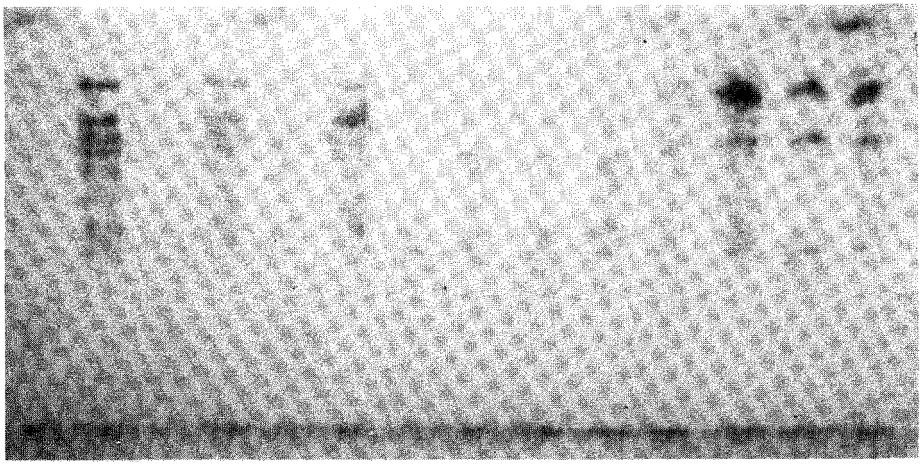


Fig. 2: banding patterns of PGM. A-a and B-b: Barbera 4n; C-c: Barbera 2n; D-d: Queen of the Vineyards; E-e: Dolcetto; F-f and G-g: Delight. Capital letters for mother plants; small letters for *in vitro* cultured plants.

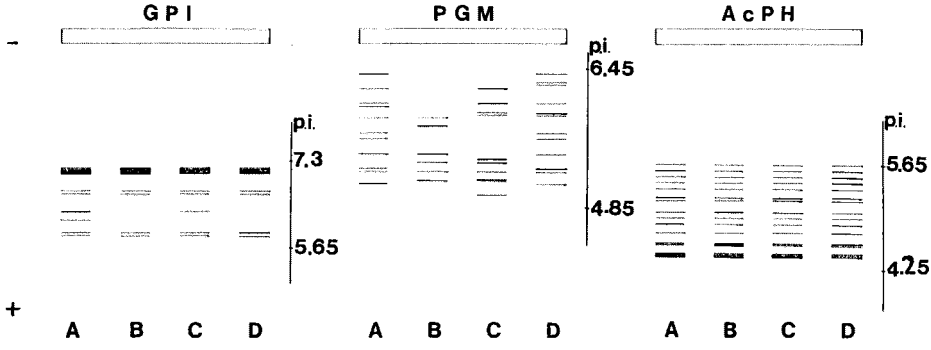


Fig. 3: Banding patterns of both *in vitro* cultured and mother plants. A) Barbera 2n and 4n; B) Queen of the Vineyards; C) Dolcetto; D) Delight.

The AcPH banding patterns showed many and quite close bands which made the comparison among the patterns difficult. Yet, the banding patterns seemed to be the same for all the cultivars considered (Fig. 4) and no differences were noticed between *in vitro* cultured and mother plants, apart from the isozyme content which was lower for the *in vitro* plants. The isoelectric range of AcPH was 4.25-5.65.

The banding patterns of PGM, GPI and AcPH are depicted in Fig. 3.

Therefore, for the three isozymes, the differences between tissue-cultured plants and mother plants were only in their amount. This can be due to the different environmental conditions affecting cellular metabolism.

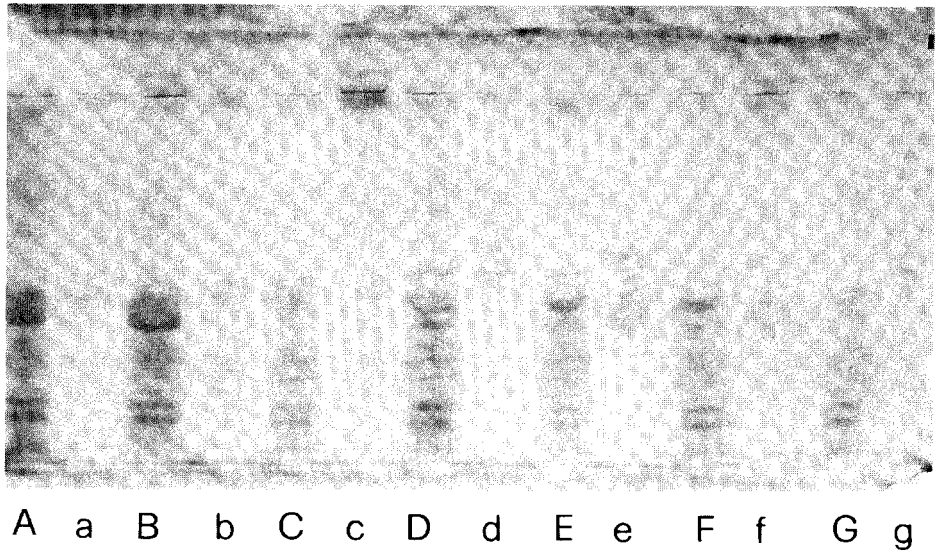


Fig. 4: Banding patterns of AcPH. For explanation of letters see legend to Fig. 2.

Literature cited

ARULSEKAR, S.; PARFITT, D. E.; 1986: Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio, and fig. HortScience **21**, 928-933.

BACHMANN, O.; BLAICH, R.; 1988: Isoelectric focusing of grapevine peroxidases as a tool for ampelography. Vitis **27**, 147-155.

BENIN, M.; GASQUEZ, J.; BESSIS, R.; 1986: Caractérisation biochimique des cépages de *Vitis vinifera* L. par analyse d'isoenzymes foliaires. 3[e] Symp. Intern. Physiologie de la Vigne, Bordeaux, 24-27 Juin, 33.

– – ; – – ; MAHFOUDI, A.; BESSIS, R.; 1988: Caractérisation biochimique des cépages de *Vitis vinifera* L. par électrophorèse d'isoenzymes foliaires: Essai de classification des variétés. Vitis **27**, 157-172.

DAL BELIN PERUFFO, A.; VARANINI, Z.; MAGGIONI, A.; 1981: Caratterizzazione di specie, varietà e cloni di vite mediante elettrofocalizzazione di estratti enzimatici fogliari. 3° Simp. Intern. Selezione Clonale Vite, Venezia, 8-12 Giugno, 31-40.

PALUDETTI, G.; CALO, G.; 1988: Elettroforesi di estratti enzimatici in *Vitis* sp.: nota metodologica. Riv. Viticolt. Enol. **41**, 365-374.

SCHAEFER, H.; 1971: Disk-elektrophoretischer Nachweis der Polyphenoloxydasen aus Rebenblättern. Vitis **10**, 31-32.

SHAW, C. R.; PRASAD, R.; 1970: Starch gel electrophoresis of enzymes. A compilation of recipes. Biochem. Genet. **4**, 297-320.

SUBDEN, R. E.; KRIZUS, A.; LOUGHEED, S. C.; CAREY, K.; 1987: Isozyme characterization of *Vitis* species and some cultivars. Amer. J. Enol. Viticult. **38**, 176-181.

WOLFE, W. H.; 1976: Identification of grape varieties by isozyme banding patterns. Amer. J. Enol. Viticult. **27**, 68-73.

Variability of must acidity in self pollinated Chardonnay progeny

P. Villa [1,3]), A. Scienza [1,2]), F. Romano [2]) and S. Stefini [3])

[1]) Istituto di Coltivazioni Arboree, Università degli Studi di Milano, Via Celoria 2, I-20133 Milano, Italy

[2]) Istituto Agrario Provinciale, Via E. Mach 1, I-38010 San Michele all'Adige, Trento, Italy

[3]) Centro Vitivinicolo Provinciale, Via Romiglia 2, I-25124 Brescia, Italy

Summary: From a total of 2,200 seedlings, obtained by self-pollination of cv. Chardonnay clone SMA 130, 250 plants were chosen and grown in a hot microclimate area. During 1986-88, morphological traits of shoot tip, leaf and bunch, as well as juice quality (sugars, pH, titratable acidity, malic and tartaric acid) were evaluated. A wide variability of the acid characteristics was noticed in the offspring. There was a significant positive correlation between total acidity and bunch size. Tartaric acid concentration was highest in medium-size bunches. A highly significant negative correlation was found between tartaric acid concentration and berry volume. More acid juice was also obtained from grapevines with a narrower apex.

Key words: self-pollination, seedling, selection, biometry, analysis, morphology, shoot, leaf, bunch, must quality, acidity.

Introduction

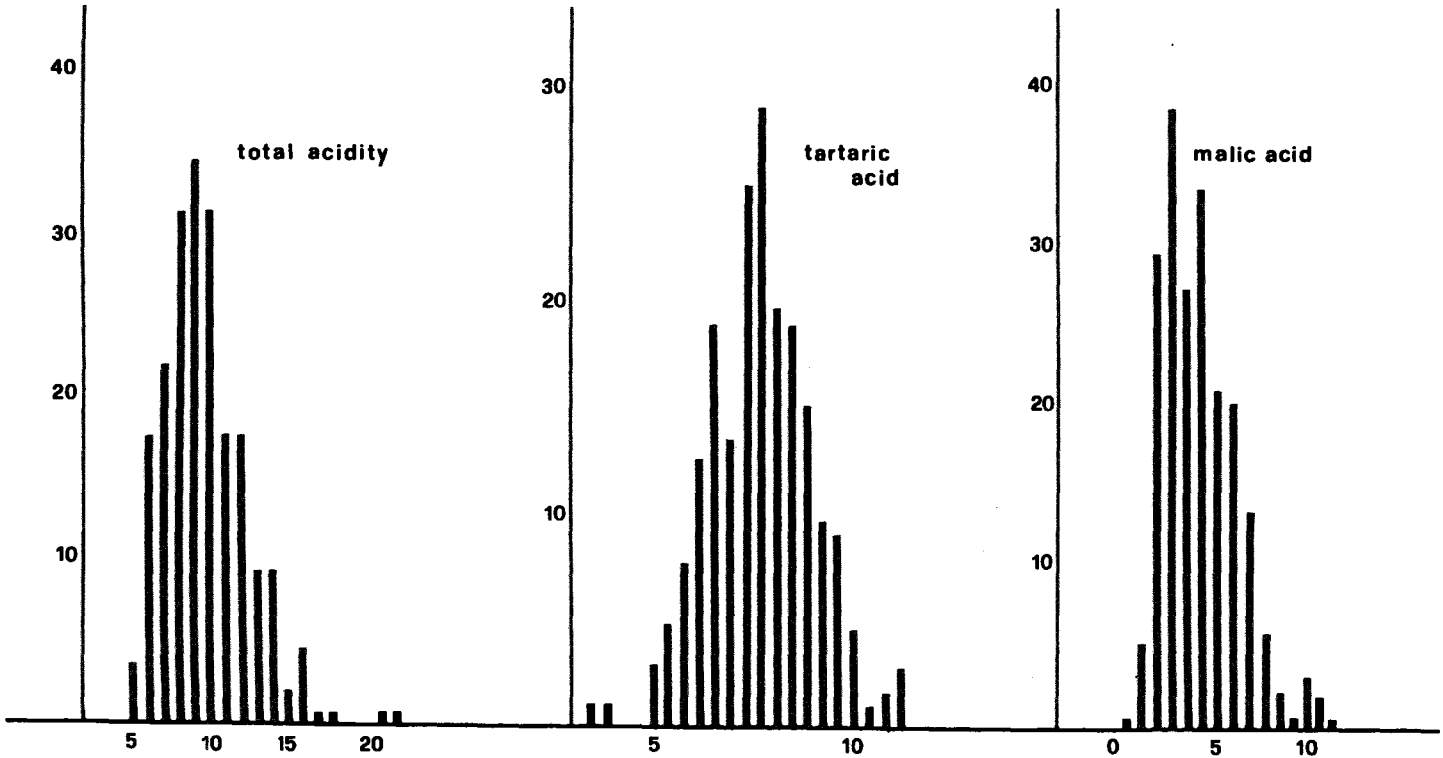
The heterozygous nature of grapevine is an interfering feature for any effective breeding program, hence the requirement for an investigation on the genetic variability of desirable traits within each clone (Fanizza and Raddi 1973; Firoozabady and Olmo 1987). One of the classical approaches for gathering information about the distribution of the genes of interest across a given population is the study of the offspring from recurrent self-pollinations (Wagner 1975; Vincourt and Gallais 1983). According to this scheme, a genetic program was undertaken with the cv. Chardonnay. The trait considered was: highly acidic must – a most needed feature for grapes to be grown in hot climates across Italy.

Material and methods

The population under investigation over the 1986-88 period included the SMA 130 Chardonnay clone and 250 fertile plants out of the about 2,200 seedlings obtained through self-pollination. The vines were planted on their own roots in the vineyard of the Centro Vitivinicolo Provinciale, Brescia, which is located in a hot microclimate area. The circumference of the trunk 20 cm above ground and the number of nodes were used to define the general vigor. A number of morphological traits (tip form, leaf size, lobe number, tooth shape, shape of petiolar sinus, cluster size, berry shape and size) were classified according to O.I.V. standards (O.I.V. 1983). The width and length of the cluster were measured and their compactness rated as: 1 = compact, 2 = well filled, 3 = loose. The fruits were crushed and total sugar content, pH, titratable acidity, malic and tartaric acid concentration of juice were determined. Data reduction was by uni- and multivariate statistics.

Results

The figure shows the frequency distribution of titratable acidity, tartaric and malic acid, as measured in the must from the offspring of self-pollinated Chardonnay. Table 1 compares the range of variability for the characters above within the parent clone and among the seedlings in 3



Frequency distribution of titratable acidity, tartaric acid and malic acid in the must from the offspring of self-pollinated Chardonnay.

Table 1: Variability range on 3 harvest years for quality traits in a Chardonnay clone and its self-pollination

	ACIDITY		MALIC ACID		TARTARIC ACID	
	parent	offspring	parent	offspring	parent	offspring
1985	4.42-6.72	5.25-9.21	2.13-5.85	3.49-7.39	2.22-3.70	2.24-3.70
1986	6.61-10.7	6.85-12.7	2.07-5.81	2.46-6.32	6.24-8.40	6.29-9.11
1987	6.90-11.6	6.10-10.8	2.36-7.10	1.38-5.44	4.02-4.81	3.51-5.99
mean	5.97-9.67	6.06-10.9	2.18-6.25	2.44-6.38	4.16-5.63	4.01-6.26

Table 2: Correlation between must acidity and morphological traits for the self-pollinated progeny from a Chardonnay clone

	ACIDITY	MALIC ACID	TARTARIC ACID
CLUSTER SIZE	0.315***	0.077	-0.139*
BERRY SIZE	-0.090	0.054	-0.356***
BERRY SHAPE	-0.071	-0.087	-0.054
NUMBER OF NODES	0.051	-0.002	-0.051
GENERAL VIGOR	0.094	0.037	-0.107
FORM OF TIP	-0.109	-0.217***	0.048
LEAF SIZE	-0.001	-0.110	-0.053

subsequent years. Only a minor improvement was observed in the seedling population, and there is little variation of the figures over the 3-year test period.

Table 2 relates some morphological traits versus titratable acidity and its components. A significant positive correlation is observed between total acidity and bunch size, while no relationship seems to exist with other parameters. An opposite trend is found for bunch size and tartaric acid, whose concentration is highest in medium-size bunches. Very significant is then the (negative) relationship of acidity versus berry solume, i. e. the increase of tartaric acid concentration with decreasing fruit size. Better (= more acidic) grapes were also obtained from plants bearing leaves with a lower number of lobes and with a narrower apex.

Conclusions

Since its establishment, the cv. Chardonnay has undergone a – direct or indirect – selection favoring the biotypes with highly acidic must, intended for the production of sparkling wine. This character is very stable within the clone, as shown by the constancy of the figures evaluated on different vintages as well as from the small standard deviation for this character among the progeny seedlings.

Also to be taken into account and further tested in any future breeding program is the finding that in this cultivar a larger bunch but a smaller berry size parallel a higher acidic content.

References

FANIZZA, G.; RADDI, P.; 1973: The heritability of fruit ripening date in *Vitis vinifera* L. Vitis **12**, 93-96.

FIROOZABADY, E.; OLMO, H. P.; 1987: Heritability and correlation studies of certain quantitative traits in table grapes, *Vitis* spp. Vitis **26**, 132-146.

O.I.V.; 1983: Codes des caractères descriptifs des variétés et espèces de *Vitis*. Office International de la Vigne et du Vin, Paris.

VINCOURT, P.; GALLAIS, A.; 1983: Sur la recherche des critères de sélection: La régression géno-phénotypique. Agronomie **3**, 827-830.

WAGNER, R.; 1975: Amélioration génétique de la vigne. Ann. Amélior. Plantes **25**, 151-175.

Wild grapevine *(Vitis vinifera* var. *silvestris)* in Italy: Distribution, characteristics and germplasm preservation - 1989 report

R. Anzani [1]), O. Failla [1]), A. Scienza [1,2]) and F. Campostrini [2])

[1]) Istituto di Coltivazioni Arboree, Università degli Studi di Milano, Via Celoria 2, I-20133 Milano, Italy

[2]) Istituto Agrario Provinciale, Via E. Mach 1, I-38010 San Michele all'Adige, Trento, Italy

Summary: Research on the distribution and characteristics of wild grapevines *(Vitis vinifera* var. *silvestris)* in Italy was started whose main goals are:

- the preservation of the germplasm by setting up plant collections;
- the furthering of biological knowledge about this plant;
- the study of relations between wild and cultivated grapevines by means of chemotaxonomic techniques;
- the assessment of the possibility of using wild plants for genetic improvement of grapevines.

The gathering of data began in 1984.

221 sites in 15 out of the 20 Italian regions have been indicated as possible locations in which wild grapevines grow. So far, 49 of these sites have been inspected. The greatest number of individuals were found in central Italy. This population is dioecious (male/female = 1.8) with few (2.0 %) hermaphrodite examples.

The leaves of the Italian wild grapevines generally have 3 lobes (57 %), but 5 to 7-lobe (36 %) and non-lobed (8.9 %) plants exist. Lower variability exists with regard to leaf shape. The study of seed morphology has classified the plants into three groupe, one of which is very numerous.

Two germplasm collections have been established with approximately 400 vines.

Key words: Vitis vinifera var. silvestris, Italy, geographical distribution, gene resources, gene bank, ampelography, biometry, analysis, sexuality, morphology, leaf, seed.

Introduction

6 years ago (Scienza 1983) research on the distribution and characteristics of wild grapevines *(Vitis vinifera* var. *silvestris)* in Italy was started whose main aims are:

- the preservation of the germplasm by setting up plant collections;
- the furthering of biological knowledge about this plant;
- the study of relations between wild and cultivated grapevines by means of chemotaxonomic techniques;
- the assessment of the possibility of using wild plants for genetic improvement of grapevines.

Initial results have already been published (Scienza *et al.* 1986 and in print). The distinguishing feature of wild European grapevines as compared to cultivated ones is their sexuality. Wild vines are mostly dioecious: the male/female ratio of Italian population is 2 : 1, with few hermaphrodite examples (2.6 %).

From the data obtained it seems likely that wild vines grow all over the country up to an altitude of 800-1000 m a.s.l.

These plants are liana-like and can achieve a remarkable development up to 15-20 m in height. They adapt to the most geologically diverse soils and can grow in different botanical associations, provided there are trees available to serve as supports. Clusters are of small dimensions, scattered or dense. Berries are small, usually spherical, with about 2 seeds each, black is the predominant colour (9 % are white). The degree of variability in sugar content, acidity, tartaric and malic acid contents is rather large, even the anthocyanin and polyphenol contents show high variability.

Chemotaxonomic studies to compare wild and cultivated vines are being carried out using pollen (Tedesco *et al.* 1990) and embryonic (Scienza *et al.* 1990) protein; and anthocyanin profiles (Mattivi *et al.* 1990).

Germplasm collections are being set up by multiplying scions from wild plants.

In this paper we report the developments achieved by this project with particular emphasis on leaf and seed morphology.

Leaf and seed morphology have been recognized as important in *Vitis* taxonomy. Leaf size and petiolar sinus angle width are important phylogenetical characteristics, indeed wild grapevines have smaller leaves with a wider sinus petiolar angle than cultivated ones (Levadoux 1956). According to Stummer (Levadoux 1956), two kinds of seeds exist: 'sativa' and 'silvestris', the first type is larger and longer, with a higher length/width ratio than the second one.

Materials and method

The gathering of data began in 1984. Based upon spot inspections and information obtained from the Corpo Forestale dello Stato (Italian Forestry Service), some geographical sites where wild grapevines are present were singled out in different regions of the country.

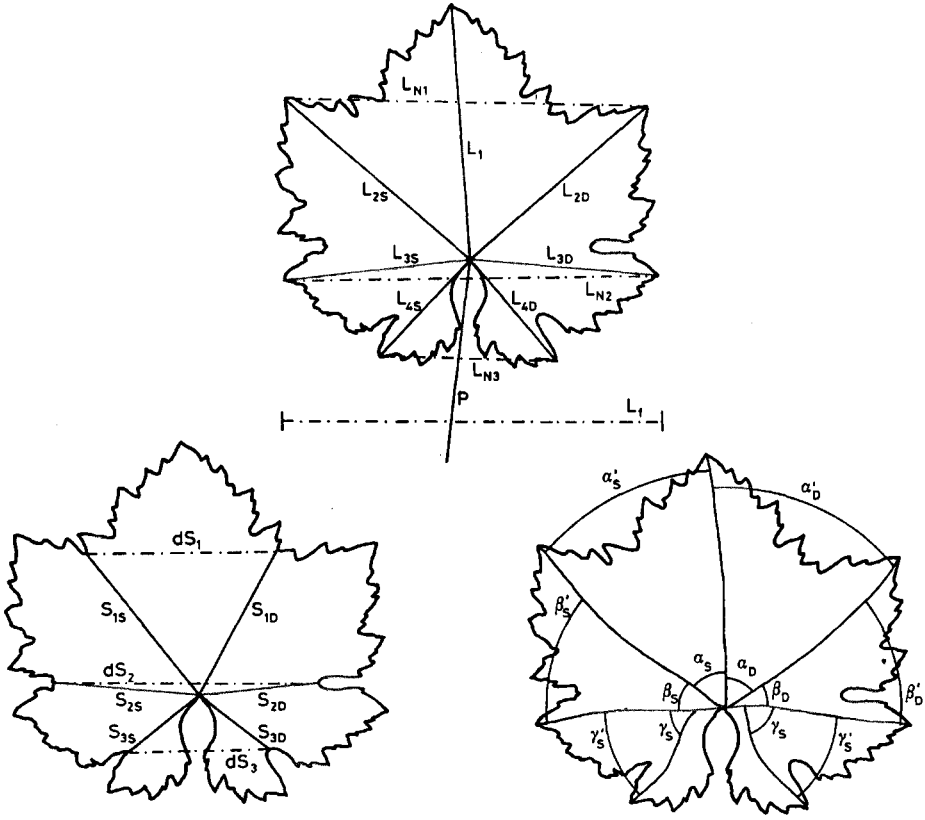


Fig. 1: Scheme of the phyllometric measures.

Whenever possible, the sex type of each individual vine was determined, and a sample made up of 10 leaves located opposite to the flower clusters was taken from each plant and measured. With male plants, a pollen sample was taken. With each female or hermaphrodite plant, samples of ripe grape clusters were collected, so that data from clusters, berries and seeds could be gathered. During winter, ligneous scions were gathered from each plant in order to multiply them in germplasm collections.

On the lower side of each leaf the following measurements were taken (Fig. 1):

L_1 = main vein length;
$L_{2D}, L_{2S}, L_{3D}, L_{3S}, L_{4D}, L_{4S}$ = superior (2), median (3) and inferior (4) lateral vein length; D = right, S = left;
L_{N1}, L_{N2}, L_{N3} = apex lateral vein distances; (1) higher, (2) median and (3) lower;
p = petiolar length;
L_f = maximum leaf width;
$S_{1D}, S_{1S}, S_{2D}, S_{2S}, S_{3D}, S_{3S}$ = superior (1), median (2) and inferior (3) sinus length; D = right, S = left;
$\alpha_D, \alpha_S, \beta_D, \beta_S, \gamma_D, \gamma_S$ = angles between vein at their insertion point or at their extremity ('); D = right, S = left.

12 phyllometric indexes were calculated:

standardized superior vein: SSN = $(L_{2S} + L_{2D}) / (2 \times L_1)$;
standardized median vein: SMN = $(L_{3S} + L_{3D}) / (2 \times L_1)$;
standardized inferior vein: AIN = $(L_{4S} + L_{4D}) / (2 \times L_1)$;
α angle = $(\alpha_D + \alpha_S) / 2$;
β angle = $(\beta_D + \beta_S) / 2$;
γ angle = $(\gamma_D + \gamma_S) / 2$;
superior lobature coefficient: SCL = $(S_{1S} + S_{1D}) / (L_{2S} + L_{2D})$;
median lobature coefficient: MLC = $(S_{2S} + S_{2D}) / (L_{3S} + L_{3D})$;
inferior lobature coefficient: ILC = $(S_{3S} + S_{3D}) / (L_{4S} + L_{4D})$;
superior lobe extension coefficient (Grenan 1984): SEC = $L_{N1} / (L_{2S} + L_{2D})$;
inferior lobe extension coefficient: IEC = $L_{N3} / (L_{4S} + L_{4D})$;
R coefficient (Galet 1956): L_1 / L_f.

These 12 coefficients and the main vein length are sufficient to describe shape and lobature characteristics of grapevine leaves (Galet 1956; Grenan 1984).

The length and maximum width of 100 seeds per vine, when possible, were measured.

Two methods of numerical taxonomy were used: discriminant and cluster analyses. Discriminant analysis was carried out using Wilks' method, step-wise procedure, minimizing the Wilks' lambda. Cluster analysis was realized with weighted pair group method centroid, using square Euclidean distances.

Results

Distribution

221 sites have been indicated as possible locations of wild grapevines, 189 of which by the Italian Forestry Service. 49 sites were inspected to ascertain the genetic nature of the vines (excluding the American species) and to collect all data and samples. On average, 3-4 vines occupy each site. Wild grapevines may occur in 15 out of 20 Italian regions, their presence has been

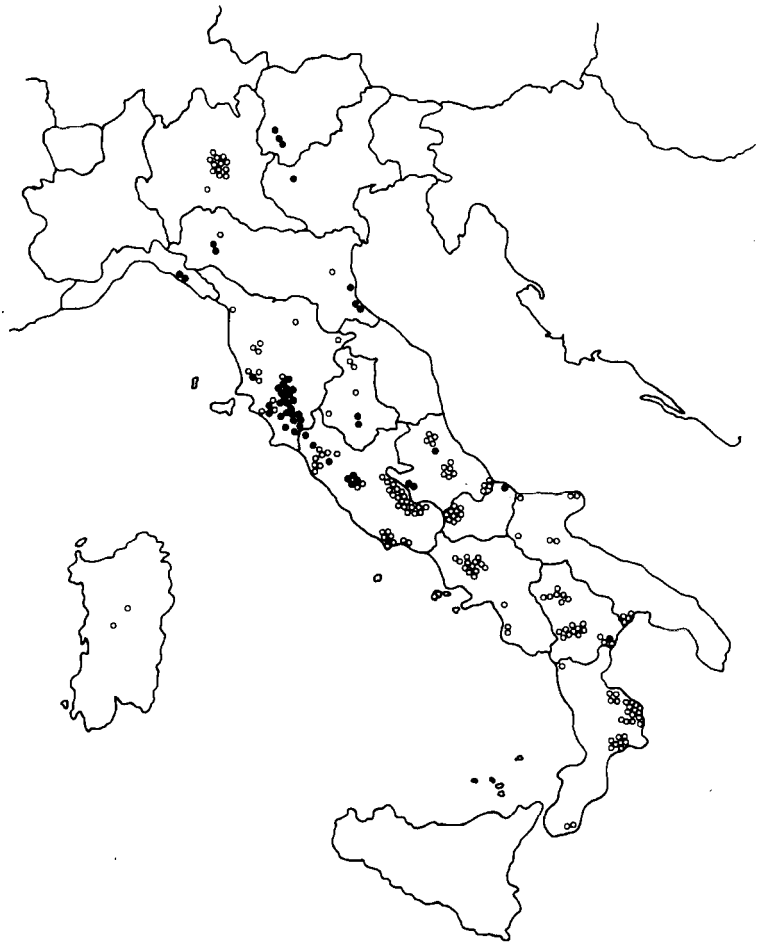


Fig. 2: Location of *Vitis vinifera* var. *silvestris* sites in some Italian regions. Indicated (o) and visited (•) sites.

ascertained in 10 until now. The greatest number of individuals have been found in central Italy (Fig. 2, Table 1).

Sex

About 65 % of the plants were studied during their flowering season. Of these the male/female ratio was approximately 1.8 while hermaphrodite plants accounted for only 2.0 % (Tables 2 and 3).

Leaf morphology

Table 4 indicates the main statistical characteristics of the 13 phyllometric indices studied.

Wild grapevine leaves are small (L_1 35-110 mm), with low length/width ratio (R 0.60-1.00), wide sinus petiolar angle ($\alpha + \beta = 65\text{-}120°$ and IEC 0.7-1.5). Leaves range from non-lobed (SLC 1) to highly lobed (SLC 0.4).

Table 1: Distribution of wild grapevine sites as located in some Italian regions

DISTRICTS	S I T E S		
	INDICATED	VISITED	VINES
TRENTINO	3	3	13
LOMBARDY	13	0	-
VENETIA	1	1	1
EMILIA	7	3	11
LIGURIA	2	2	3
TOSCANY	37	24	92
UMBRIA	6	2	3
LATIUM	45	8	23
ABRUZZO	16	2	7
MOLISE	11	1	2
APULIA	10	0	-
CAMPANIA	18	0	-
BASILICATE	21	1	6
CALABRIA	29	0	-
SARDINIA	2	0	-
T O T A L	221	49	161

Table 2: Ratio distribution of wild vines in relation to their sex

TOTAL		MALES		FEMALES		HERMAPHRODITES	
INDIVIDUALS	%	INDIVIDUALS	%	INDIVIDUALS	%	INDIVIDUALS	%
101	100	64	63.4	35	34.6	2	2.0

Table 3: Sex characteristics of the wild vines sampled in Italy

TOTAL		PROBABLY POST-CULTURAL*		MALES		FEMALES		HERMAPHRODITES		UNCLASSIFIED		FEMALES OR HERMAPHRODITES	
INDIVIDUALS	%	INDIV.	%	INDIV.	%	INDIV.	%	INDIV.	%	INDIV.	%	INDIV.	%
161	100	8	5.0	64	39.8	35	21.7	2	1.2	32	19.9	20	12.4

* ACCORDING LEVADOUX (1956)

Table 4: Main characteristics of the wild grapevine leaves

	AVERAGE	S.D.	MIN.	MAX
MAIN VEIN LENGTH mm	73.3	19.2	28	169
STAND. SUPERIOR VEIN	0.86	0.08	0.59	1.12
STAND. MEDIAN VEIN	0.61	0.09	0.34	0.94
STAND. INFERIOR VEIN	0.35	0.07	0.17	0.67
SUPERIOR LOBATURE COEFF.	0.71	0.15	0.25	0.97
MEDIAN LOBATURE COEFF.	0.84	0.10	0.38	1.10
INFERIOR LOBATURE COEFF.	0.88	0.06	0.60	1.18
α ANGLE	45.5	6.90	26.5	70.0
β ANGLE	48.0	7.43	27.5	75.5
γ ANGLE	47.0	7.36	21.5	70.5
SUPERIOR LOBE EXTENSION COEFF.	0.63	0.08	0.41	0.96
INFERIOR LOBE EXTENSION COEFF.	1.07	0.21	0.17	1.71
R COEFF.	0.80	0.10	0.53	1.12

Cluster analysis carried out on the lobature coefficients (SLC, MLC and ILC) of the average leaf of each plant classified the wild grapevines in four groups (Fig. 3, Table 5): A1 (38 individuals) 5 to 7-lobe leaves, A2 (7 individuals) 5 to 7 marked-lobe leaves, B1 (11 individuals) light-lobe leaves and B2 (59 individuals) 3-lobe leaves.

Cluster analysis carried out on shape coefficients (SSN, SMN, SIN, α, β, γ, SEC, IEC, and R) of the average leaf of each plant classified the wild grapevines in four groups (Fig. 4, Table 6): C1 (1 individual) very close sinus petiolar angle, C2 (15 individuals) close sinus petiolar angle, D1 (3 individuals) very wide sinus petiolar angle and D2 (97 individuals) wide sinus petiolar angle.

The validity of the results of the cluster analyses has been verified with discriminant analysis. These analyses were carried out using all the collected leaves, after having classified the vines according to cluster analysis groups, and using the same phyllometric indexes as used in the respective cluster analyses.

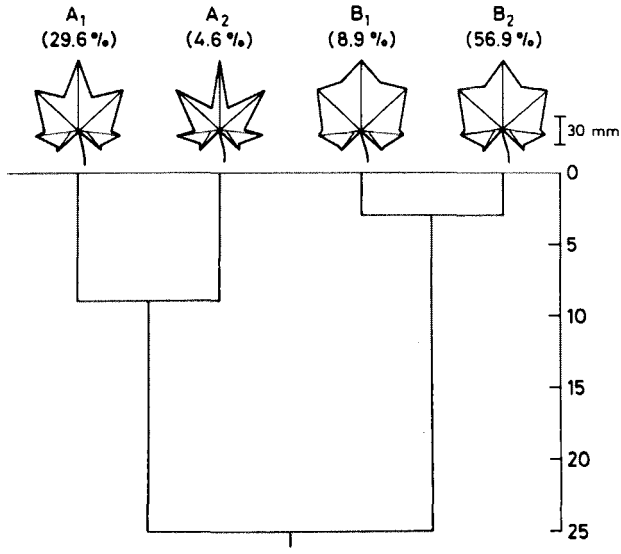


Fig. 3: Dendrogram and average leaf types of the cluster analysis relative to lobature characteristics of the wild vine leaves.

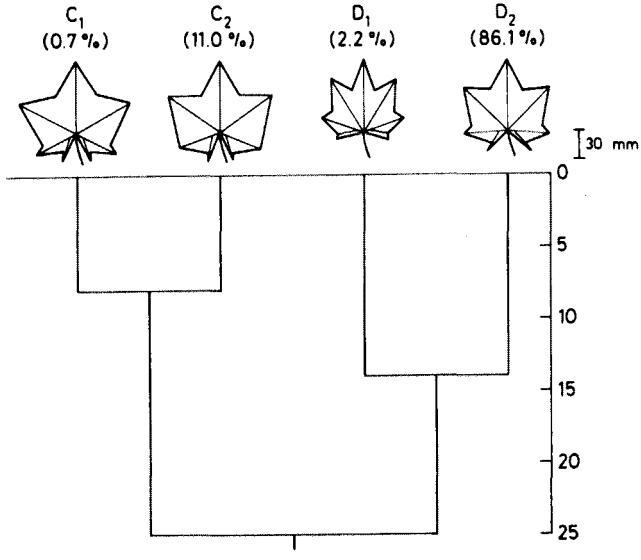


Fig. 4: Dendrogram and average leaf types of the cluster analysis relative to shape characteristics of the wild vine leaves.

Table 5: Average lobature coefficients of the four lobature cluster groups

GROUP	SUPERIOR	MEDIAN	INFERIOR
B2	0.76	0.87	0.89
B1	0.87	0.91	0.91
A2	0.38	0.58	0.82
A1	0.61	0.80	0.86

Table 6: Average angles of the four shape cluster groups

GROUP	ANGLES		
	α	β	γ
D2	44.8	47.1	46.5
D1	32.1	37.2	38.2
C2	52.6	56.6	53.0
C1	60.4	55.7	44.3

The discriminant analysis regarding the lobature groups (Fig. 5, Table 7) indicates that superior and median lobes are of greater importance than inferior ones, 68 % of cases correctly classified show a high variability in leaf lobature both within plants and groups.

The discriminant analysis regarding the shape groups (Fig. 6, Table 8) indicates the greater importance of the angles α, β and γ. The results of grouped cases correctly classified (76 %) show a satisfactory difference between the groups.

Leaf lobature is different in northern, central and southern Italy. In northern Italy, type A1 (5 to 7-lobe leaves) is prevalent; in southern Italy, type B2 (3-lobe leaves) is prevalent, in central Italy both types are present (Table 9).

Leaf morphology is related to plant sex. Leaves in male plants are smaller than in female ones, of analogous and petiolar sinus angle. Hermaphrodite plants show greater leaf size and a less open petiolar sinus angle (Table 10).

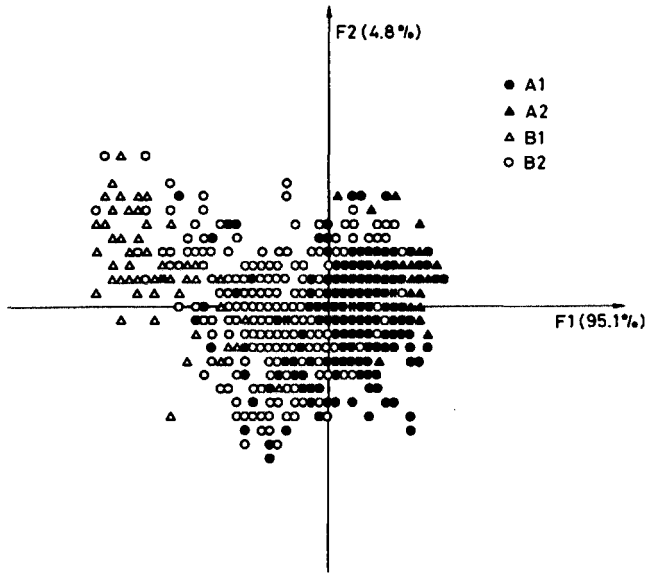


Fig. 5: Diagram of the discriminant analysis relative to lobature characteristics of the wild vine leaves.

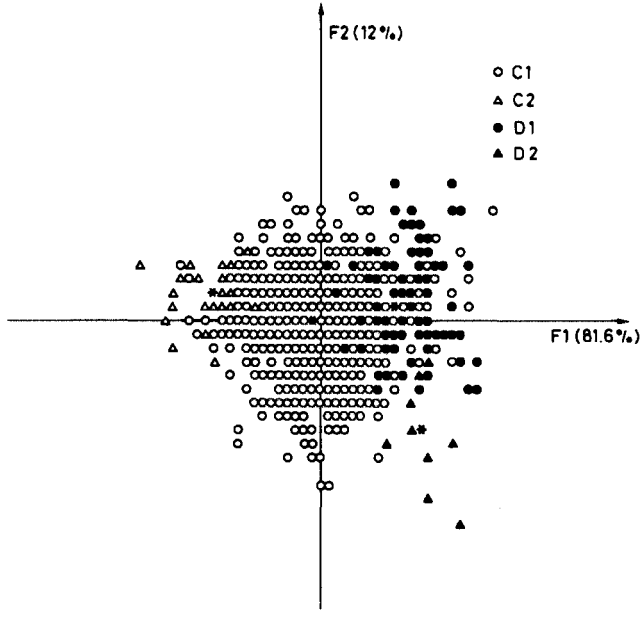


Fig. 6: Diagram of the discriminant analysis relative to shape characteristics of the wild vine leaves.

Table 7: Discriminant analysis about lobature cluster groups

CANONICAL DISCRIMINANT FUNCTIONS

FUNCTION	EIGENVALUE	% OF VARIANCE	CANONICAL CORRELATION	AFTER FUNCTION	D.F.	WILKS' LAMBDA
				0	9	0.38***
1	1.44	95.1	0.77	1	4	0.93***
2	0.07	4.8	0.26			

STANDARDIZED CANONICAL DISCRIMINANT FUNCTIONS COEFFICIENTS

	FUNC. 1	FUNC. 2
SUPERIOR LOBATURE COEFF.	**0.71**	0.87
MEDIAN LOBATURE COEFF.	**0.42**	-1.13
INFERIOR LOBATURE COEFF.	0.07	0.36

POOLED WITHIN - GROUPS CORRELATION BETWEEN DISCRIMINATING VARIABLES AND CANONICAL DISCRIMINANT FUNCTIONS

	FUNC. 1	FUNC. 2
SUPERIOR LOBATURE COEFF.	**0.92**	0.35
MEDIAN LOBATURE COEFF.	**0.79**	-0.59
INFERIOR LOBATURE COEFF.	0.25	0.07

CLASSIFICATION RESULTS

ACTUAL GROUP	N° OF CASES	PREDICTED GROUP MEMBERSHIP (%) A2	B1	A2	A1
B2	779	**55.8**	29.7	0.9	13.6
B1	122	9.0	**91.0**	0.0	0.0
A2	63	0.0	0.0	**81.0**	19.0
A1	406	20.2	4.7	12.1	**63.1**

PERCENT OF "GROUPED" CASES CORRECTLY CLASSIFIED : 62.3%

Seed morphology

Seed characteristics and variability are shown in Table 11. Cluster and discriminant analyses were used in the same way as in the leaf morphology study.

Cluster analysis carried out on length, width and length/width ratio of the average seed of each of the 30 vines of which the seeds could be measured classified the wild grapevines in three groups (Fig. 7, Table 12): A (25 individuals) average seed type, B (2 individuals) small and round seeds; C (3 individuals) large and long seeds.

Table 8: Discriminant analysis about shape cluster groups

FUNCTION	EIGENUALUE	% OF VARIANCE	CANONICAL CORRELATIION	AFTER FUNCTION	D.F.	WILK'S LAMBDA
				0	27	0.59***
1	0.55	81.6	0.60	1	16	0.89***
2	0.08	12.0	0.27			

STANDARDIZED CANONICAL DISCRIMINANT FUNCTION COEFFICIENTS

	FUNC. 1	FUNC. 2
STAND. SUPERIOR VEIN	-0.18	-0.08
STAND. MEDIAN VEIN	0.14	1.64
STAND. INFERIOR VEIN	-0.62	0.00
α ANGLE	0.47	0.10
β ANGLE	0.43	0.15
γ ANGLE	0.18	0.44
SUPERIOR LOBE EXTENSION COEFF.	-0.08	-0.37
INFERIOR LOBE EXTENSION COEFF.	-0.49	-0.30
R COEFF.	0.03	1.60

POOLED WITHIN-GROUPS CORRELATION BETWEEN DISCRIMINATING VARIABLES AND CANONICAL DISCRIMINANT FUNCTIONS

	FUNC. 1	FUNC. 2
α ANGLE	**0.75**	-0.26
β ANGLE	**0.69**	0.22
γ ANGLE	**0.44**	**0.40**
SUPERIOR LOBE EXTENSION COEFF.	**0.48**	**-0.66**
INFERIOR LOBE EXTENSION COEFF.	**-0.57**	0.02
STAND. INFERIOR VEIN	-0.10	0.32
R. COEFF.	-0.10	0.05
STAND. MEDIAN VEIN	-0.15	0.12
STAND. SUPERIOR VEIN	-0.14	0.31

CLASSIFICATION RESULTS

ACTUAL GROUP	N° OF CASES	PREDICTED GROUP MEMBERSHIP (%) D2	D1	C2	C1
D2	1179	**74.7**	8.6	14.3	2.4
D1	30	3.3	**96.7**	0.0	0.0
C2	151	15.9	0.0	**78.8**	5.3
C1	10	0.0	0.0	0.0	**100.0**

PERCENT OF "GROUPED" CASES CORRECTLY CLASSIFIED : 75.84%

Discriminant analysis (Fig. 8, Table 13) indicates the great importance of seed length in grouping the vines. 72% of grouped cases correctly classified show a satisfactory difference between the groups.

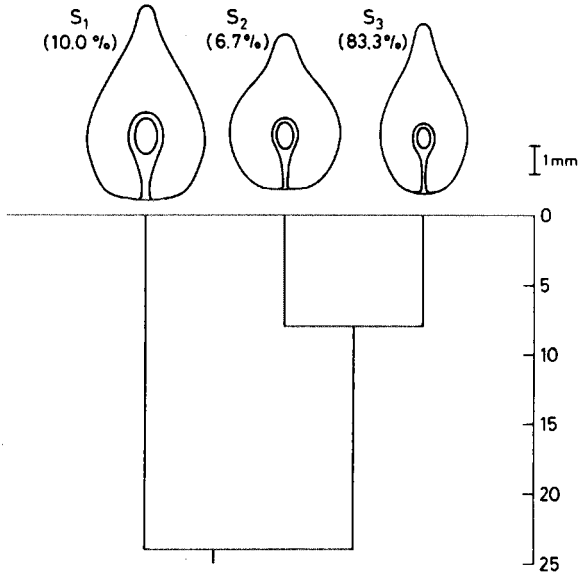


Fig. 7: Dendrogram and average leaf types of the cluster analysis relative to seed morphology of the wild vines.

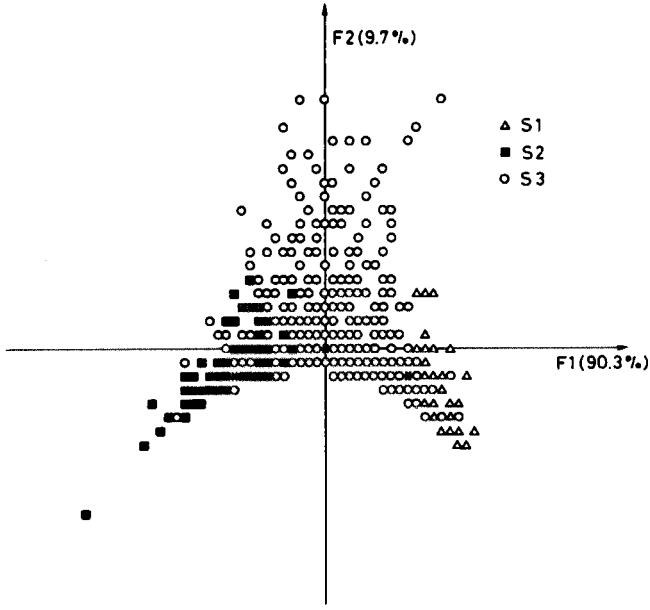


Fig. 8: Diagram of the discriminant analysis relative to seed morphology of the wild vines.

Table 9: Distribution of the four lobature cluster groups in relation to grographical location of the wild vines

LOCATION	N° OF VINES	LOBATURE CLUSTER GROUP			
		A1	A2	B1	B2
NORTH	16	**62.4**	18.8	0.0	18.8
CENTER	93	**29.0**	4.3	11.8	**54.9**
SOUTH	6	16.7	0.0	0.0	**83.3**

Table 10: Leaf characteristics in relation to plant sex

	ANGLES			INFERIOR EXTENSION COEFFICIENT	MAIN VEIN LENGTH
	α	β	γ		
MALES	45.1a	47.6a	46.9a	1.11a	70a
FEMALES	45.5a	48.0a	47.0a	1.03b	76b
HERMAPHRODITES	51.8b	55.8b	49.9b	0.79c	95c

Table 11: Main characteristics of wild grapevine seeds

	AVERAGE	S.D.	MIN.	MAX.
LENGTH mm	6.05	0.57	3.70	7.80
WIDTH mm	4.12	0.36	3.00	5.30
LENGTH/WIDTH	1.48	0.16	1.04	2.06

Table 12: Average dimension and shape of the three seed cluster groups

GROUP	N° OF CASES	LENGTH mm	WIDTH mm	LENGTH/WIDTH
S3	158	6.96	4.32	1.61
S2	177	5.06	3.93	1.29
S1	2206	6.06	4.12	1.48

Table 13: Discriminant analysis about seed cluster groups

CANONICAL DISCRIMINANT FUNCTIONS

FUNCTION	EIGENVALUE	% OF VARIANCE	CANONICAL CORRELATIION	AFTER FUNCTION	D.F.	WILK'S LAMBDA
				1	6	0.59***
1	0.59	90.3	0.61	0	2	0.94***
2	0.06	9.7	0.24			

STANDARDIZED CANONICAL DISCRIMINANT FUNCTION COEFFICIENTS

	FUNC.1	FUNC.2
LENGTH	0.05	-7.13
WIDTH	1.12	7.97
LENGTH/WIDTH	1.26	9.49

POOLED WITHIN - GROUPS CORRELATION BETWEEN DISCRIMINATING VARIABLES AND CANONICAL DISCRIMINANT FUNCTIONS

	FUNC.1	FUNC.2
LENGTH	0.99	-0.13
LENGTH/WIDTH	0.52	0.08
WIDTH	0.26	-0.09

CLASSIFICATION RESULTS

ACTUAL GROUP	N° OF CASES	PREDICTED GROUP MEMBERSHIP (%) S3	S2	S1
S3	158	**88.0**	0.0	12.0
S2	177	0.0	**92.0**	7.9
S1	2206	17.5	12.8	**69.7**

PERCENT OF "GROUPED" CASES CORRECTLY CLASSIFIED : 72.37

Germplasm conservation

Germplasm collections are being established by multiplying scions from wild plants. Thanks to the collaboration of European institutes, in these collections wild grapevines of different European, North-African and Asiatic regions are being gathered in addition to Italian wild grapevines (Table 14).

Table 14: Number of genotypes and vines gathered in the germplasm collection

COLLECTION	GENOTYPES	VINES
SIENA		
ITALIAN	33	106
OTHER	36	160
TRENTO		
ITALIAN	48	90
OTHER	32	85

Conclusion

The research studies are helping to precisely define the distribution and characteristics of wild grapevines in Italy.

From the data obtained and the recent information from the Italian Forestry Service, we can assert that wild vines grow all over the country from 0 to 800 m a.s.l.

This population is dioecious (male/female = 1.8) with few (2.0 %) hermaphrodite examples.

The leaves generally have 3 lobes (57 %) but 5 to 7-lobe (36 %) and non-lobed (8.9 %) plants exist. Lower variability exists with regard to leaf shape: 86.1 % of the vines were classified in the same cluster group.

Due to the increase in sampled vines, the differences between the leaves of male and female plants have become less important with respect to our previous observations (Scienza *et al.* 1988); instead, the differences between the leaves of hermaphrodite and unisexual plants have been confirmed.

The study of seed morphology has classified the plants into three groups, one of which is very numerous (83.3 %).

Investigations on the unvisited sites should confirm these results.

Furthering leaf and seed morphology studies will compare wild and cultivated plants. The germplasm collection will be useful for the comparison.

Literature cited

Galet, P.; 1956: Cépages et Vignobles de France. I. Les Vignes Américaines. Dehan, Montpellier.

Grenan, S.; 1984: Polymorphisme foliaire consécutif à la culture *in vitro* de *Vitis vinifera* L. Vitis **23**, 159-174.

LEVADOUX, L.; 1956: Les populations sauvages et cultivées de *Vitis vinifera*. Ann. Amélior. Plantes **1**, 59-118.

MATTIVI, F.; SCIENZA, A.; FAILLA, O.; VILLA, P.; ANZANI, R.; TEDESCO, G.; GIANAZZA, E.; RIGHETTI, P.; 1990: *Vitis vinifera* – a chemotaxonomic approach: Anthocyanins in the skin. Proc. 5th Intern. Symp. Grape Breeding, 12-16 Sept. 1989, St. Martin/Pfalz, FRG.

SCIENZA, A.; 1983: Notizie ed importanza di alcuni recenti ritrovamenti di *Vitis vinifera silvestris* GMELIN in Italia. Not. Ortoflorofrutticolt. **6**, 297-310.

– – ; ANZANI, R.; FAILLA, O.; MATTIVI, F.; VILLA, P. L.; TEDESCO, G.; RIGHETTI, P. G.; ETTORI, C.; MAGENES, S.; GIANAZZA, E.; 1990: *Vitis vinifera* – a chemotaxonomic approach: Seed storage proteins. Proc. 5th Intern. Symp. Grape Breeding, 12-16 Sept. 1989, St. Martin/Pfalz, FRG.

– – ; FAILLA, O.; ANZANI, R.: Wild grapevine (*Vitis vinifera* L. *silvestris* GMEL.) in Italy: Diffusion, characteristics and germplasm preservation. Proc. Germplasm Preservation of Horticultural Crops: Wild and Cultivated Plants, 1-3 Sept. 1988, Bejing, Chin. Pop. Rep. (in print).

– – ; PROTTI, A.; CONCA, E.; ROMANO, F.; 1986: Diffusione e caratteristiche della *Vitis vinifera silvestris* GMELIN in Italia. Proc. 4th Intern. Symp. Grape Breeding, 13-18 April 1985, Verona, Italia.

TEDESCO, G.; CARGNELLO, G.; ZANARDINI, E.; VILLA, P.; MANINI, F.; GIANAZZA, E.; 1990: *Vitis vinifera* – a chemotaxonomic approach: Pollen wall proteins. Proc. 5th Intern. Symp. Grape Breeding, 12-16 Sept. 1989, St. Martin/Pfalz, FRG.

Vitis vinifera - a chemotaxonomic approach: Pollen wall proteins

G. Tedesco, G. Cargnello, E. Zanardini, P, Villa, F. Manini and E. Gianazza

Dipartimento di Biologia Luigi Gorini, Sezione di Botanica Sistematica, Università degli Studi, Via Celoria 26, I-20133 Milano, Italy

Summary: The electrophoretic pattern of the pollen wall proteins from clones of cv. Nebbiolo grown in different areas show a geographical clustering. Vermentino, Pigato and Favorita are found virtually identical both by morphological and biochemical criteria.

Key words: pollen, cell wall, protein, Vitis vinifera, variety of vine, clone, ampelography, Italy.

Introduction

Pollen wall proteins as extracted by 0.2 M glycine give on pH 3.5-10 focusing range complex banding patterns with tens of components clustered in the acidic to neutral portion of the gradient (Cargnello *et al.* 1988). For cvs Cabernet and Merlot, the IEF pattern has been shown to be specific to individual clones and independent from the environment as well as from the growing conditions (Cargnello *et al.* 1988; Tedesco *et al.* 1989). The above approach was thus applied when addressing two problems in *Vitis* systematics.

Materials and Methods

For each sample 0.2 M glycine buffer extracts were obtained with 1:20 (w/v) ratio and adjusted to ca. 20-30 μg/lane.

Isoelectric focusing under native conditions of the pollen wall extracts was performed on a non-linear pH 3.5-10 gradient. Proteins were stained with silver nitrate according to Merril *et al.* (1981). Zymograms were developed for esterase (Coates *et al.* 1975), phosphoglucomutase (Spencer *et al.* 1964), acid phosphatase (Swallow and Harris 1972), alcohol dehydrogenase (Smith *et al.* 1971) and peroxidase (Taketa 1987).

After reduction with 2-mercaptoethanol, the subunits of the storage protein from the seed endosperm were resolved on a pH 4-6 gradient in presence of 8 M urea (Gianazza *et al.* 1989).

Results

Cultivar Nebbiolo is widely grown in the north-eastern part of Italy (Piemonte through Lombardia). When the banding patterns of the pollen wall proteins for clones from different areas were compared, a geographical clustering was obvious: Sassella, Valgella and Inferno from Valtellina gave identical bands, as did Bolla, Michet, Lampia and Rosé from Asti. Grumello was found to be more similar to the former group, Gattinara to the latter (Fig. 1). The same relationships were observed when the subunits of the seed storage proteins were analyzed by IEF in presence of 8 M urea after reduction with 2-mercaptoethanol (Fig. 2).

Cvs Vermentino, Pigato and Favorita are not easily distinguished by ampelographic criteria. Likewise, the banding patterns of their pollen wall proteins are very similar, with some quantitative variations only for the minor components (Fig. 3 A). Virtually identical results for the three samples are also obtained for the seed components – storage proteins (Fig. 3 B) as well as a number of

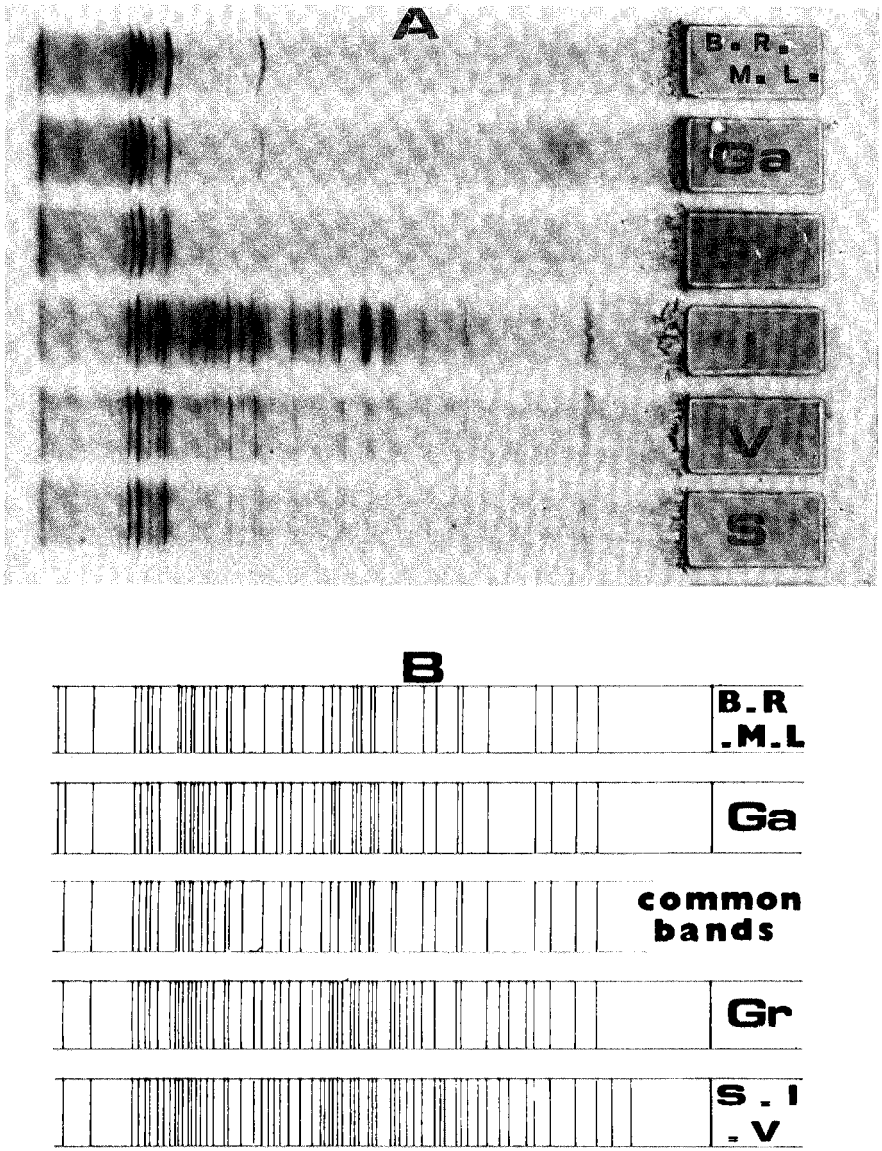


Fig. 1: IEF pattern of the pollen wall proteins o a non-linear pH gradient (range 3.5-10) under native conditions. Samples: Nebbiolo cultivars. Sassella (S), Valgella (V), Inferno (I), Grumello (GR), Gattinara (GA), Bolla (B), Rosé (R), Lampia (L), Michet (M), Barolo (BA). – A) Original gel. B) Schematic drawing.

enzymes (Fig. 3 C-G). These findings are in agreement with the hypothesis that the three clones do not correspond to distinct cultivars but to different names given to a single cultivar in different areas.

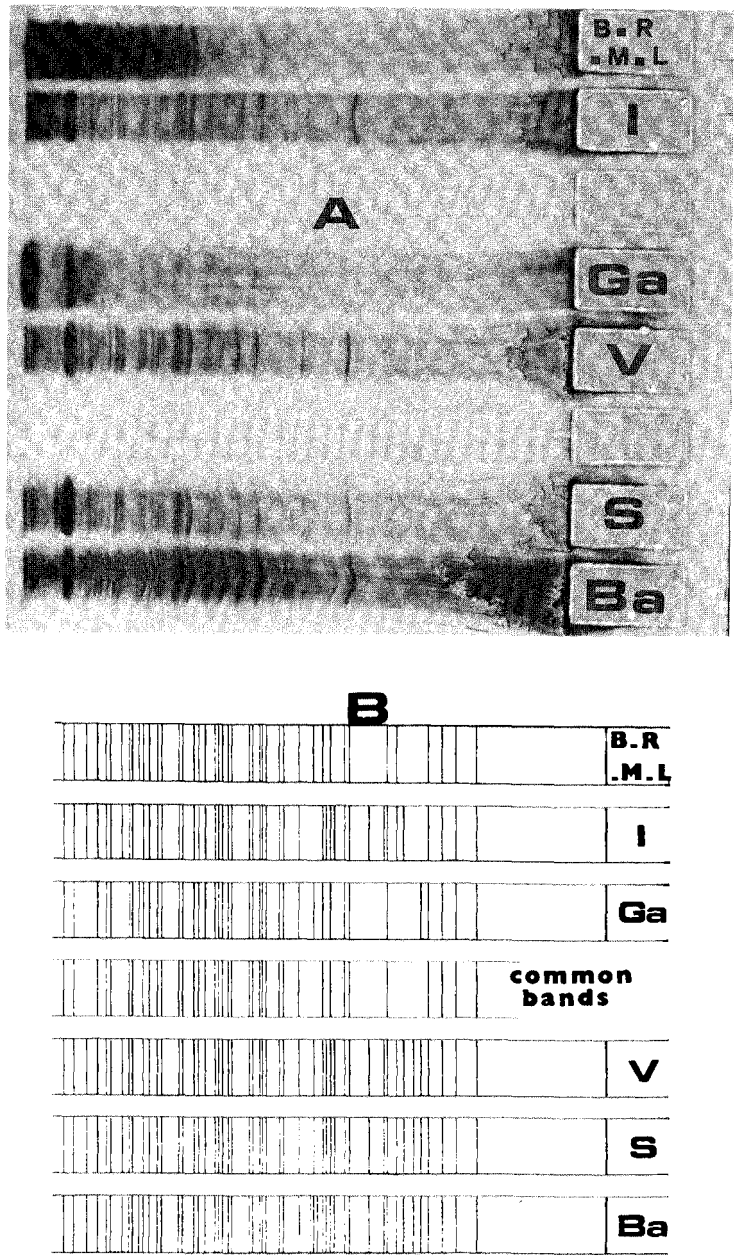


Fig. 2: IEF pattern of the subunits of pollen wall proteins on a non-linear pH gradient (range 3.5-10) in 8 M urea. Sample abbreviations as in Fig. 1. – A) Original gel. B) Schematic drawing.

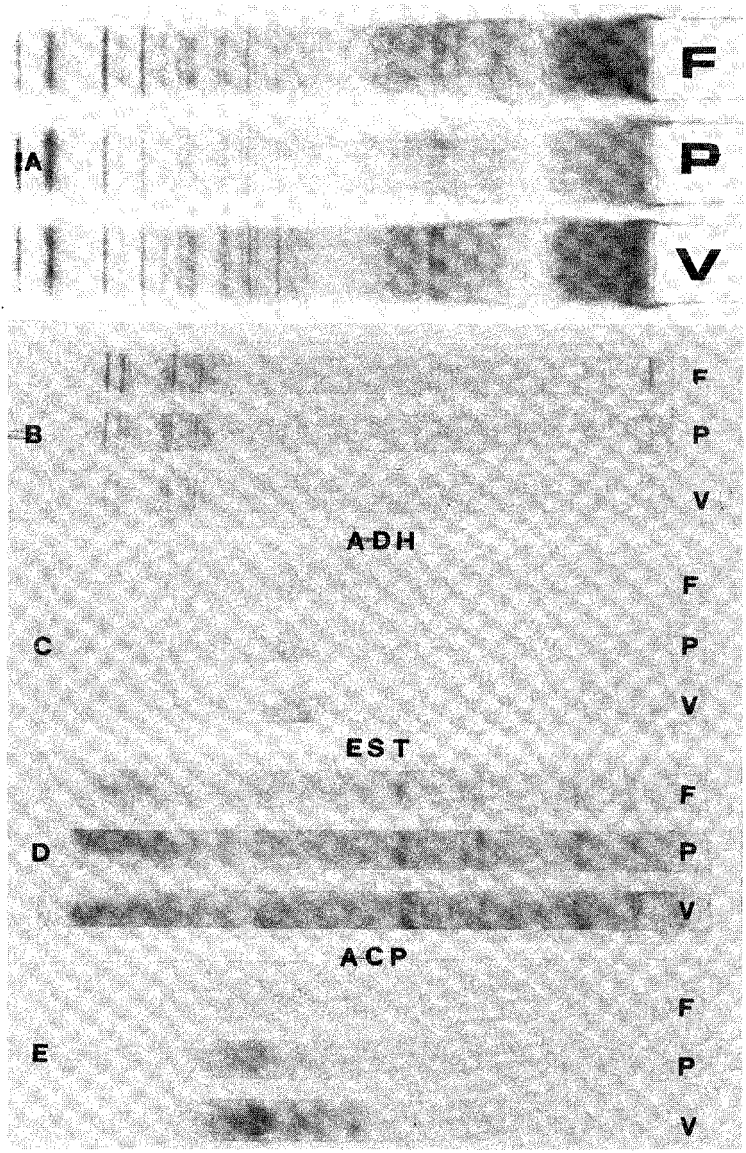


Fig. 3: Samples: Favorita (F), Pigato (P), Vermentino (V). – A) IEF pattern of the pollen wall proteins on a non-linear pH gradient (range 3.5-10). – B) IEF pattern of the subunits of seed proteins on a non-linear pH gradient (range 4-6) in 8 M urea. – C-G) IEF pattern of isoenzymes for seed extracts: C) Alcohol dehydrogenase (ADH) on pH range 4-10. d) Esterase (EST) on pH range 4-10. E) Acid phosphatase (ACP) on pH range 4.5-7. F) Peroxidase (POD) on pH range 4-10. G) Phosphoglucomutase (PGluM) on pH range 4-10. (Continued overleaf)

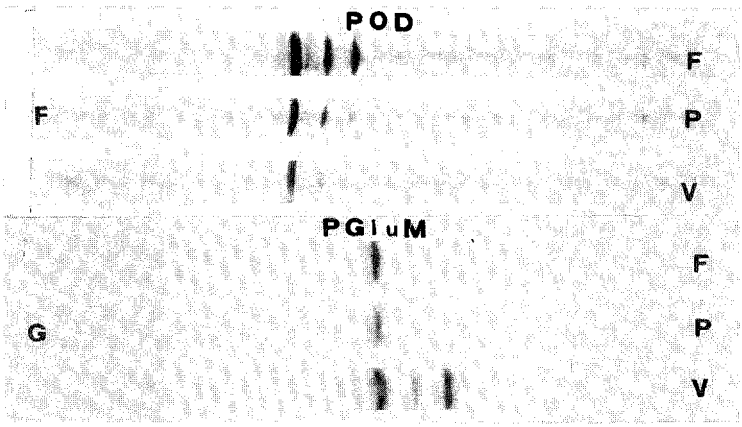


Fig. 3 (continued)

References

CARGNELLO, G.; GIANAZZA, E.; TEDESCO, G.; CAPPELLA, M.; GEROLA, F. M.; 1988: Wall proteins of *Vitis vinifera* pollen. I. Constancy of the phenotype. Vitis **27**, 47-55.

COATES, P. M.; MESTRINER, M. A.; HOPKINSON, D. A.; 1975: A preliminary genetic interpretation of esterase isozymes of human tissues. Ann. Hum. Genet. **39**, 1-8.

GIANAZZA, E.; TEDESCO, G.; VILLA, P.; SCIENZA, A.; CARGNELLO, G.; RIGHETTI, P. G.; OSNAGHI, A.; 1989: Proteins from *Vitis vinifera* seeds. Plant Sci. **62**, 73-81.

MERRIL, C. R.; GOLDMAN, D.; SEDMAN, S. A.; EBERT, M. H.; 1981: Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science **211**, 1437-1438.

SMITH, M.; HOPKINSON, D. A.; HARRIS, H.; 1971: Developmental changes and polymorphism in human alcohol dehydrogenase. Ann. Hum. Genet. **34**, 251-256.

SPENCER, N.; HOPKINSON, D. A.; HARRIS, H.; 1964: Phosphoglucomutase polymorphism in man. Nature **204**, 742-745.

SWALLOW, D.; HARRIS, H.; 1972: A new variant of the placental acid phosphatase. Ann. Hum. Genet. **37**, 31-34.

TAKETA, K.; 1987: A tetrazolium method for peroxidase staining. Electrophoresis **8**, 409-414.

TEDESCO, G.; GIANAZZA, E.; ARRIGOTTI, S.; CARGNELLO, G.; 1989: Wall proteins of *Vitis vinifera* pollen. II. Influence of environment and rootstock on the electrophoretic pattern. Vitis **28**, 65-72.

Vitis vinifera - a chemotaxonomic approach: Anthocyanins in the skin

F. Mattivi [1]), A. Scienza [1,2]), O. Failla [2]), P. Villa [2]), R. Anzani [2]), G. Tedesco [3]), E. Gianazza [4]) and P. Righetti [4])

[1]) Istituto Agrario Provinciale, Via E. Mach 1, I-38010 S. Michele all'Adige, Trento, Italy

[2]) Istituto di Coltivazioni Arboree, Università di Milano, Via Celoria 2, I-20133 Milano, Italy

[3]) Dipartimento di Biologia, Sezione di Botanica Sistematica, Università di Milano, Via Celoria 26, I-20133 Milano, Italy

[4]) Facoltà di Farmacia, Dipartimento di Scienze e Tecnologie Biomediche, Università di Milano, Via Celoria 2, I-20133 Milano, Italy

Summary: The gaining of new knowledge about varietal differences in grapevines can be useful for the designing of genetic improvement programs. More and more, chemical methods complement ampelographic ones in the study of variability in grapevines.

This work is aimed at the anthocyanin profiling of red-coloured grapes, of which ca. 120 cultivars were sampled; among these there were a high number of old Italian vines and 30 *Vitis vinifera* ssp. *silvestris* originating from different areas of Italy. Anthocyanins were HPLC separated and quantified with the aid of an inverse phase microbore column and a photodiode detector.

Grapevines were numerically separated in groups using as indexes the percentage of the 5 monoglucosides present, the summations of: acetic esters; malvidin-3-monoglucoside-caffeoate plus all 5 p-coumaric esters; as well as a series of relations correlated to certain enzymatic activities necessary for the esterification of glucosides, hydroxylation and methylation in the biosynthesis of several anthocyanins. Data derived from the study of indexes of varietal enzymatic activity enable us to qualify differences between grapevines linked to the synthesis of anthocyanins. The stability of anthocyanic profiles within the same grape variety enables the use of this technique for taxonomic purposes. This research study discusses the use of this technique for classification and analysis of grape phylogenesis. An in-depth look into the relations between cultivated and wild varieties is given.

Key words: Vitis vinifera, variety of vine, Italy, berry, skin, anthocyanin, glucoside, ester, analysis, statistics, ampelography, taxonomy.

Introduction

Wulf and Nagel (1978) developed the first method of separation of pigments in Cabernet Sauvignon grapes skin by means of high pressure liquid chromatography (HPLC). Since then, anthocyanins analysis proved to be useful in grapevine classification and chemotaxonomy.

The technique has been improved further, as shown by several studies on the gaining of the first analytical data (Piergiovanni and Volonterio 1981; Di Stefano and Corino 1984; Bakker and Timberlake 1985) and also on the interpretation of anthocyanin metabolism (Roggero *et al.* 1986; Darné 1988 b).

The strong discriminating power of this technique was demonstrated by the first classifications, based on direct observation of chromatograms or parts of chromatograms: groups were assembled according to similarity of monoglucoside profiles (Wenzel *et al.* 1987).

Some of the studies were aimed at developing statistical procedures, in order to obtain more complete and systematic utilization of the data derived from analysis of grape skin anthocyanins and of the individuation of the variables-set more suitable to these purposes (Scienza *et al.* 1985; Roggero *et al.* 1988).

From these works the unanimous opinion that the anthocyanin profile of grape skins can complement ampelographic methods in the study of grapevine variability was derived. This knowledge can be very useful for the development of genetic improvement programs.

Lately, an analogous application was suggested for anthocyanins of leaves of *Vitis* genus and *Vitis vinifera* varieties (DARNÉ 1988 a; DARNÉ et GLORIES 1988).

In Italy, at S. Michele Institute, a databank containing analyses of skin anthocyanins of many grape varieties was constituted and these data were used for chemotaxonomic and phylogenetic studies on some red-coloured grapes typical of Trentino Alto-Adige (SCIENZA *et al.* 1989).

This report is aimed at the presentation of the analytic and methodologic work on which the classification technique is based.

For a further examination of the taxonomic and phylogenetic implications of this report, and of its connections with other chemotaxonomic techniques, please refer to other parts of this research work (SCIENZA *et al.* 1989).

Materials and methods

In order to obtain a classification of anthocyanins we sampled technologically ripe grapes from approx. 120 varieties (Table 1). The samples were chosen among the most significant varieties from the taxonomic point of view, and they included a high percentage of old Italian vines. Approx.

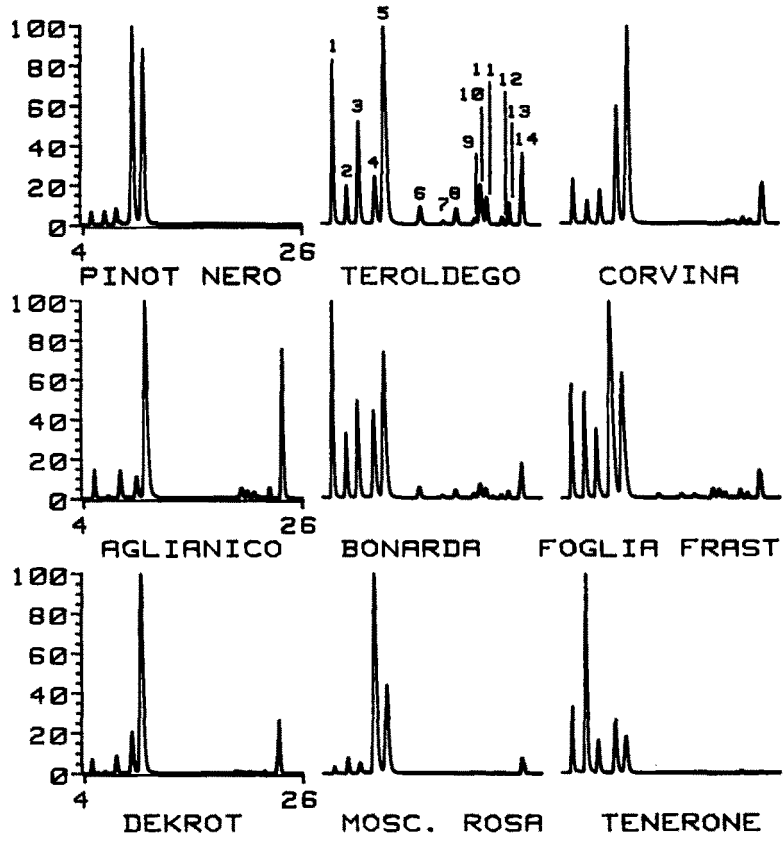


Fig. 1: Chromatographic patterns of grape skin anthocyanins of some cultivars. Time as min; 1 = delphinidin-3-glucoside, 2 = cyanidin-3-glucoside, 3 = petunidin-3-glucoside, 4 = peonidin-3-glucoside, 5 = malvidin-3-glucoside, 6-10 = (1-5)-acetates, 11-14 = (1-5)-p-coumarates.

30 *V. vinifera* ssp. *silvestris* grapevines originating from various Italien regions were also sampled. We studied a total number of 500 samples harvested in the years 1986, 1987 and 1988 primarily from North Italian ampelographic collections. Some samples were taken directly from country vineyards.

These samples were subjected to spectrophotometric determination of total skin anthocyanins, and anthocyanins were HPLC separated and quantified.

The skins of 20 frozen berries were extracted in two phases for 12 h with 100 and 50 ml of methyl alcohol, HCl 0.1 %. The extract was evaporated to dryness in a rotary evaporator at 36 °C and redissolved with a solution suitable for injection in HPLC.

The determination of total anthocyanins was made spectrophotometrically at 520 nm, with the method based on differences in pH.

The separation of single anthocyanins was made by means of gradient elutions using a chromatograph Hewlett Packard 1090M with diode-array detector HP 1040 and column type C18 Hypersil ODS (5 μm, 200 x 2.1 mm). Eluants were: A = perchloric acid 0.3 %, B = methanol. Identification was made according to retention times and UV-VIS spectra of each peak. Quantification was made on areas at 520 nm (Fig. 1).

Data thus obtained were statistically processed using the statistic package SPSS-X.

Results and discussion

A classification of cultivated varieties was thus obtained; as a second step we also evaluated the resemblance of wild varieties to cultivated ones.

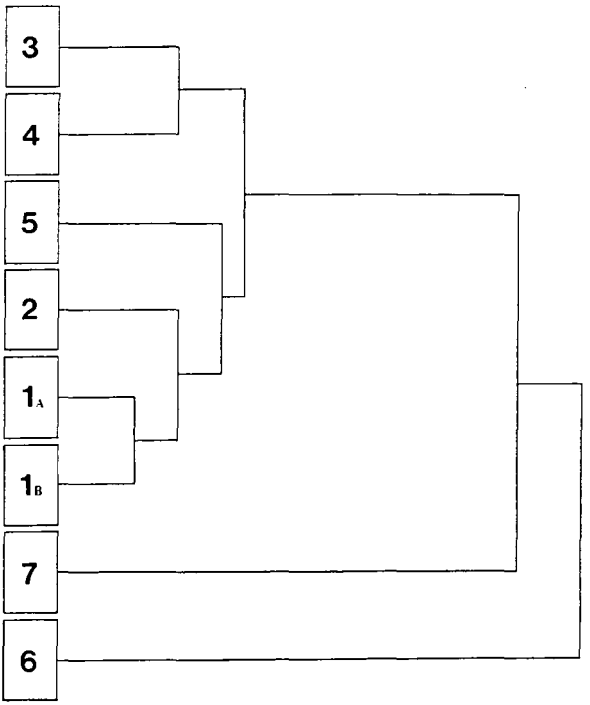


Fig. 2: Classification of cultivated varieties obtained by cluster analysis.

Table 1: List of varieties subjected to the present classification. They are divided into groups according to results of cluster analysis

GROUP 0: Pinot nero, Pinot grigio, Pinot*Dekrot, Pinot tete de negre.
GROUP 1-a: Ancellotta, Barbera, Bombino, Braubana, Cabernet franc, Cabernet sauvignon, Cabrusina, Codelonghe, Colorino pisano, Croatina, Fortana, Fumat, Fumin, Givan, Lagrein, Lambrusco di Sorbara, Lambrusco grasparossa, Lambrusco maestri, Lambrusco salamino, Malbo gentile, Malvasia di Casorzo, Malvasia nera di Lecce, Malvasia nera di Pisa, Mariabino, Marzemino, Merlot, Negrat, Nera grossa, Petit Verdot, Refoscone, Ribolla nera, Teroldego, Vien de nus, 107-2 (Merlot x Marzemino), 107-3 (Merlot x Marzemino), 95-5(Cab.Franc x Merlot).
GROUP 1-b: Aleatico, Bonamico, Burghisana, Canaiolo, Cesanese comune, Ciliegiolo, Colorino, Corvina, Fortana nera (Brugnola), Gamay, Grillone, Kolor, Lambrusco di Alessandria, Lambrusco marani, Moscato violetto, Mourvedre, Negrara, Neyret, Pomela schiava, Rafosal, Rondinella, Rossara, Uvarosa, 200-496.
GROUP 2: Aglianico, Albanina, Aramon, Balsamina, Canena, Cornacchia, Groppello ruberti, Malbech, Negretto, Pavana, Schiava lombarda, Syrah, Tosca, Turca, Incrocio Bruni 147.
GROUP 3: Bonarda, Brugnola, Casetta, Corvino, Cuneute, Denela, Dindarella, Forgiarin, Jagodinka, Lambrusco oliva, Molinara, Oseleta, Pelara, Picolit nero, Pignul, Quaiara, Rossetta di montagna, Rossiola, Simesara, Sangiovese (Brunello), Sangiovese (Prugnolo), Sangiovese (Chianti g.n.), Sangiovese (Chianti p.), Uva d'oro, Vercluna.
GROUP 4: Cianorie, Colorino di Lucca, Foglia frastagliata, Forselina, Groppello, Malvasia nera di Brindisi, Rossignola.
GROUP 5: Dekrot, Tocai rosa.
GROUP 6: Mammolo pisano, Moscato d'Adda, Moscato rosa, Muscat rouge, Nebbiolo, Schiava gentile, Schiava grossa, Trollingher.
GROUP 7: Tenerone.

Among the varieties studied, the Pinot group was singled out as the only one different from the qualitative point of view, as this cultivar lacks esterificated anthocyanins. For this reason, this variety was not included in further elaborations.

Table 2: The 6 canonical discriminant functions

FUNCTION	EIGENVALUE	PERCENT OF VARIANCE	CUMULATIVE PERCENT	CANONICAL CORRELATION
1*	12.05263	72.57	72.57	0.9609303
2*	2.84243	17.11	89.68	0.8600860
3*	1.31966	7.95	97.63	0.7542566
4*	0.34797	2.10	99.72	0.5080804
5*	0.04530	0.27	99.99	0.2081786
6*	0.00114	0.01	100.00	0.0337203

STRUCTURE MATRIX:
POOLED WITHIN-GROUPS CORRELATIONS BETWEEN DISCRIMINATING VARIABLES AND CANONICAL DISCRIMINANT FUNCTIONS
(VARIABLES ORDERED BY SIZE OF CORRELATION WITHIN FUNCTION)

	FUNC 1	FUNC 2	FUNC 3	FUNC 4	FUNC 5	FUNC 6
V4	0.63193*	0.40868	-0.16058	0.44765	-0.23703	-0.34903
V2	0.28959	-0.82135*	-0.47218	0.03277	-0.02424	-0.08845
SCINNAM	-0.41314	0.49538	-0.65091*	-0.25071	-0.04214	0.30588
V3	-0.02890	-0.41004	0.35193	-0.66091*	0.03317	0.08413
V1	0.00087	-0.45684	0.32305	-0.64191*	-0.10481	0.51300
V5	-0.36503	0.17682	0.39852	0.63652*	-0.18336	-0.46652
SACILATI	-0.10245	0.04917	0.11299	-0.23219	0.81604*	0.41544

UNSTANDARDIZED CANONICAL DISCRIMINANT FUNCTION COEFFICIENTS

	FUNC 1	FUNC 2	FUNC 3	FUNC 4	FUNC 5	FUNC 6
V1	0.5944021	0.1188802	0.1547636	1.600972	-0.1666806	2.478363
V2	0.5883325	-0.3051396E-01	-0.1163867	1.675948	0.7740529E-01	2.257265
V3	0.6668733	0.2861497	0.6093347E-01	1.258827	0.1842856	1.942293
V4	0.7505337	0.2667792	0.5683841E-01	1.569709	0.1818205E-01	2.267260
V5	0.5054739	0.1614975	0.9598790E-01	1.664238	0.1458670E-01	2.266669
SACILATI	0.6041821	0.1926011	0.7046074E-01	1.571663	0.2705803	2.242297
SCINNAM	0.5232926	0.2710039	-0.8984168E-01	1.583276	-0.1125723E-01	2.379620
(CONSTANT)	-58.26904	-19.06001	-5.104143	-158.0238	-2.546739	-226.0329

Classification of cultivated varieties

Classification was made according to the seven following variables: the five monoglucoside concentrations (expressed as percentage chromatographic area at 520 nm), the summation of acetic esters and the summation of p-coumaric esters. The summations of the two kinds of esters were utilized, according to the hypothesis – verified as far as acetic esters are concerned (Wenzel *et al.* 1987) – that the esters syntheses rate from the anthocyanin-3-monoglucosides varies only slightly.

We obtained a mean anthocyanic profile for every grapevine variety from which we had available analyses over different years, origins and clones.

These data were processed in order to make a research on typologies. Classification was obtained by means of cluster analysis, following the method 'average linkage between groups'. As to the proximity measures, we used the squares of Euclidean distances.

The cultivars were thus divided into 7 groups. Their classification obtained is shown in Table 1 and in the corresponding dendrogram (Fig. 2).

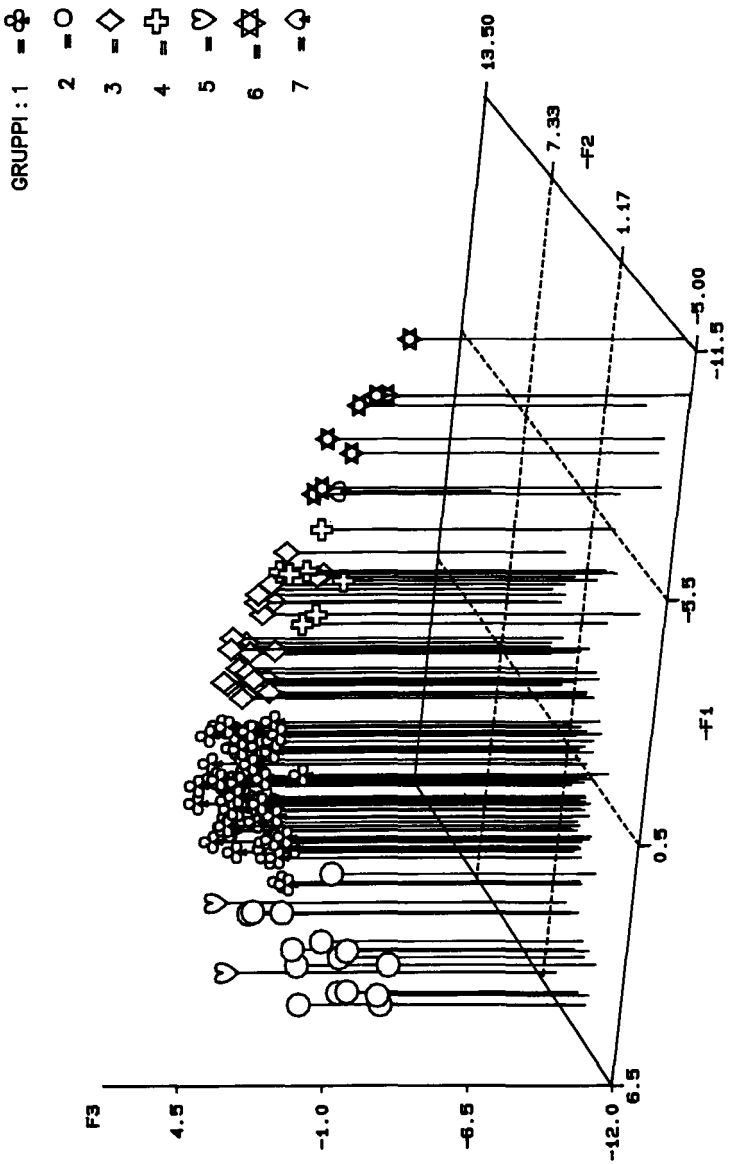


Fig. 3: Subdivision of cultivated varieties into 7 groups by means of discriminant analysis. Each symbol represents one cultivar.

Table 3: Classification results for cases not selected for use in the analysis

ACTUAL GROUP		NO. OF CASES	PREDICTED GROUP MEMBERSHIP 1	2	3	4	5	6	7
GROUP	1	170	159	4	1	3	3	0	0
			93.5%	2.4%	0.6%	1.8%	1.8%	0.0%	0.0%
GROUP	2	17	1	15	0	0	1	0	0
			5.9%	88.2%	0.0%	0.0%	5.9%	0.0%	0.0%
GROUP	3	219	21	0	190	0	2	0	6
			9.6%	0.0%	86.8%	0.0%	0.9%	0.0%	2.7%
GROUP	4	13	1	0	1	11	0	0	0
			7.7%	0.0%	7.7%	84.6%	0.0%	0.0%	0.0%
GROUP	5	2	0	0	0	0	2	0	0
			0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%
GROUP	6	10	0	0	0	1	0	9	0
			0.0%	0.0%	0.0%	10.0%	0.0%	90.0%	0.0%
GROUP	7	1	0	0	0	0	0	0	1
			0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%

PERCENT OF "GROUPED" CASES CORRECTLY CLASSIFIED: 89.58%

The subdivision into groups obtained in this way was further confirmed by discriminant analysis. The discriminant analysis was carried out following the 'stepwise' method based on Wilks' lambda; we used the seven parameters formerly used for the cluster analysis. With this method we obtained six linear canonic discriminant functions. Some of the most important characteristics of this elaboration are reported in Table 2:

The first three functions can account for 97.6 % of total variance. The first discriminant function (F1) accounts for 72.6 % of variance and is well correlated (0.632) to peonidin-3-monoglucoside.

The second discriminant function (F2) explains 17.1 % of variance, and is inversely correlated (-0.821) to cyanidin-3-monoglucoside.

The third function (F3) explains 7.9 % of variance and is inversely correlated (-0.651) to the summation of p-coumaric esters.

The six canonic discriminant functions thus obtained confirm 95.8 % (i. e. in 113 cases out of 118) of the subdivision obtained using cluster analysis.

The distribution of the cultivars in the space defined by the first three canonic discriminant functions is shown in Fig. 3.

The classification of the 118 cultivars into 7 groups explained above was obtained using mean anthocyanin profiles. We decided to evaluate reliability of these results by assigning the 432 samples stored in our databank to these seven groups. The division into seven typologies was confirmed in 89.6 % of the cases (i. e. in 387 cases out of 432), as shown in Table 3.

Table 4: Mean composition parameters of Sangiovese grapes sampled in the years 1987 and 1988 in different areas of Tuscany. The single anthocyanins are expressed as percentage areas at 520 nm: the total anthocyanins appear as malvidin diglucoside chloride (mg/100 g of grapes)

	BRUNELLO (N=53)		PRUGNOLO (N=46)	
Parameter	Mean Conc.	Standard Deviation	Mean Conc.	Standard Deviation
Dp	11.22	3.13	13.54	3.42
Cy	21.82	6.28	18.92	5.31
Pt	12.99	2.42	15.15	2.66
Pn	18.63	5.21	14.66	4.94
Mv	33.74	7.83	36.19	6.03
Sum acet.	00.28	0.10	00.26	0.10
Sum coum.	01.24	0.45	01.21	00.27
Total conc.	110.4	66.9	132.0	81.2
	CHIANTI P. (N=26)		CHIANTI G.N. (N=67)	
Parameter	Mean Conc.	Standard Deviation	Mean Conc.	Standard Deviation
Dp	12.40	2.22	11.83	3.29
Cy	18.88	4.18	18.10	7.05
Pt	14.60	1.51	14.08	2.14
Pn	15.85	2.73	15.79	3.61
Mv	36.59	6.54	38.58	11.01
Sum acet.	00.22	00.07	00.21	00.08
Sum coum.	01.41	00.62	01.35	00.64
Total conc.	50.9	12.6	126.0	89.1

This outcome proved that classification obtained through mean anthocyanin profiles is sufficiently valid even for identification of single samples.

A distinguishing characteristic of each cultivar is the variability of anthocyanin profiles between individual samples. In order to illustrate this difference in behaviour, we show in Fig. 4 the classification of 63 samples belonging to the Teroldego variety (this grapevine is cultivated in a circumscribed and homogeneous area) and 194 samples of Sangiovese (cultivated in an area much wider both from the geographical and climatic point of view). This figure clearly shows that the variability range of Sangiovese is much wider than that of Teroldego.

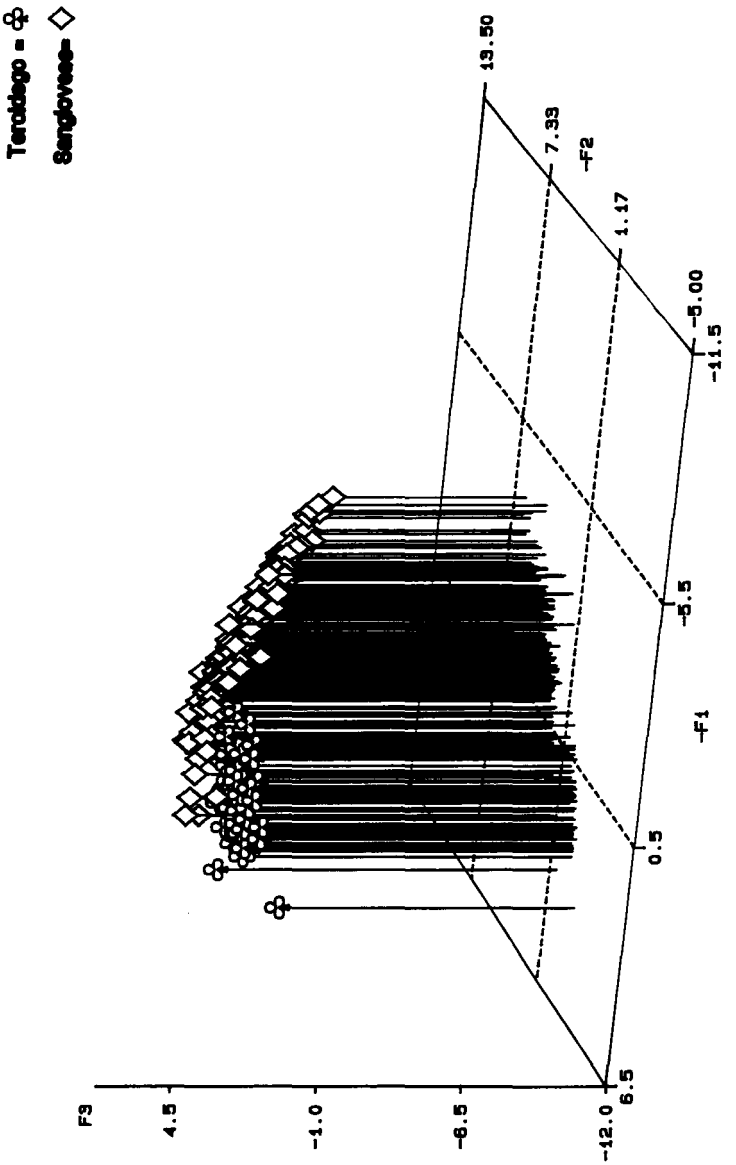


Fig. 4: Variability range of anthocyanin profiles of cvs Sangiovese and Teroldego. F1, F2 and F3 are the first three discriminant functions utilized for classification of cultivated varieties.

Table 4 shows how a sufficiently high number of samples can lead to average anthocyanin profiles extremely similar even when working on different clones of the same cultivar. This table refers to Sangiovese grapes sampled in the years 1987 and 1988 in different areas of Tuscany. This grape underwent various selections over the years, which resulted in a remarkable polymorphism and to the consequent attribution of different names (Brunello, Montepulciano, Prugnolo, Sangiovese, Sangioveto).

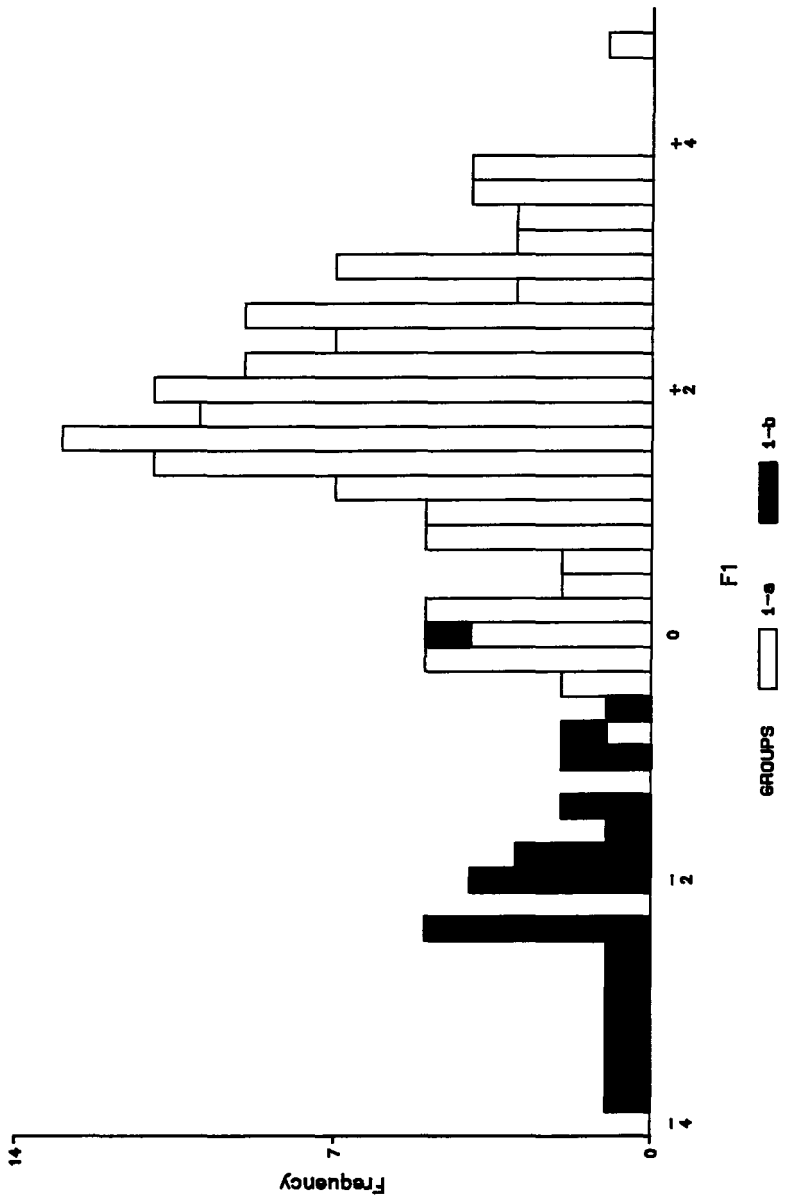


Fig. 5: Separation of groups 1-a and 1-b by means of linear discriminant analysis.

The similarity of these samples is such that they have been assigned to the same group (No. 3), as shown in Table 1, and in the cluster analysis of mean profiles these cultivars are placed as nearest neighbours.

Further subdivision can be obtained by studying the groups singled out one by one.

For example, a cluster analysis was performed on the cultivars belonging to group No. 1, the largest among the 8 groups identified so far (7 plus Pinot).

We were able to further divide this group into two sub-groups, shown in Table 1 with the codes 1-a and 1-b. Discriminant analysis of these two sub-groups resulted in a correct classification in 97.7 % of the samples (i. e. 166 out of 170), as shown in Fig. 5.

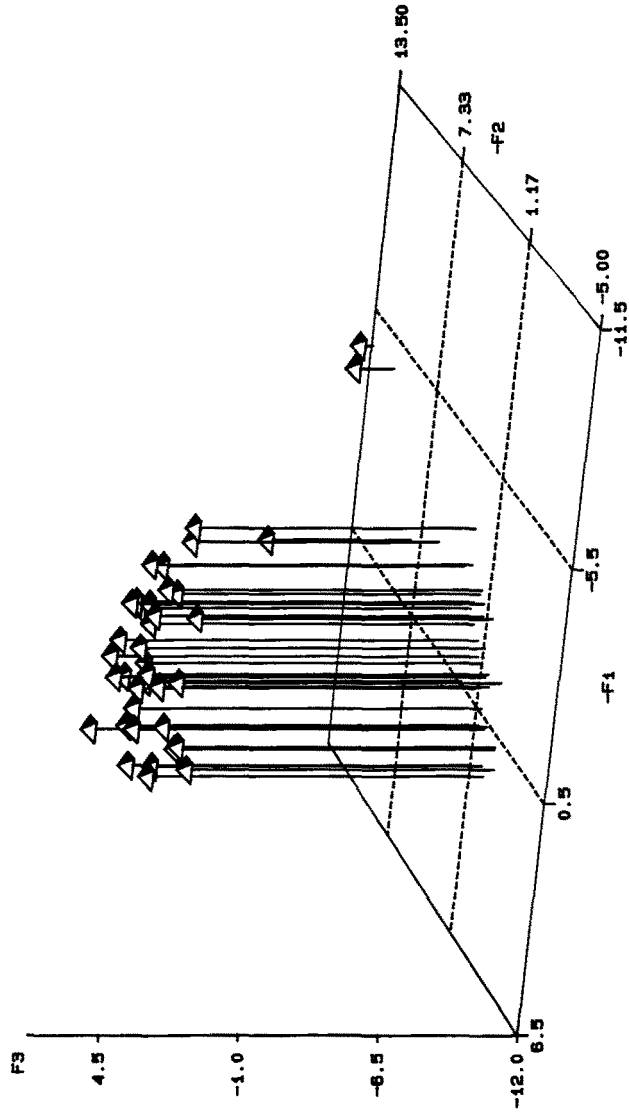


Fig. 6: Distribution of *Vitis vinifera* ssp. *silvestris* samples originating from various Italian regions in the space defined by the first three discriminant functions. The functions are the same as utilized for classification of cultivated varieties.

Vitis vinifera ssp. *silvestris*

The analysis of anthocyanins in wild grape samples originating from different areas of Italy revealed a wide range of anthocyanic profiles. At present, not the whole range shown in cultivated varieties is covered by wild varieties, but this is probably due to the fact that the number of wild samples examined is considerably lower than that of cultivated ones.

47 samples coming from 30 *V. vinifera* ssp. *silvestris* were plotted within the space defined from the three canonic discriminant functions previously calculated. Their distribution covers a considerable space, as shown in Fig. 6.

Conclusions

This research on anthocyanin profiles of approx. 500 samples belonging to about 120 cultivated varieties and 30 *V. vinifera* ssp. *silvestris* resulted in a subdivision of samples into 9 groups.

The utilization of percentages (instead of absolute quantities) reduces the influence of variability due to phenotype on classification (synthesis of different absolute quantities connected to ripening phase and year).

The utilization of percentages also allows a better verification of similarities between varieties belonging to the same family, often very different from one another as far as the absolute quantities of anthocyanins are concerned, but with similar profiles (see Moscati).

The seven variables suggested are homogeneous and consequently a standardization is not necessary. This procedure allows avoidance of possible loss of information consequent to standardization.

In Table 5 the mean composition of the parameters of the groups studied is shown. In this table, besides the percentage composition and the total anthocyanins, a series of relations supposed to be correlated to certain enzymatic activities necessary for the esterification of glucosides (Ratio 2 and Ratio 5), hydroxylation (Ratio 1) and methylation (Ratio 3 and Ratio 4) in the biosynthesis of several anthocyanins are shown.

These relations, within each one of the 9 typologies, show a dispersion of values higher than that of the initial concentrations from which they derived, depending on the way groups were constituted.

It can be clearly seen that the two 'methylation indexes' of tri- and di-substituted, although they have different absolute values, are generally covariant.

The formation of acetic esters and p-coumaric esters seems to be independent from one another, as can be inferred from the remarkable variability of the ratio between the two esters (Ratio 2).

The examination of the values of the variables peonidin-3-monoglucoside, cyanidin-3-monoglucoside and summation of p-coumaric esters shows the strong correlation between these factors and our first three discriminant functions, and consequently their importance as differentiating factors.

Literature cited

BAKKER, J.; TIMBERLAKE, F.; 1985: The distribution of anthocyanins in grape skin extracts of port wine cultivars as determined by HPLC. J. Sci. Food Agricult. **36**, 1315-1324.

DARNÉ, G.; 1988 a: Peut-on envisager de distinguer les espèces du genre *Vitis* au moyen des anthocyanes des feuilles?. Connaiss. Vigne Vin **22**, 85-87.

-- --; 1988 b: Evolution des différentes anthocyanes des pellicules de Cabernet Sauvignon au cours du développement des baies. Connaiss. Vigne Vin **22**, 225-231.

Table 5: Mean composition parameters of the groups. The single anthocyanins are expressed as percentage areas at 520 nm; the total anthocyanins appear as malvidin-diglucoside chloride (mg/100 g of grapes)

	GROUP 0 (N=4)			GOUP 1-a (N=36)		
Parameter	Mean Conc.	Std. Dev.	Notes	Mean Conc.	Std. Dev.	Notes
Dp	03.16	1.08		14.34	3.75	(++)
Cy	02.09	0.83	(-)	03.66	1.93	
Pt	05.12	1.37		11.53	2.91	(++)
Pn	35.07	10.31	(+)	08.57	3.95	(-)
Mv	54.55	11.00	(++)	38.45	4.05	
Sum acet.	00.00		(---)	08.90	5.04	(+++)
Sum coum.	00.00		(---)	14.17	4.12	(+)
Total conc.	106.9	94.1		214.7	101.0	
Ratio 1						
(tri)/(di)	01.94	1.13		06.51	3.37	(+)
Ratio 2						
(Acet/Coum.)	***	***		00.68	0.47	(+++)
Ratio 3						
(Mv/Dp)	19.16	8.28	(++)	02.94	1.08	(-)
Ratio 4						
(Pn/Cy)	17.75	3.73	(++)	2.68	1.20	(-)
Ratio 5						
(Esters/Free)	00.00	0.00	(---)	00.31	0.13	(++)

	GROUP 1-b (N=24)			GROUP 2 (N=15)		
Parameter	Mean Conc.	Std. Dev.	Notes	Mean Conc.	Std. Dev.	Notes
Dp	05.56	2.28		04.55	2.02	
Cy	02.95	1.53		00.63	0.22	(--)
Pt	06.77	2.73		05.78	2.14	
Pn	19.09	6.51		04.60	1.66	(---)
Mv	48.49	4.93	(+)	43.89	6.98	
Sum acet.	02.75	2.37		06.49	3.36	(++)
Sum coumar.	13.65	5.13		32.62	7.50	(+++)
Total conc.	096.7	68.4		106.6	77.4	
Ratio 1						
(tri)/(di)	03.09	1.19		11.36	3.85	(++)
Ratio 2						
(Acet/Coum.)	00.20	0.16		00.21	0.12	(--)
Ratio 3						
(Mv/Dp)	10.64	5.43		11.74	6.12	
Ratio 4						
(Pn/Cy)	08.28	5.53		07.84	2.83	
Ratio 5						
(Esters/Free)	00.20	0.09		00.69	0.22	(+++)

(Continued overleaf)

Table 5 (continued)

	GROUP 3 (N=25)			GROUP 4 (N=7)		
Parameter	Mean Conc.	Std. Dev.	Notes	Mean Conc.	Std. Dev.	Notes
Dp	16.14	5.07	(+++)	07.41	3.64	(-)
Cy	14.33	6.39	(++)	09.73	4.37	(+)
Pt	12.32	2.32	(+++)	06.57	2.43	(-)
Pn	18.77	5.13		38.19	5.63	(++)
Mv	28.28	6.99		27.06	4.76	(-)
Sum acet.	03.64	3.56	(+)	02.22	1.46	
Sum coumar.	06.26	2.97		08.47	2.88	
Total conc.	167.6	123.3		124.4	90.3	
Ratio 1 (tri)/(di)	01.84	0.58		00.87	0.18	(-)
Ratio 2 (Acet/Coum.)	00.55	0.48	(++)	00.28	0.18	(+)
Ratio 3 (Mv/Dp)	02.00	0.93	(--)	05.90	6.77	
Ratio 4 (Pn/Cy)	01.77	1.32	(--)	06.75	8.57	
Ratio 5 (Esters/Free)	00.11	0.07		00.12	0.04	

	GROUP 5 (N=2)			GROUP 6 (N=8)		
Parameter	Mean Conc.	Std. Dev.	Notes	Mean Conc.	Std. Dev.	Notes
Dp	02.27	0.44	(--)	01.55	0.98	(---)
Cy	00.39	0.20	(---)	07.99	3.86	
Pt	03.91	0.56	(--)	03.03	1.30	(---)
Pn	07.48	4.69	(--)	62.54	6.78	(+++)
Mv	68.20	1.19	(+++)	18.00	8.85	(--)
Sum acet.	01.36	0.65	(-)	01.42	1.05	
Sum coumar.	15.68	3.77	(++)	05.23	2.34	(-)
Total conc.	105.8	92.5		052.2	34.2	
Ratio 1 (tri)/(di)	11.73	7.31	(+++)	00.33	0.16	(---)
Ratio 2 (Acet/Coum.)	00.08	0.02	(---)	00.24	0.16	
Ratio 3 (Mv/Dp)	30.67	6.45	(+++)	13.72	6.58	(+)
Ratio 4 (Pn/Cy)	18.50	2.63	(+++)	10.39	6.39	(+)
Ratio 5 (Esters/Free)	00.21	0.07	(+)	00.07	0.04	(-)

(Continued overleaf)

Table 5 (continued)

	GROUP 7 (N=1)		
Parameter	Mean Conc.	Std. Dev.	Notes
Dp	13.25		(+)
Cy	47.50		(+++)
Pt	09.09		(+)
Pn	15.65		
Mv	13.10		(---)
Sum acet.	00.24		(--)
Sum coumar.	01.10		(--)
Total conc.	020.8		
Ratio 1 (tri)/(di)	00.56		(--)
Ratio 2 (Acet/Coum.)	00.22		(-)
Ratio 3 (Mv/Dp)	00.99		(---)
Ratio 4 (Pn/Cy)	00.33		(---)
Ratio 5 (Esters/Free)	00.01		(--)

-- ; GLORIES, Y.; 1988: Les anthocyanes des feuilles de différentes variétés de *Vitis vinifera* L. entre la véraison des raisins et la chute des feuilles. Vitis **27**, 71-78.

DI STEFANO, R.; CORINO, L.; 1984: Terpeni ed antociani di alcune uve rosse aromatiche. Riv. Viticolt. Enol. **10**, 581-593.

PIERGIOVANNI, L.; VOLONTERIO, G.; 1981: Studio della frazione antocianica delle uve. Nota I. Vignevini **8**, 49-53.

ROGGERO, J. P.; COEN, S.; RAGONNET, B.; 1986: High performance liquid chromatography survey on changes in pigment content in ripening grapes of Syrah. An approach to anthocyanin metabolism. Amer. J. Enol. Viticult. **37**, 77-83.

-- ; LARICE, J. L.; ROCHEVILLE-DIVORNE, C.; *Archier*, P.; COEN, S.; 1988: Composition anthocyanique des cépages. Rev. Franç. Oenol. **112**, 41-48.

SCIENZA, A.; ANZANI, R.; FAILLA, O.; MATTIVI, F.; VILLA, P. L.; TEDESCO, G.; RIGHETTI, P. G.; ETTORI, C.; MAGENES, S.; GIANAZZA, E.; 1990: *Vitis vinifera* – a chemotaxonomic approach: Seed storage protein. Proc. 5th Intern. Symp. Grape Breeding, 12-16 Sept., St. Martin/Pfalz, FRG.

-- ; MATTIVI, F.; VILLA, P. L.; FAILLA, O.; 1989: Rapporti filogenetici tra il Teroldego, alcuni vitigni trentini ed altri appartenenti a zone geografiche diverse. Atti del Convegno: Il Teroldego Rotaliano, S. Michele a/A, Trento, Italia.

-- ; PIERGIOVANNI, L.; VISAI, C.; CONCA, E.; ROMANO, F.; 1985: Il profilo antocianico delle uve quale mezzo tassonomico per il riconoscimento dei vitigni rossi. Atti del Convegno di Genetica della Vite, Verona. Vignevini **13** Suppl. al (12), 75-81.

-- ; VERSINI, G.; MATTIVI, F.; 1989: Il profilo aromatico ed antocianico dell'uva e del vino di Moscato rosa. Atti della Tornata della Accademia Italiana della Vite e del Vino, Tornata di Parenzo, Istria.

WENZEL, K.; DITTRICH, H. H.; HEIMFARTH, M.; 1987: Die Zusammensetzung der Anthocyane in den Beeren verschiedener Rebsorten. Vitis **26**, 65-78.

WULF, L. W.; NAGEL, C. W.; 1978: High-pressure liquid chromatographic separation of anthocyanins of *Vitis vinifera*. Amer. J. Enol. Viticult. **29**, 42-49.

Inheritance of isoenzymes and soluble proteins in grape varieties and F_1 hybrids

P. KOZMA [1]), A. H. NAGY [2]) and O. JUHASZ [1])

[1]) Department of Viticulture, University of Horticulture and Food Industry, Budapest, Hungary

[2]) Department of Genetics, Eötvös Loránd University, Budapest, Hungary

S u m m a r y : The aim or our experiments was to verify the existence of a genetically interpretable molecular polymorphism in several grape varieties and their F_1 hybrids, which we can employ for genetical and ampelographical characterization. In addition, we also programmed the progress of investigation methods.

The authors present protein and enzyme analysis of two pairs of parents, Pearl of Csaba x S. V. 12375 and Saperavi x Blaufränkisch, and of ten other cultivars and several F_1 progenies.

The best experimental results for genetic markers can be gained when shoot and callus samples are collected at the end of winter, in February, at the same time. Spring shoot collection is less effective because of high chlorophyll contents, and must and wine samples are less suitable due to their microbial contamination.

The esterase enzyme group gives well reproducible, characteristically differentiated patterns. The enzyme patterns of parental varieties typically segregate in individual progenies, hence they prove to be good markers.

K e y w o r d s : genetics, ampelography, protein, enzyme, analysis, variety of vine, hybrid, Hungary, shoot, callus, must, wine.

Introduction

After developing investigation methods for plant proteins and enzymes in the last two decades, these methods are widely used for the ampelographical and genetical characterization and segregation of grape varieties. The proteins and isoenzymes are the most acceptable and simple gene markers which are co-dominant in hereditary processes.

For the analysis of protein and enzyme samples in the grape, leaves (SCHAEFER 1969, 1970; WOLFE 1976; DAL BELIN PERUFFO *et al.* 1981; FALLOT *et al.* 1985; ARULSEKAR and PARFITT 1986; BENIN *et al.* 1988), pollen grains (SAMAAN and WALLACE 1981; LOUKAS *et al.* 1983; STAVRAKAKIS and LOUKAS 1983; CARGNELLO *et al.* 1988), shoots (SUBDEN *et al.* 1987), internodal phloem of shoots (BACHMANN and BLAICH 1988), juice of berry (DRAWERT and GÖRG 1974; CORREA *et al.* 1988) have been used. From the samples, proteins and enzymes were separated and determined with gel electrophoresis and isoelectronic focusing methods. Among enzymes, α-esterase, acidic phosphatase, glutamate-oxaloacetate transaminase, catechol oxydase, phenol oxydase, indophenol oxydase, leucine aminopeptidase, alcohol dehydrogenase, peroxydases, etc. have been detected.

The results of our experiments made possible ampelographical and genetical characterization of a great number of grape varieties.

Materials and methods

The experiments were carried out in 1987 and 1988. Our task was to resolve protein and enzyme analysis first on several typical grape varieties of three convarietates of *Vitis vinifera* L. and next on parental varieties of two hybrid families and their F_1 progenies. We also decided to detect genetically interpretable molecular polymorphism.

Grapevine material

Vitis silvestris GMEL.

convarietas *occidentalis*:
- Pinot gris B. 10
- Welschriesling B.20
- Traminer
- Müller-Thurgau

convarietas *pontica*:
- Noble Furmint
- Noble Kadarka

convarietas *orientalis*:
- Chasselas blanc
- Afuz Ali

Other varieties:
- S. V. 18315
- Berlandieri x Riparia T 5C
- CsFT 3166 (composed species hybrid)

Hybrid families:
- S.V. 12375 x Pearl of Csaba (KOZMA P.)
  - CsFT 175
  - CsFT 194
  - CsFT 195
- Pearl of Zala (CSIZMAZIA, BEREZNAI)
- Saperavi x Blaufränkisch (= Limberger) (KOZMA P.)
  - CsV 463
  - CsV 525
  - CsV 528
- Hindognü x Blaufränkisch (KOZMA P.)
  - CsV 420

Sampling

In 1987 the following samples were used: at the end of winter, shoots from field-grown vine-stocks; shoots collected in June; in the autumn freshly made and preserved must; in March fermented new wine. Shoots were collected on 6th November, 1987; 6th February, 9th March, 11th April, 1988. Two-bud cuttings of the latter sample were sprouted at 26 °C and fresh callus tissues were also used.

Extraction

a) Shoots deep-frozen at -30 °C, vine-stocks and callus tissues derived from them were sliced into 5 mm pieces and broken in a special disc grinder. Extraction buffer solutions coded to below 0 °C were added at concentrations of 1 : 5 (w/v). The composition of buffers was the following:

1. 0.2 M HEPES buffer, pH = 6.5
   0.02 M $MgCl_2$
   1 % (v/v) NP-40
   0.005 M 2-mercapto-ethanol
2. 0.05 M Tris-HCl buffer, pH = 6.5
   0.5 mM NaCl
   2 % (v/v) LiDS
   5 % (v/v) 2-mercapto-ethanol

After blending, the mixture for enzyme detection was frozen to -20 °C for 24 h and the samples that contained ionic detergent were kept in water bath at 50 °C for 3 h.

The samples were then centrifuged, and after dissolving them in 20 % (v/v) saccharose they were stored at -20 °C until electrophoresis.

b) Preparation of wine and must samples: Into 1 ml centrifuged must and wine the following components were measured: 20 mg LiDS, 50 ml 2-mercapto-ethanol, 200 mg saccharose. After solution of the components, the samples were kept in water bath for 3 h at 50 °C. Afterwards the samples were stored at -20 °C until electrophoresis.

Electrophoresis, staining

a) Separation and detection of enzymes: 8 x 8 x 0.2 cm polyacrylamide gel slabs were used. The monomer concentration was 30 % (stock solution), the concentration gradient of polymer was

4-10 %. Solidity pH = 6.0, separation pH = 7.0, electrode buffer: 0.01 M Tris, 0.03 M 5,5'-diethyl-barbituric acid. The electrophoresis has run for 1.5 h at 200 V. The hydrolase activity possessing protein components, the esterase isoenzymes, were made visible in 0.01 M phosphate buffer (pH = 6.5) by applying α-naphtyl-acetate as substrate, and diazonium salt dye.

b) Separation of proteins on the basis of molecular weight: The separations were performed on 8 x 8 x 0.2 cm polyacrylamide gel slabs. The monomer concentration was 30 % (stock solution), the gel contained 0.2 % ionic detergent (LiDS). Solidity pH = 6.5, separation pH = 8.9, the composition of electrode buffer: 0.05 M Tris, 0.4 M glycine, 0.1 % (v/v) SDS. The relative molecular masses were determined by parallel running of Pharmacia's molmass standard. The molecular masses of the calibration series were as follows:

	Molecular mass (kD)
phosphorylase-b	94
albumine	67
ovalbumine	43
carboxylic acid anhydrase	30
trypsin inhibitor	20.1
α-lactalbumine	14.4

Results

Esterase isoenzymes of shoot samples

The enzyme patterns originated from different samples of a certain variety were the same, consequently they can be considered as an adequate biochemical characteristic.

Fig. 1 shows enzyme patterns of the parental varieties: (1) Pearl of Csaba, (2) S. V. 12375; of their F_1 progenies: (3) CsFT 194, (4) CsFT 195, (5) Pearl of Zala. We could establish that parental patterns appear in additive manner in hybrids and that hybrids also differ from each other. It is recognizable that: 1. sample (3) (CsFT 194) is similar to sample (1) (Pearl of Csaba) in band number; 2. the bands of S.V. 12375 sample dominate in all hybrid samples, although less intensively; 3. the characteristic bands of Pearl of Csaba can be detected in samples (4) and (5).

Fig. 2 presents enzyme patterns from shoot samples of seven grape varieties. Significant alterations could also be found among them. Pinot gris B. 10 (11) and Welschriesling B. 20 (12) which belong to convar. *occidentalis* group, are characteristic of identical intensive upper (anodic) bands, while the others are different. Noble Furmint (14) and Noble Kadarka (15) originated from convar. *pontica* have both identical and divergent bands. Chasselas (13) (convar. *orientalis* subconvar. *caspica*) has less intensively stained bands, but the pattern corresponds to (11) or more to (12) sample. The enzyme patterns of (10) and (16) samples are markedly different from those of the others. A possible explanation may be that these hybrids are descendants of North American *V. vinifera* varieties.

Esterase isoenzymes of callus samples

Enzyme patterns of callus origin are generally more complicated, differentiated and have more bands than those of shoot samples. But in our opinion, they are more suitable for isoenzyme analyses, because they more accurately show the heredity of parental features in F_1 progenies. The alterations among varieties and their descendants are well recognizable as is the additivity of the markers in the hybrids.

It can be seen in Fig. 3 that the bottom and upper bands of Pearl of Csaba (1) are similar to those of CsFT 194 (3). S. V. 12375 (2) upper band and middle band of (1) and (3) are equivalent

Parental polypeptide components of Pearl of Csaba and S. V. 12375 hybrids in shoot extracts

parent / hybrid	Polypeptides /kD/ Pearl of Csaba	S.V. 12375
194	19.8	59.5
	19.2	58.5
	18.5	31.0
	--	30.5
175	30.5	59.5
	19.8	58.5
	19.2	--
	18.5	--
Pearl of Zala	30.5	59.5
	19.8	58.5
	19.2	--
	18.5	--

with Pearl of Zala (5) bands. Bands of Pearl of Csaba appear also in CsFT 195 (4) sample, but less intensively.

In Fig. 4 shoot callus esterase patterns are shown from varieties Blaufränkisch (6) and Saperavi (7) with those of their hybrid, CsV 525 (8) and of Hindognü x Blaufränkisch hybrid, CsV 420 (9). The patterns of parents are essentially different but in hybrids they appear additive.

Fig. 5 presents esterase patterns of four *V. vinifera* cultivars. All of them have identical and non-identical bands.

Results of protein analyses

We separated proteins by their molecule masses isolated from shoots and calli with ionic detergents, and investigated the protein composition of enzymatic isolates. The results of shoot analyses in 1987 and 1988 are shown in the table and Figs. 6 and 7.

Saperavi and Blaufränkisch have easily distinguishable polypeptides of molecular masses at 58.5 and 59.0 kD, respectively. In their hybrids, these two components appear in additive manner and form two-banded patterns in the region in question, as Fig. 6 shows. This is also characteristic of CsV 463, CsV 525 and CsV 528.

Fig. 1.

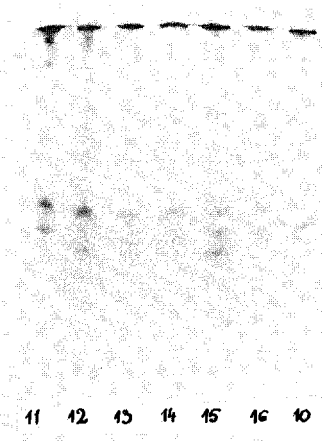


Fig. 2.

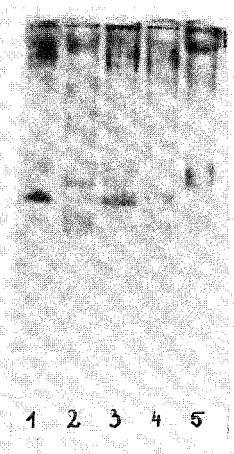


Fig. 3.

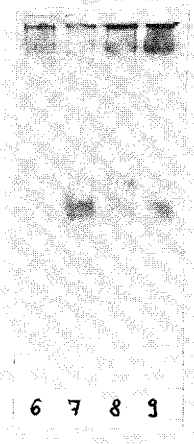


Fig. 4.

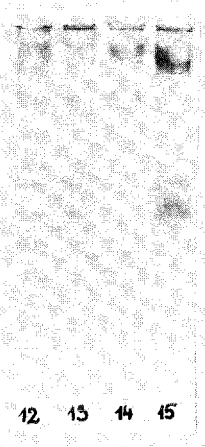


Fig. 5.

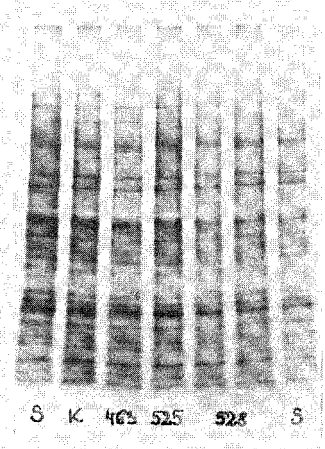


Fig. 6.

Fig. 1: Zymogram for shoot esterases of Pearl of Csaba (1) and S. V. 12375 (2) parental varieties and CsFT 194 (3), CsFT 195 (4), Pearl of Zala (5) hybrids.

Fig. 2: Esterase enzymes of Pinot gris B. 10 (11), Welschriesling B. 20 (12), Chasselas (13), Noble Furmint (14), Noble Kadarka (15), S. V. 18315 (16) and Berlandieri x Riparia T 8B (10) shoot samples.

Fig. 3: Esterase patterns of calli from Pearl of Csaba (1) and S. V. 12375 (2) parental varieties and CsFT 194 (3), CsFT 195 (4) and Pearl of Zala (5) hybrids.

Fig. 4: Esterase patterns of shoot calli from Blaufränkisch (6) and Saperavi (7) parental varieties and CsV 525 (8) and CsV 420 (9) hybrids.

Fig. 5: Esterase patterns of shoot calli from Welschriesling (12), Chasselas (13), Noble Furmint (14) and Noble Kadarka (15).

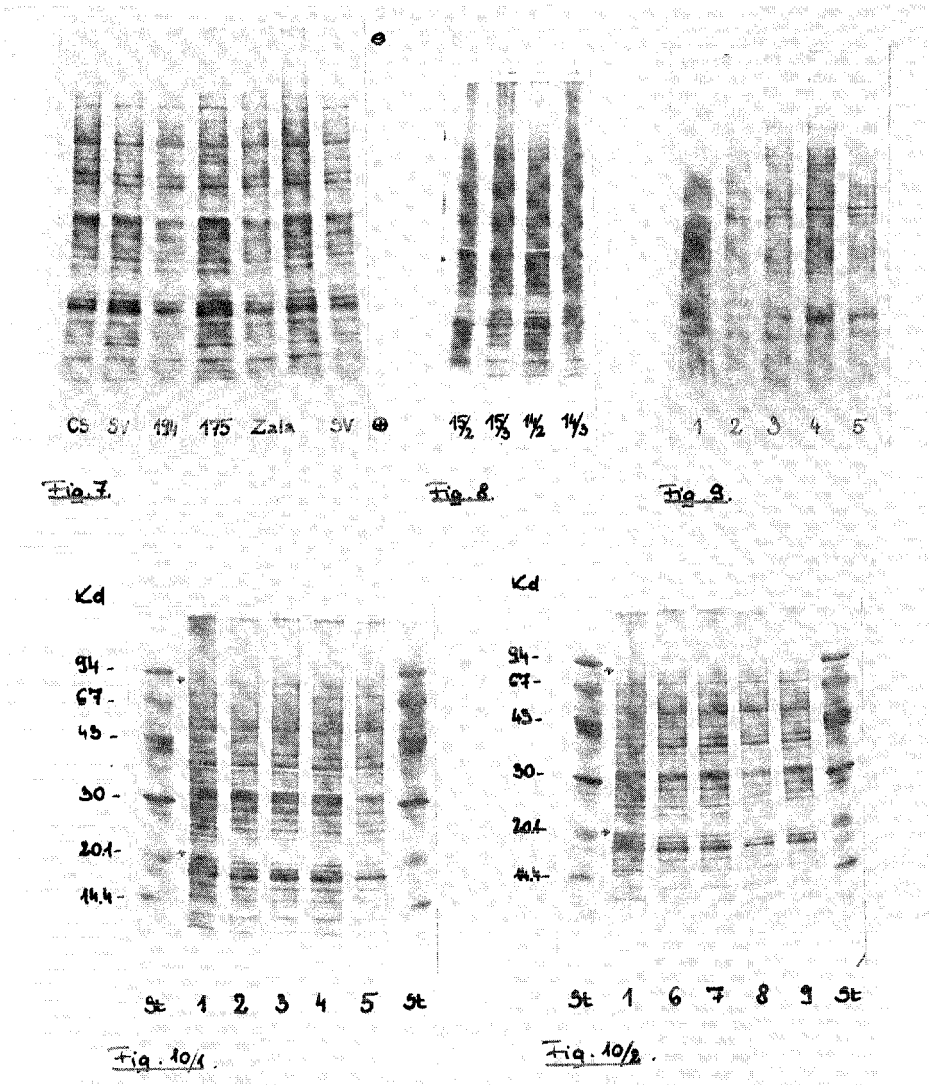


Fig. 6: Polypeptide range of shoot extract from Saperavi and Blaufränkisch varieties, as well as of their hybrids.

Fig. 7: Polypeptide range of shoot extract from Pearl of Csaba, S. V. 12375, and of their hybrids.

Fig. 8: Polypeptide patterns of Noble Furmint (14) and Noble Kadarka (15) shoots collected on 6th February (2) and 9th March (3), 1988.

Fig. 9: Callus protein composition of Pearl of Csaba (1), S. V. 12375 (2) parents and CsFT 194 (3), CsFT 195 (4) and Pearl of Zala (5) hybrids.

Fig. 10: Callus proteins from *Vitis silvestris* species (1), Pinot gris B.10 (2), Welschriesling B.20 (3), Chasselas blanc (4), Afuz Ali (5), Noble Furmint (6), Noble Kadarka (7) varieties and S. V. 12375 (8), CsFT 3166 (9) species hybrids.

In the hybrid family of Pearl of Csaba x S. V. 12375 progenies (CsFT 175, 194, Pearl of Zala), the patterns are more complicated. The differences observable among polypeptides of two varieties appear in various degrees: some regions are similar to patterns of the one parent, some to those of the other parent (Fig. 7, Table).

Fig. 8 shows comparative electrophorograms of protein extracts coming from shoots sampled in February, March and April 1988. Significant differences in the polypeptide compositions are visible between winter and spring samples with regard to the relative amount and number of components. It can be stated therefore, that samples for characterization of some varieties, hybrid lines, clones, etc. must be collected at identical times.

The results on soluble proteins extracted from calli are demonstrated in Fig. 9 by examination of parents and their hybrids. In contrast with shoots, the protein range of parental calli turned to be considerably divergent. Hybrids are also well distinguishable from each other and from parents. Several characteristic bands of Pearl of Csaba (on upper part and lower third part of photo) come out in progenies: most intensively in CsFT 195 and Pearl of Zala. Nevertheless, generally S. V. 12375 is dominant.

In Fig. 10, protein patterns are seen belonging to six cultivars of *V. silvestris* and *V. vinifera* and two species hybrids. Pinot gris (2) and Welschriesling (3) belong into convar. *occidentalis*, Chasselas (4) and Afuz Ali (5) to convar. *orientalis*, Noble Furmint (6) and Noble Kadarka (7) to convar. *pontica*. *V. silvestris* has alterations in 80-85 kD interval, and in a 21 kD component in comparison to the other samples. The differences found among varieties are in number, width and intensity of the bands.

Results of spring collection

The samples collected had high chlorophyll contents which may affect the method applied. The protein ranges of green samples differ significantly from those of winter samples. It is probable that major photosynthetic components which are present in each of green plants should also be considered.

Polypeptide analysis of wines and musts

The results of our experiments indicate that the protein patterns of wine and must are divergent from those of shoot and callus samples. Considering the existence of microbial proteins in must and wine samples, these are less suitable for detection of varietal divergencies. For this reason, we will review our results on this topic.

Conclusions

On the basis of our 2-year investigations we can draw the following conclusions.

1. Genetically and ampelographically useful markers can be obtained from esterase isozymes and protein ranges from shoots and from calli originated from shoots.
2. Both the shoot and the callus samples result in well reproducible, characteristically different, variety specific enzyme patterns. On zymograms, the simple and additive appearances of parental markers can be traces in their hybrids. The 18 varieties examined show characteristically identical and definite bands related to their origin and taxonomic status.
3. On the basis of non-specific protein staining results, the protein patterns of shoot change very sensitively parallel with the physiological condition of the given plant, and the differences among varieties are less characteristic. For this reason, the method is applicable only to samples collected at the same point of physiological development. As for calli, protein composition separated by molecular mass may also be variety or clone specific.

4. For each type of analyses in comparative studies identical sampling (e. g. timing, physiological condition, localization on plant specimen) must be performed. Within a given time interval, the same day and, moreover, the same time of day is important for collection of samples and, in addition, equivalent storage conditions are also necessary. For callus samples, light and dark periods during its culture may also be important.
5. Must and especially wine samples are less suitable for the comparison of varieties because of microbial influences.

References

ARULSEKAR, S.; PARFITT, D. E.; 1986: Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio, and fig. HortScience **21**, 928-933.

BACHMANN, O.; BLAICH, R.; 1988: Isoelectric focusing of grapevine peroxidases as a tool for ampelography. Vitis **27**, 147-155.

BENIN, M.; GASQUEZ, J.; MAHFOUDI, A.; BESSIS, R.; 1988: Caractérisation biochimique des cépages de *Vitis vinifera* L. par électrophorèse d'isoenzymes foliaires: Essai de classification des variétés. Vitis **27**, 157-172.

CARGNELLO, G.; GIANAZZA, E.; TEDESCO, G.; CAPPELLA, M.; GEROLA, F. M.; 1988: Wall proteins of *Vitis vinifera* pollen. I. Constancy of the phenotype. Vitis **27**, 47-55.

CORREA, J.; POLO, M. C.; AMIGO, L.; RAMOS, M.; 1988: Séparation des protéines des moûts de raisin au moyen de techniques électrophorétiques. Connaiss. Vigne Vin **22**, 1-9.

DAL BELIN PERUFFO, A.; VARANINI, Z.; MAGGIONI, A.; 1981: Caratterizzazione di specie, varieta e cloni di vite mediante elettrofocalizzazione di estratti enzimatici fogliari. C. R. 3e Symp. Intern. Sélection Clonale de la Vigne, Venise, 8-12 Juin 1981, 31-40.

DRAWERT, F.; GÖRG, A.; 1974: Über die elektrophoretische Differenzierung und Klassifizierung von Proteinen. III. Disk-Elektrophorese und isoelektrische Fokussierung in Polyacrylamid-Gelen von Proteinen und Enzymen aus Trauben verschiedener Rebsorten. Z. Lebensm.-Untersuch.-Forsch. **154**, 328-338.

FALLOT, J.; MAURO, M. CL.; AMBID, C.; NEF, C.; LELIEVRE, J. M.; BUIS, R.; BARTHOU, H.; PHILIPPE, I.; ALBERTINI, L.; PETITPREZ, M.; 1985: Amélioration de *Vitis vinifera* var. Cabernet-Sauvignon. Méthode d'évaluation précoce de la variabilité. Moët-Hennessy, Colloq. Amélioration la Vigne et Culture *in Vitro*, Paris, 83-99.

LOUKAS, M.; STAVRAKAKIS, M. N.; KRIMBAS, C. B.; 1983: Inheritance of polymorphic isoenzymes in grape cultivars. J. Heredity **74**, 181-183.

SCHAEFER, H.; 1969: Untersuchungen zur Methodik der Extraktion und Disk-Elektrophorese der Blatteiweiße der Gattung *Vitis*. Wein-Wiss. **24**, 205-232.

-- --; 1970: Über die Isolierung und Disk-Elektrophorese der Isoenzyme der Peroxidase aus den Blättern der Gattung *Vitis*. Wein-Wiss. **25**, 277-282.

-- --; 1971 a: Enzympolymorphismus in Rebenblättern. Phytochemistry **10**, 2601-2607.

-- --; 1971 b: Vergleichende disk-elektrophoretische Untersuchungen über die Eiweiße der Blätter und Triebspitzen der Gattung *Vitis*. Wein-Wiss. **26**, 57-74.

-- --; 1971 c: Vergleichende Untersuchungen über das Auftreten von Isoenzymen der Peroxidase in den Blättern und Triebspitzen der Rebe. Wein-Wiss. **26**, 112-134.

-- --; 1971 d: Disk-elektrophoretischer Nachweis der Polyphenoloxidasen aus Rebblättern. Vitis **10**, 31-32.

STAVRAKAKIS, M.; LOUKAS, M.; 1983: The between- and within-grape-cultivars genetic variation. Sci. Hort. **19**, 321-334.

SUBDEN, R. E.; KRIZUS, A.; LOUGHEED, S.C.; CAREY, K.; 1987: Isoenzyme characterization of *Vitis* species and some cultivars. Amer. J. Enol. Viticult. **38**, 176-181.

WOLFE, W. H.; 1976: Identification of the grape varieties by isozyme banding patterns. Amer. J. Enol. Viticult. **27**, 68-73.

The use of isozymes for characterization of Spanish *Vitis* cultivars

J. M. Ortiz, H. Altube, J. R. Lissarrague and V. Sotés

Departamento de Biología Vegetal, E.T.S.I. Agrónomos, Universidad Politécnica de Madrid, E-28040 Madrid, Spain

Abstract: Cuttings and leaves from *Vitis* cultivars were sampled and extracted in a buffered medium throughout the growing season. From the extracts, several isozyme systems were electrophoretically separated in polyacrylamide slab gels and stained with adequate solutions. The isozymes studied were: catechol oxidase (CO), acid phosphatase (ACPH), esterase (EST), peroxidase (PER), malate dehydrogenase (MDH), glucophosphate isomerase (GPI), and glutamate-oxaloacetate transaminase (GOT). The results of the analysis indicate the usefulness of isozymes for distinction among *Vitis* species and cultivars for the studied varieties. The utilization of these analyses as a standard method for characterization of grape cultivars and rootstocks is discussed.

The anthocyanins of grapevine leaves and their taxonomic importance

G. Darné

Université de Bordeaux I, Laboratoire de Physiologie Végétale et Ampélologie, F-33405 Talence, France

Abstract: The anthocyanin composition of red leaves of some *Vitis* species was determined by HPLC at berry maturity. Chromatograms show important variations between species:

- Asiatic species *(Vitis amurensis, V. coignetiae* and *V. thunbergii)* have a lot of cyanidin monoglucoside (MG-Cy ≥ 90 % of total anthocyanins). They also have diglucosides of cyanidin and delphinidin, but no acylated anthocyanins.
- American species *(V. berlandieri, V. riparia* and *V. rupestris)* have no anthocyanins in the leaf blade, but they have some traces of these pigments in the petioles.
- Two groups occur in *V. vinifera* cultivars: The first group (Sultanine rouge, Merlot noir ...) has a preponderance of hydroxylated anthocyanins (cyanidin and delphinidin monoglucosides) over related methoxylated (paeonidin and malvidin monoglucosides), and has some traces of acylated anthocyanins. The second group (Gamay Fréaux, Malbec, Cabernet Sauvignon ...) has a preponderance of methoxylated anthocyanins over related hydroxylated, and large amounts of acylated anthocyanins.

Section 2: Genetic improvement of grapevine form or function

Plant breeders' rights for vine varieties based on the International Convention for the Protection of New Varieties of Plants

R. BECHER

Bundessortenamt, Prüfstelle Haßloch, Neustadter Straße 42, D-6733 Haßloch, F. R. Germany

S u m m a r y : Plant breeders' rights offer an economic stimulant for the creation of new and improved varieties. Only the holder of the right is authorized to commercialize the protected variety. The UPOV Convention contains the necessary rules for the grant of protection. The following conditions must be fulfilled before plant breeders' rights can be granted: distinctness, homogeneity, stability, novelty and an acceptable variety denomination. At present is is being discussed as to how far the new developments in plant breeding, especially in biotechnology, necessitate a revision of the UPOV Convention.

K e y w o r d s : law, plant breeders' rights, UPOV Convention, variety of vine, characteristic, distinctness, homogeneity, stability, novelty, variety denomination, biotechnology, patent.

Introduction

It is a well known fact that man's food supply and farmer's operating income depend to a high degree on plant breeder's efficiency. But the creation of new varieties of plants requires considerable investment in qualified staff, suitable fields, buildings and technical equipment. Therefore, plant breeding can only be economically interesting for a breeder if the commercialization of a new variety depends on his prior authorization in such a way that he can get the remuneration necessary for further investments.

Based on this background, in 1961 the International Convention for the Protection of New Varieties of Plants was created. The purpose of the Convention is to recognize and ensure the proprietary right of plant breeders which is clearly defined in the rules of the Convention. Today 18 states of all five continents apply the rules of the Convention. These states form the International Union for the Protection of New Varieties of Plants (UPOV).

Rules of the Convention

With regard to vine varieties the following rules of the UPOV Convention are important:

1.– It is immaterial whether a variety is the result of systematic breeding activities or it is a mutant of an existing variety which has been discovered. In principle all genera and species of plants fall under the rules of the Convention.

2.– The prior authorization of the holder of the right is required before propagating material of a protected variety can be produced for the purpose of commercial marketing, offered for sale or marketed.

3.– No authorization of the holder of the right is required if his protected variety is used as a basic source for the creation of a new variety (breeder's privilege). However, such authorization is required when, for instance, the repeated use of a protected inbred line is necessary for the commercial production of a hybrid, e. g. maize or sunflower.

4.– The minimum period of protection for grapevine varieties lasts 18 years. In the Federal Republic of Germany the right is granted for 30 years. No prolongation is possible.

5.– Conditions required for plant breeders' rights

5.1 Test for distinctness, homogeneity and stability

Each new grapevine variety has to undergo a test for distinctness, homogeneity and stability. In the Federal Republic of Germany this test is conducted at a testing station of the Bundessortenamt in Neustadt-Mußbach. At least 2 years of observation on fully developed plants are necessary to test grapevine or rootstock varieties. 24 or 20 plants, respectively, are needed for the examinations. In the Federal Republic of Germany all grapevine varieties protected or registered in the variety list or already in test serve as a reference collection.

Distinctness, homogeneity and stability are judged by characteristics which are coded in up to 9 different states of expression in order to facilitate data processing and international communication. In cooperation with professional organizations and experienced scientists UPOV has established test guidelines for grapevine varieties. They contain 77 different characteristics of which 38 are observed in the Federal Republic of Germany. These characteristics are important in the sense of the UPOV Convention for distinguishing varieties. They are also used for the description of the variety.

Example of characteristic coding:

Young shoot: intensity of anthocyanin coloration of tip

State of expression	Note
absent or very weak	1
very weak to weak	2
weak	3
weak to medium	4
medium	5
medium to strong	6
strong	7
strong to very strong	8
very strong	9

To receive protections the following test criteria must be fulfilled:

5.1.1 Distinctness

Every variety has to be clearly distinguishable by at least one important characteristic from any other variety whose existence is a matter of common knowledge. For that purpose a minimum distance is applied to each characteristic.

5.1.2 Homogeneity

The new variety must be sufficiently homogeneous, taking into account the particular features of its sexual or vegetative reproduction. It is evident that an allogamous species like rye cannot be as homogeneous as an autogamous species like pea or a vegetatively propagated species like grapevine. Dependent on the number of plants observed, a tolerance for the number of off-type plants is fixed.

5.1.3 Stability

The new variety must be stable in its essential characteristics and must correspond to ist initial description after repeated reproduction.

5.2 Novelty

At the date of application for plant breeders' rights the variety has to be new, i. e. in the case of grapevine varieties commercialization with the agreement of the breeder must not date back more than 1 year in the state where the application was filed and must not date back more than 6 years in any other state.

5.3 Variety denomination

Every variety needs an acceptable denomination which enables the variety to be clearly identified.

Current statistics

1.– Number of applications for plant breeders' rights between July 1, 1988 and July 1, 1989

UPOV member states	> 5000
Federal Republic of Germany	
total	> 1000
grapevine varieties	7
rootstock varieties	2

2.– Number of protected varieties in the Federal Republic of Germany on July 1, 1989

total	3405
grapevine varieties	47
rootstock varieties	5

Final remarks

In the light of the new development in plant breeding especially in biotechnology it is being discussed within UPOV as to how far the rules of the UPOV Convention have to be strengthened. Three of the most important points for grapevine varieties are mentioned below:

1.– At present, only propagating material falls under the scope of protection. Especially in respect to tissue culture, it is discussed if all material of the protected variety should fall under the scope of protection. Every material means all material out of which the variety can be reproduced.

2.– In order to avoid plagiate breeding with the help of easy mutations or quick changes by biotechnological means, it is being discussed if a system of dependent rights should be introduced into the UPOV Convention. But this only when the new variety is mainly based on an already existing variety.

3.– In respect to patent legislation it is common understanding in most countries that new procedures in plant breeding can be patented as process patents. On the other hand it is also common understanding that artificial genes can be patented as product patents. These two examples indicate that conflicts may arise by an overlapping of patent legislation and plant breeders' rights.

Therefore on an international basis groups of patent experts and plant breeders' rights experts are deliberating on workable interfaces between these two sectors.

Possible changes of the present UPOV Convention in these and many other questions are expected to be made during a diplomatic conference in 1991. Meanwhile, a discussion has also

started in Brussels to establish a common plant breeders' rights system for the 12 member states of the European Economic Community. It is the declared intention of the Community to establish a system which is in full conformity with the content of the present and any future UPOV Convention.

Investigations about the influence of some physiological and phenological characteristics on quality and their heredity

R. EIBACH

Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, F. R. Germany

S u m m a r y : The analysis of the offspring of 6 crossings with a total of 360 genotypes revealed that, of a total of 13 characteristics taken into account by the investigations, only the yield or the yield components, the beginning of berry ripening and the degree of botrytis infection have a significant influence on the sugar content of the must. About 60 % of the fluctuations in the sugar content of the must could be explained by these, whereby at approx. 35 % the beginning of berry ripening accounted for the highest percentage. The coefficient of determination for the three yield parameters which were investigated was between 12 and 19 %. The weight of the berries was the most important factor, while the number of clusters per shoot was nearly neutral with respect to its effect on the sugar content of the must. Consideration of these relations when selecting seedlings may, if only to a limited extent, lead to an increase in the success of selection for varieties with a high sugar content. It should be mentioned that these results, which were reached under the climatic conditions of Central Europe, cannot necessarily be applied to wine growing areas in other climates.

Some of the heritability levels determined differ considerably for the individual characteristics. While a low heritability coefficient was ascertained for the number of clusters per shoot, the single berry weight and the beginning of berry ripening indicate a high percentage of additive genetic effect, i. e. their degree in the offspring can be influenced to a great extent by the selection of the parents for crossing combinations.

By taking into account both the heritability levels when making the crossing combinations and the relations between these characteristics and the sugar content of the berries when selecting, a breeding programme to increase the sugar content is likely to make rapid progress. In addition, the selection criteria pointed out may be useful in clonal selection with regard to better quality. Last but not least, values for combining ability, which can be calculated by a similar model of variance analysis, may help by the identification of varieties.

K e y w o r d s : genetics, heritability, crossing, selection, biometry, yield, must quality, maturation, botrytis.

Introduction

The quality of the must is not only affected by external factors such as climate or methods of cultivation, but also by the yield. As early as 1927 SARTORIUS identified this negative correlation between yield and the sugar content of the must, known as a quantity/quality ratio. Results of previous research (BÄDER 1979) showed that this quantity/quality ratio was not identical for every variety. It was also shown that the sugar content tends to correlate more negatively to the weight of the cluster than to the number of clusters per plant (ALLEWELDT and KOEPCHEN 1978). It is assumed that greater knowledge of the influence of the different yield parameters on the sugar content of the must and other substances such as acid and aroma compounds could mean an improvement of selection criteria within seedlings.

In continuation of previous research (ALLEWELDT and KOEPCHEN 1978) these investigations are primarily concerned with the influence of the yield parameters on the sugar content of the must.

Material and methods

Genotypes from 6 populations were studied in 1987 and 1988. For each population the sugar content of the must, the yield per plant as well as the yield parameters of approx. 60 individual plants were determined. Further characteristics which might have an effect on the sugar content were scored. These include the density of the clusters and the degree of botrytis infection at the time of the harvest, as well as phenological characteristics such as the beginning of flowering and berry ripening. The number of shoots per plant, which is hardly affected by genetic factors, is mainly

determined by pruning. For this research it was standardized by retaining 10 buds per plant. A total of approx. 360 genotypes were included in the investigations.

In addition, the collected data was used to estimate the heritability coefficients for characteristics which influence the sugar content of the must. The design of the experiment corresponds to a diallel crossing model and enables one to estimate the variance components according to the variance analysis model with nested classification (WEBER 1978; WRICKE and WEBER 1986).

Results and discussion

By using multiple regression analysis the influence of all recorded parameters on the sugar content of the must can be determined. First of all, only the total yield per plant is taken into account instead of the individual yield parameters. The summarized results in Fig. 1 show that the only characteristics which have a significant effect on the sugar content of the genotypes used in the research are the yield per plant, the beginning of berry ripening and the degree of botrytis infection.

Variations of these characteristics explain 51 % of the fluctuations in the sugar content of the berries. It is at first surprising that the total yield accounts for only 8 % of the differences in the sugar content of the must, while the percentage for the beginning of berry ripening is about 4 times as high at 34 %. If the yield is replaced by the individual yield parameters for the statistical analysis, the results are as follows (Fig. 2): The single berry weight accounts for 8 % of the variations of the sugar

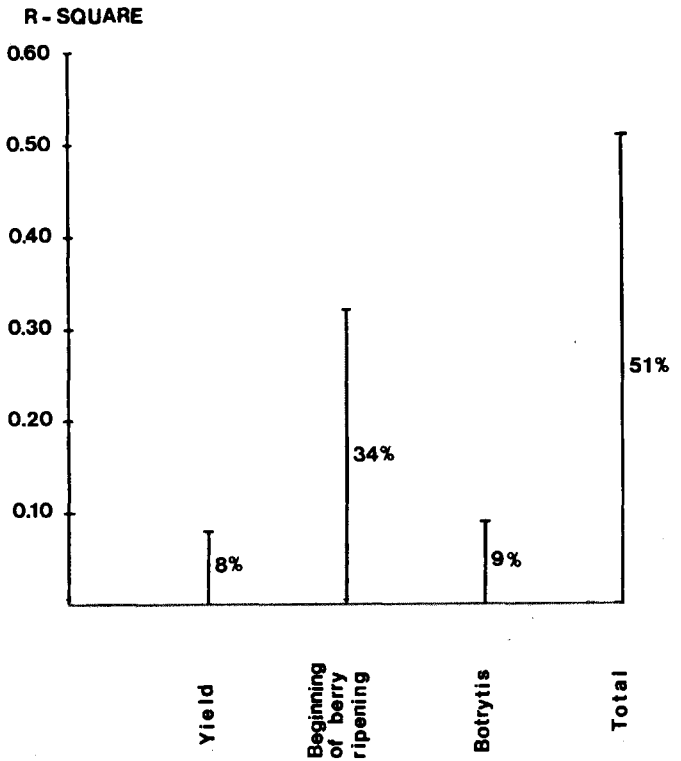


Fig. 1: Yield and other characteristics affecting the sugar content in berries (n = 357); probability of F > 0.05.

content of the berries, thus reaching the same level as the total yield in Fig. 1. The sum of the single coefficients of determination for all yield parameters now reaches 14 %. These results show that a considerably higher percentage of the fluctuations in the sugar content can be explained by the individual yield components than by the total yield per plant. It also becomes evident that the individual yield parameters affect the sugar content of the must to varying degrees. Because of the existing but more or less weak positive correlations between yield per plant and yield parameters, and negative correlations between the different yield parameters, this analysis can imply only tendencies. These weak correlations can be explained by the yield structures of the seedlings, some of which vary considerably.

As an example of this, Fig. 3 shows the number of berries per cluster compared to the total yield. The mass of points indicates the relation between the two characteristics. However, it covers a large area and there are many stray points. Genotypes which lie within a very limited area and thus have a high correlation between the number of berries per cluster and the total yield were now chosen for further calculations. For these genotypes the variation of the yield per plant depends mainly, but not exclusively, on the variation of the number of the berries per cluster. For such selected genotypes as these a correlation coefficient of $r > 0.98$ between the number of berries and the yield as well as a minimum of 55 genotypes was fixed. By shifting this marked area parallel to

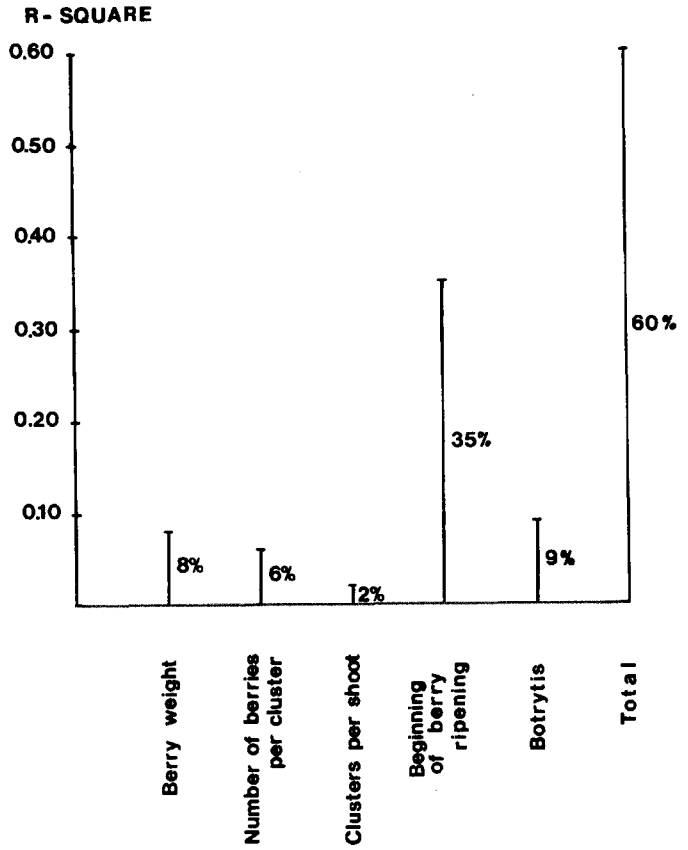


Fig. 2: Yield parameters and other characteristics affecting the sugar content in berries (n = 357); probability of $F > 0.05$.

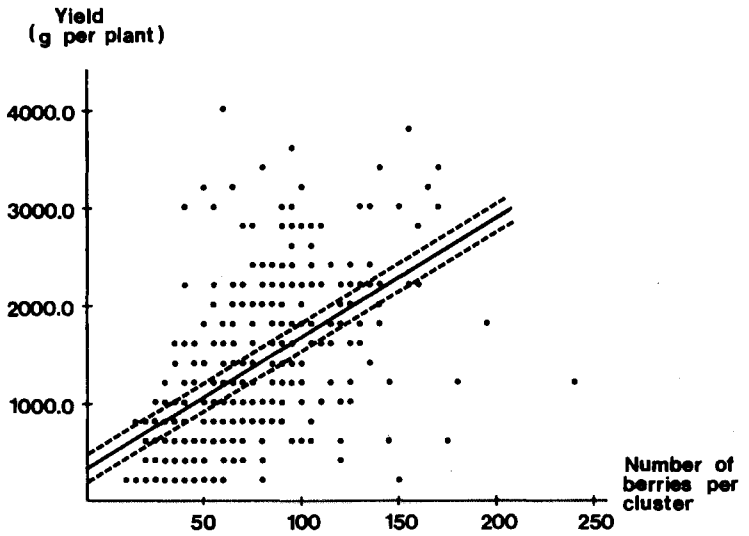


Fig. 3: Number of berries per cluster plotted against total yield per plant with corresponding regression line (n = 357).

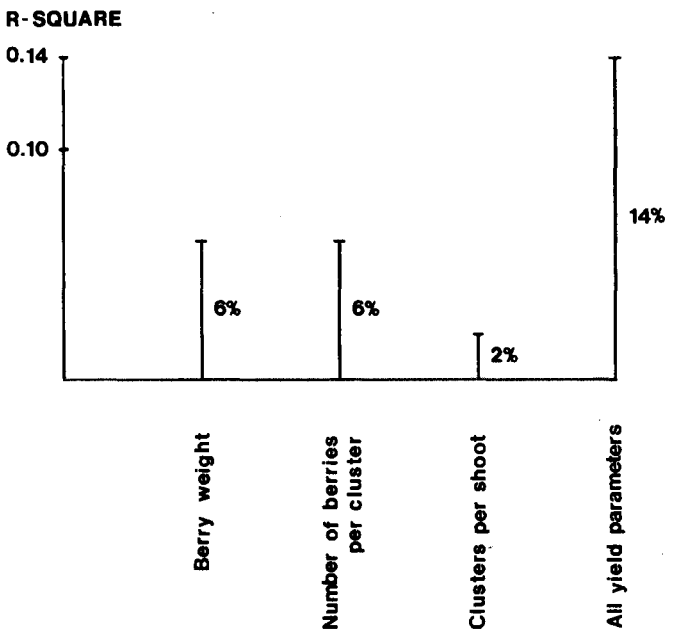


Fig. 4: Influence of yield parameters on sugar content of berries; selected genotypes with r › 0.98 between total yield and number of berries per cluster (n > 55).

the regression line, groups of genotypes can be found which show various yield levels, but still meet the requirements mentioned. It was possible to form between 4 and 6 groups for each yield parameter. The multiple correlation coefficients were calculated for each group.

Fig. 4 shows the means from these evaluations for the number of berries per cluster. When an increase in yield is mainly the result of the number of berries per cluster thus maximizing the variation of the number of berries and minimizing the variation of the other yield parameters, the fluctuations in the sugar content of the must can be explained as follows: 6 % by the number of berries per cluster, 6 % by the single berry weight and 2 % by the number of clusters per shoot. If the same principle is used for the single berry weight, which is maximizing the variation for this yield parameter, the results shown in Fig. 5 are reached: Now the single berry weight is responsible for 12 % of the fluctuations in the sugar content of the must. At 6 % the coefficient of determination for the number of berries is 50 % lower and the number of clusters per shoot only reaches 1 %.

Fig. 6 shows the summarized results when the variation of the number of clusters per shoot is maximized. Despite the increase in yield due primarily to the number of clusters per shoot, only 3 % of the fluctuations in the sugar content of the must are subject to this parameter, whereas the figure doubles for fluctuations due to the single bery weight.

In addition to the practical consequences for the selection of seedlings which can be derived from these relations, the type of heredity of these characteristics is of special interest to the breeder. With knowledge of the coefficients of heritability, he might be able to influence the degree of the characteristics within the offspring by selecting suitable parents. In so doing, he might be able to increase the success of selection.

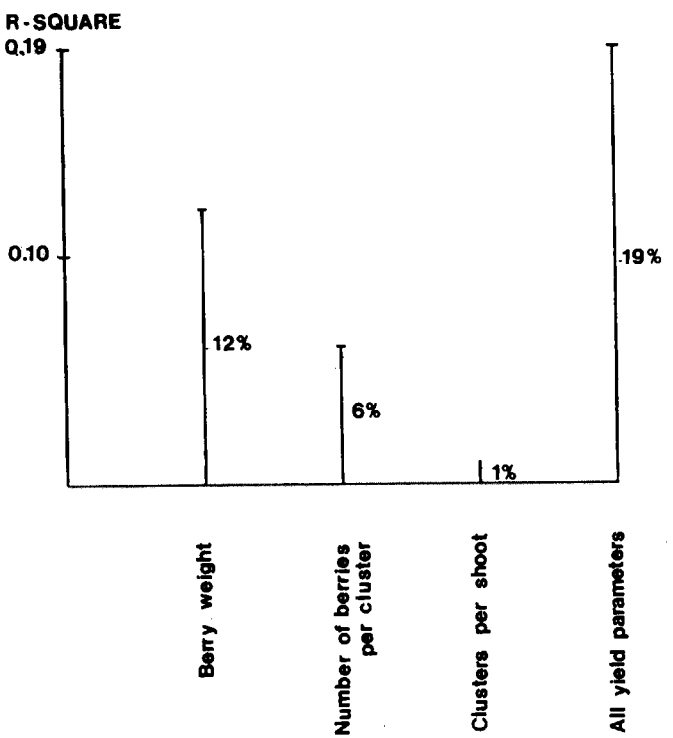


Fig. 5: Influence of yield parameters of sugar content of berries; selected genotypes with $r > 0.98$ between total yield and berry weight ($n > 55$).

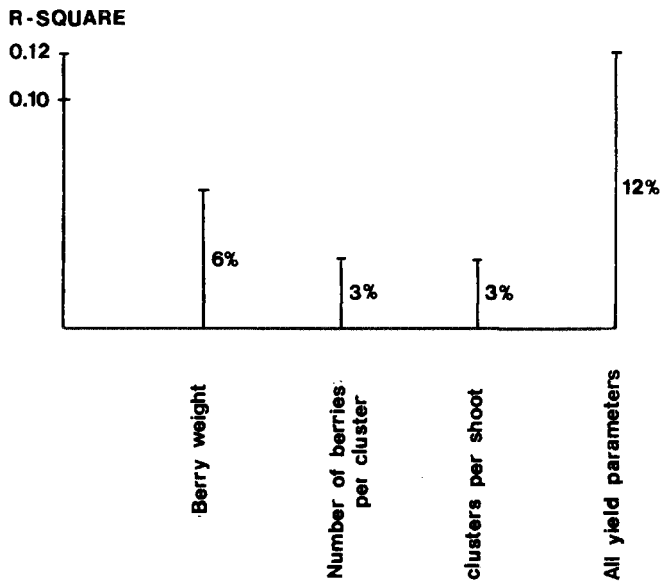


Fig. 6: Influence of yield parameters on sugar content of berries; selected genotypes with r > 0.98 between total yield and clusters per shoot (n > 55).

The most important parameter for cross-breeding with regard to polygenic characteristics (such characteristics are being dealt with exclusively here) is the heritability in the narrow sense. In this case only additive genetic effects are taken into account while dominant gene effects, which reduce the breeding value of a variety, are omitted.

For the investigations the same 6 populations were used. The populations were progenies of two female parents, Sirius and Gf. Ga-54-14, both fungus resistant varieties of BFAR - each crossed with the male parents Maréchal Joffre, Vidal blanc and Bellandais noir.

Fig. 7 shows the frequency of the single berry weight in each population. The range of variation extends from 0.5 g to 3.1 g per berry. The low single berry weight of Maréchal Joffre is evident in the offspring of this variety, where the frequency distribution deviates to the left in each case. None of the genotypes produced from a crossing with this variety had a single bery weight of more than 2.0 g. When the male parents Bellandais noir and Vidal blanc are compared, there are traces of a frequency distribution of the crossing with Bellandais noir which deviates somewhat towards a higher single berry weight. This corresponds to the somewhat higher single berry weight of this variety compared to Vidal blanc. There are no significant differences between the female parents, which is evidenced by their nearly equivalent single berry weight. The horizontal bar drawn across the frequency distribution shows the range of variation of the parent varieties. On the one hand, the single berry weights of the offspring are also concentrated within this area but, on the other hand, the deviations to the left and to the right are nearly equal, thus indicating a relatively high percentage of additive genetic effects. One would expect a clearer deviation of the frequency distribution either to the left or to the right if the degree of the characteristics in the parent varieties was mainly subject to dominant genes.

Contrarily to the single berry weight, a differentiation of frequency distributions for the number of clusters per shoot both between the male parents within a female parent and between the female parents is nearly impossible (Fig. 8). The curve is very similar for all the crossings.

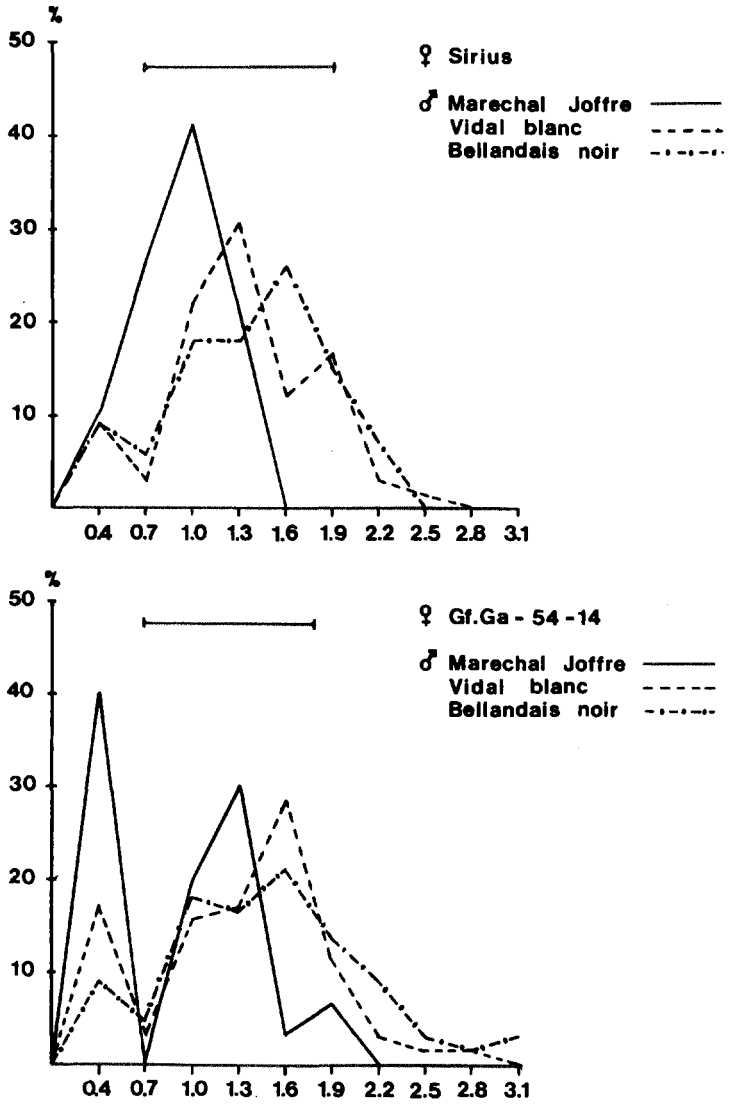


Fig. 7: Distribution of frequency of mean berry weight for some selected cross populations.

Particularly in the crossings with Gf. Ga-54-14 the frequency distributions of the offspring deviate clearly from the range of variation of the parent varieties. The Gf. Ga-54-14 variety, which is characterized by a high average number of clusters per shoot, does not seem to transmit this characteristic well.

The calculated heritability coefficients for both the yield per plant and the individual yield components are shown in Table 1. The mean for the heritability coefficients over the 2 years of research for the yield per plant is 50 %. At 69 % the single berry weight has the highest heritability coefficient of the yield parameters and at 12 % the number of clusters per shoot has the lowest.

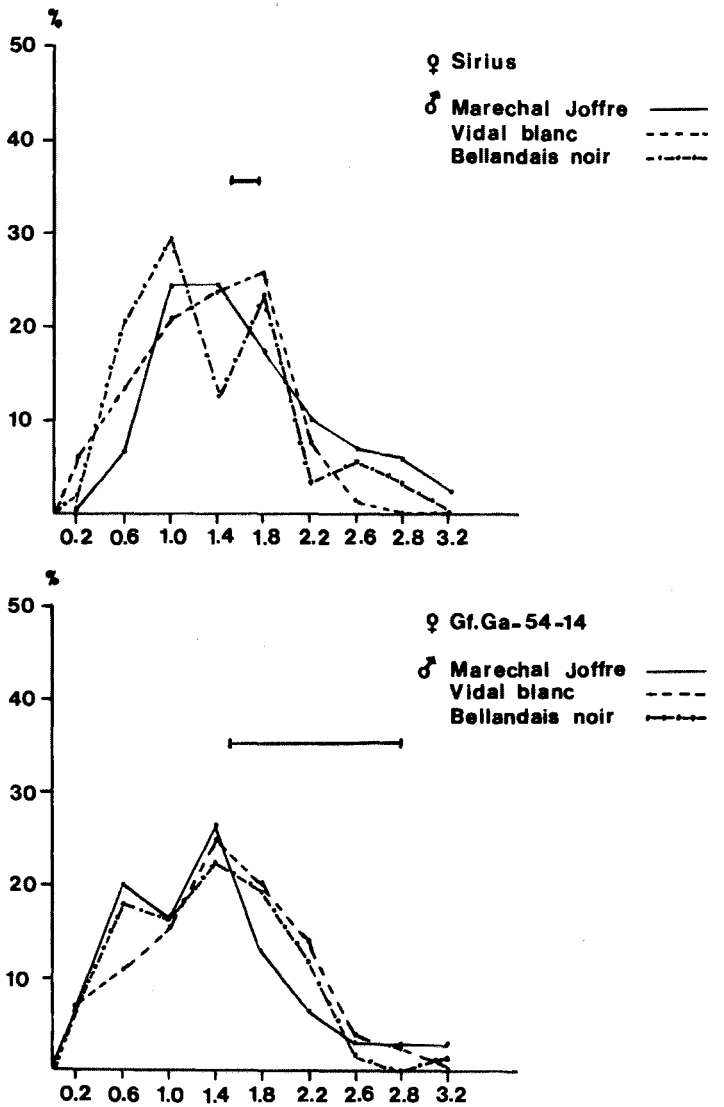


Fig. 8: Distribution of frequency of clusters per shoot for some selected cross populations.

According to this, the markedness of the characteristic of single berry weight in the offspring is the most easily influenced characteristic when selecting the parents for the crossing combination. In contrast, the high number of clusters per shoot is apparently due to a great degree to dominant genetic effects. Therefore, for varieties with a high number of clusters per shoot, a low breeding value for this characteristic can be expected.

As mentioned at the outset, the sugar content of the berries is not only influenced by the yield parameters, but also by the extent of the botrytis infection and, above all, by the beginning of berry ripening. There is no point in calculating the heritability of the resistance to botrytis infection since it

Table 1: Calculated coefficients of heritability for yield and components of yield

	heritability (%)		
Characteristic	*1987*	*1988*	*Mean*
yield	46	54	50
mean berry weight	73	65	69
number of berries	31	38	35
clusters per shoot	10	13	12

Table 2: Calculated coefficients of heritability for begin of berry ripening and density of cluster

	heritability (%)		
Characteristic	*1987*	*1988*	*Mean*
beginning of berry ripening	58	44	51
density of cluster	25	28	27

strongly correlates to the stage of ripening, and it is not possible to standardize. However, the evaluations show that the density of the clusters, in particular, strongly influences the degree of botrytis infection, which is reflected in the highly significant correlation of $r = 0.42$. In addition to the yield parameters, Table 2 shows the heritability levels for the beginning of berry ripening and for the density of the clusters. A medium heritability coefficient of 51 % was reached for the beginning of berry ripening. According to this, this characteristic is inherited quite well. The low dependency of the density of the clusters on the environment and the relatively low heritability in the narrow sense at 27 % imply a high percentage of dominant genetic effect and, therefore, a type of heredity which is not very much influenced by the selection of the crossing parents.

Literature

ALLEWELDT, G.; KOEPCHEN, W.; 1978: Criteria of breeding for quality. Grapevine Genetics and Breeding. Proc. 2nd Intern. Symp. Grapevine Breeding, Bordeaux, 14-18 June; 387-396.

BÄDER, G.; 1979: Einfluß des Anschnittes auf Menge und Güte des Ertrages bei den wichtigsten Rebsorten auf 128 Standorten der deutschen Weinbaugebiete. Diss. Univ. Gießen.

SARTORIUS, O.; 1927: Zur Rebenselektion unter besonderer Berücksichtigung der Methodik und der Ziele auf Grund von 6-14jährigen Beobachtungen an einem Klon. Z. Pflanzenzücht. **12**, 31-74.

WEBER, E.; 1978: Mathematische Grundlagen der Genetik. VEB Gustav Fischer Verlag, Jena.

WRICKE, G.; WEBER, E. W.; 1986: Quantitative Genetics and Selection in Plant Breeding. Walter de Gruyter, Berlin.

The heritability of methyl anthranilate and total volatile esters in *Vitis* spp. hybrids

K. H. Fisher [1]), T. Fuleki [1]) and A. G. Reynolds [2])

[1]) Horticultural Research Institute of Ontario, Vineland Station, Ontario, Canada

[2]) Agriculture Canada Research Station, Summerland, British Columbia, Canada

Summary: Two grapevine seedlings, V. 72181 and V. 72182, selected for extremely high methyl anthranilate (MA) and total volatile esters (TVE) content, were selfed to create families 8020 and 8021 respectively, to test the inheritance of these two components of labrusca flavour character. Reynolds *et al.* (1982) had postulated a three-gene, dominant and complementary system (M, A, F) for MA and a two-gene dominant and complementary system (T, V) for TVE. Families 8020 and 8021 segregated 3 : 1 for MA, indicating only one heterozygous locus for MA in the parents. This would question Reynolds' assignment of genotypes for the grandparents of these two families and would suggest a more complex environmentally influenced system. The TVE segregation patterns followed Reynolds' hypothesis and segregated 3 : 1 for one heterozygous locus.

Key word: genetics, heritability, flavour, methyl anthranilate, volatile esters, biometry, sensory rating, breeding.

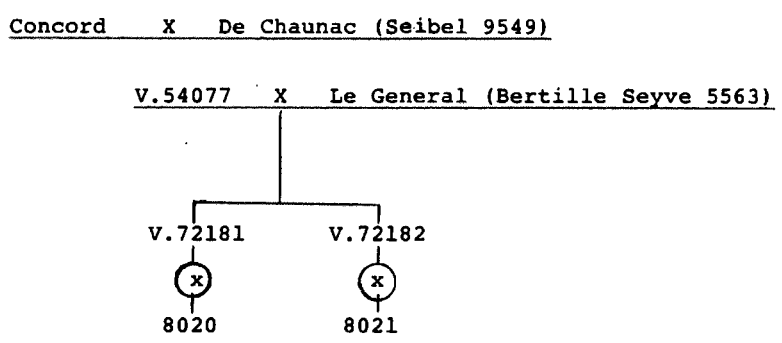
Introduction

Since 1913, the grape breeding programme at the Horticultural Research Institute of Ontario (HRIO) has been striving to produce wine grapes of suitable hardiness for the climatic conditions in southern Ontario, Canada. Flavour and colour requirements have changed markedly since that time and the breeding programme has been altered to meet the new requirements. With more recent demands for non-labrusca flavoured varieties, the heritability of labrusca flavour components has become quite critical.

Since 1970, Dr. T. Fuleki of this Institute has been surveying the HRIO variety collection as well as the seedlings resulting from the HRIO breeding programme for compounds thought to be instrumental in the perception of labrusca flavour, i. e. methyl and ethyl anthranilate (MA) and total volatile esters (TVE). He later used these components to develop the Vineland Grape Flavour Index (VGFI) that became the objective flavour selection criterion for the grape seedling populations at the Institute (Fuleki 1982).

The analysis of grape seedling selections for the above compounds became routine procedure, but perhaps more important was the impact on the choice of parents in the breeding programme for subsequent generations. The MA and TVE survey values were examined to assess the potential labrusca flavour of seedlings according to the MA/TVE/VGFI values of the parents. Based on the hypothesis that heritability could be predicted from these values, Reynolds studied three seedling families, 7216, 7218 and 7219, which had high and low labrusca flavoured parents. The seedling populations of these three families were examined in 1977 and 1978 and Reynolds postulated genotypes for MA and TVE synthesis for their parents. He also identified two seedlings, V. 72181 and V. 72182, with extraordinarily high MA and TVE values (Reynolds 1980). These two seedlings were maintained in the HRIO vineyard and were selfed in 1980 to create families 8020 and 8021, respectively (Fig. 1).

Reynolds postulated that MA synthesis was governed by three dominant and complementary genes (M, A and F), with the F locus necessary in the dominant condition for MA synthesis to proceed and M and A loci both dominant for excessive MA production. The presence of M and/or A in the recessive condition would result in lower amounts of MA, below the organoleptic threshold of labrusca flavour influence, and the presence of the F locus in the recessive



[a] Fisher, Ann. Rept. HRIO, 18-20, 1981.

Fig. 1: Genealogy of grape vine cultivars/seedlings used in MA/TVE inheritance studies [a].

```
Mm Aa Ff  X  MM Aa ff  = (excess MA)  3:5  (low or no Ma)
   Tt Vv  X  tt Vv     = (excess TVE) 3:5  (low TVE)
```

[a] Reynolds et al, Am . J. Enol. Vitic., 33:1:14-19, 1982.

Fig. 2: Segregation ratios for MA and TVE for Family 7218 (V. 54077 x Le Général) [a].

condition would preclude any MA synthesis. Reynolds also postulated that TVE was governed by two dominant complementary genes (T and V) and that either in the recessive condition would constitute TVE production below the organoleptic level of labrusca flavour influence (Reynolds *et al.* 1982).

These hypotheses led to the genotypic assumptions presented in Fig. 2.

Since seedlings V. 72181 and V. 72182 were selected for their exceptionally high MA and TVE, it is assumed that they would have the genotypic make-up for excess MA and TVE production. Based on Reynolds' genotypic assignments for the parents, the following genetic combinations could be assigned to the above seedlings, as outlined in Table 1.

In order to confirm the ratios postulated in Table 1, the following study was initiated.

Method

Plant material

Parental lines were planted in the vineyard as individuals or as populations of vegetatively propagated clones. Their genealogy is illustrated in Fig. 1.

Selfed populations were created by bagging flower clusters of the appropriate parental vines prior to capfall. Seedling populations (8020, 8021) were planted as a group in the seedling block of the main vineyard.

Table 1: Proposed genotypes and segregation ratios for MA and TVE for F_2 populations derived from V. 72181 and V. 72182[a] crosses

Excess MA	Segregation Ratio		Excess TVE	Segregation Ratio	
MM AA Ff	L/NL[b]	3:1	Tt VV	L/NL	3:1
MM Aa Ff MM aA Ff mM AA Ff	L/NL	9:7	Tt Vv Tt vV	L/NL	9:7
mM Aa Ff mM aA Ff	L/NL	27:37			

[a] Reynolds et al, Am . J. Enol. Vitic., 33:1:14-19, 1982
[b] L = labrusca, NL = non-labrusca flavour character

Table 2: Threshold values for organoleptic detection of labrusca flavour characters

Methyl anthranilate	0.10 ppm	(Nelson et al, 1977)
Total volatile esters	12.0 ppm	(Fuleki, 1982)

Harvest

At maturity, the total crop was harvested from each seedling vine (total crop was sub-sampled from a parent vine). Grapes were washed, removed from the stems, frozen and maintained at -30 °C until analysis. Analyses for MA and TVE were carried out within 3 months of the harvest date. Data are presented for the harvests of 1986, 1987 and 1988 in Tables 3-6.

To obtain representative samples for analyses, frozen grape samples were ground without breaking the seeds. A household and a 5 HP commercial meat grinder (Butcher Boy, Model A 42.50, Laser Mfg. Co. Inc., Los Angeles, CA, USA) were used for smaller and larger lots respectively. The ground frozen material was thoroughly mixed and two 50 g aliquots were removed for steam distillation.

Steam distillation

Samples were placed in an all-glass distillation apparatus (Cat. No. JD-2115, SGA Sci., Bloomfield, N.J., USA). The distillation was carried out on duplicate 50 g samples, collecting 100 ml in 15 min. The distillate was used to determine both the MA and the TVE. Glass distilled water was steam distilled under the same conditions as the grapes to produce blanks for the MA and TVE analyses.

Determination of MA

A highly sensitive fluorometric method was used (Casimir *et al.* 1976). The measurements were carried out on a ZFM4 fluorescence attachment of a Zeiss DMR21 recording spectrophotometer (C. Zeiss, Oberkochen, Germany). The exciting radiation was isolated with a

Table 3: Methyl anthranilate values and equivalent labrusca/non-labrusca codes for Family 8020 (1986 to 1988)

Seedling Number	Methyl anthranilate (ppm)					
	1986	Code[a]	1987	Code	1988	Code
1	1.776	L	7.446	L	2.776	L
2	0.136	L	-	-	-	-
3	-	-	5.096	L	4.596	L
4	0.618	L	0.560	L	-	-
5	-	-	17.218	L	14.746	L
6	0.290	L	0.482	L	0.767	L
7	0.244	L	0.676	L	2.164	L
8	-	-	-	-	8.546	L
9	-	-	-	-	0.037	NL
10	0.143	L	0.042	NL	0.052	NL
11	-	-	1.786	L	1.950	L
12	-	-	6.446	L	11.326	L
13	-	-	-	-	0.541	L
15	0.866	L	0.039	NL	1.654	L
16	4.076	L	4.076	L	5.386	L
17	5.436	L	5.556	L	-	-
19	-	-	0.022	NL	0.079	NL
20	0.163	L	0.239	L	0.261	L
21	0.358	L	0.565	L	0.882	L
23	0.093	NL	0.479	L	1.733	L
24	-	-	0.106	L	0.192	L
25	7.956	L	9.836	L	6.819	L
26	1.746	L	4.876	L	2.626	L
27	0.096	NL	0.301	L	0.354	L
28	-	-	-	-	2.167	L
29	0.006	NL	0.006	NL	0.014	NL
30	0.079	NL	0.471	L	-	-
31	3.436	L	-	-	-	-
32	0.366	L	1.356	L	1.119	L
33	4.166	L	12.606	L	11.796	L
34	-	-	0.065	NL	0.070	NL
35	2.936	L	2.826	L	6.071	L
37	0.000	NL	0.011	NL	0.031	NL
38	-	-	-	-	0.992	L
39	0.013	NL	0.032	NL	0.044	NL
40	0.032	NL	0.018	NL	0.015	NL
42	0.000	NL	0.006	NL	0.044	NL
43	0.036	NL	-	-	0.060	NL
44	-	-	3.046	L	2.583	L
45	10.096	L	15.776	L	7.826	L

[a] L = labrusca, NL = non-labrusca flavour character

365 nm monochromatic filter from the line spectrum of a mercury lamp. The 4MQIII monochromator of the spectrophotometer was set at 425 nm to measure the fluorescence emitted by the MA. The fluorometer reading was related to the MA concentration by using a standard curve. To eliminate interference from fluorescent volatile substances other than methyl and ethyl anthranilate, the reading taken on a *Vitis vinifera* L. cultivar corresponding to a few ppb MA was subtracted from each measurement.

Table 4: Methyl anthranilate values and equivalent labrusca/non-labrusca codes for Family 8021 (1986 to 1988)

Seedling Number	Methyl anthranilate (ppm)					
	1986	Code[a]	1987	Code	1988	Code
1	0.176	L	1.526	L	2.370	L
2	0.179	L	0.816	L	0.72	L
3	-	-	0.037	NL	0.087	NL
4	2.726	L	7.516	L	2.836	L
5	0.189	L	1.436	L	0.931	L
6	-	-	0.655	L	0.530	L
8	0.386	L	0.024	NL	0.097	NL
10	0.748	L	-	-	0.511	L
11	0.023	NL	0.361	L	1.346	L
12	0.336	L	-	-	1.137	L
13	0.082	NL	0.075	NL	0.066	NL
14	0.081	NL	0.121	L	0.172	L
15	3.696	L	15.676	L	11.776	L
17	-	-	17.696	L	13.966	L
18	3.976	L	8.216	L	2.832	L
19	0.077	NL	0.221	L	-	-
20	0.136	L	0.053	NL	0.202	L
21	0.471	L	1.112	L	2.206	L
23	0.452	L	0.630	L	0.571	L
24	0.020	NL	0.026	NL	0.029	NL
25	0.034	NL	0.137	L	0.140	L
27	0.251	L	0.683	L	0.436	L
28	0.627	L	1.416	L	1.715	L
29	1.236	L	2.776	L	4.055	L
30	0.730	L	1.876	L	-	-
31	-	-	-	-	2.919	L
32	0.028	NL	0.384	L	0.213	L
33	0.480	L	1.424	L	0.639	L
34	1.236	L	2.546	L	2.366	L
35	0.451	L	0.752	L	0.925	L
38	1.326	L	2.705	L	2.266	L
39	0.484	L	1.276	L	1.074	L
40	2.836	L	3.766	L	5.207	L
42	2.566	L	3.586	L	6.336	L
43	-	-	-	-	0.038	NL
44	-	-	0.018	NL	3.266	L
45	-	-	0.924	L	0.920	L
46	0.559	L	1.626	L	0.694	L
47	-	-	0.051	NL	0.081	NL
48	0.159	L	0.096	NL	0.193	L
50	0.010	NL	0.063	NL	0.043	NL
54	-	-	-	-	5.206	L

[a] L = labrusca, NL = non-labrusca flavour character

Determination of TVE

A modification of THOMPSON's method (1950) was used. The reaction of esters with hydroxylamine in aqueous alkaline solution forms hydroxamic acids, which react with the ferric ion to form red ferric hydroxamate complexes.

Table 5: Total volatile esters values and equivalent labrusca/non-labrusca codes for Family 8020 (1986 to 1988)

Seedling Number	Total volatile esters (ppm)					
	1986	Code[a]	1987	Code	1988	Code
1	56	L	66	L	59	L
2	128	L	-	-	-	-
3	-	-	90	L	456	L
4	170	L	28	L	-	-
5	-	-	81	L	197	L
6	233	L	223	L	126	L
7	3	NL	5	NL	9	NL
8	-	-	-	-	20	L
9	-	-	-	-	2	NL
10	212	L	6	NL	162	L
11	-	-	19	L	15	L
12	-	-	22	L	61	L
13	-	-	-	-	266	L
15	92	L	70	L	70	L
16	150	L	141	L	208	L
17	288	L	58	L	-	-
19	-	-	2	NL	3	NL
20	235	L	20	L	42	L
21	5	NL	8	NL	21	L
23	2	NL	2	NL	4	NL
24	-	-	62	L	297	L
25	127	L	32	L	48	L
26	239	L	75	L	134	L
27	-	-	144	L	255	L
28	-	-	-	-	36	L
29	20	L	22	L	21	L
30	3	NL	2	NL	-	-
31	116	L	-	-	-	-
32	89	L	58	L	95	L
33	52	L	51	L	181	L
34	-	-	20	L	166	L
35	45	L	39	L	66	L
37	1	NL	1	NL	1	NL
38	-	-	-	-	3	NL
39	80	L	36	L	72	L
40	4	NL	62	L	186	L
42	1	NL	2	NL	2	NL
43	4	NL	-	-	2	NL
44	-	-	40	L	152	L
45	194	L	154	L	119	L

[a] L = labrusca, NL = non-labrusca flavour character

Fresh alkaline hydroxylamine solution was prepared by mixing equal volumes of 6 M hydroxylamine hydrochloride and 10.5 N sodium hydroxide. 2 ml of this solution and 20 ml of the distillate were added to a 25 ml volumetric flask. These were mixed thoroughly and allowed to stand for 5 min. Then, 1 ml of concentrated hydrochloric acid was added, followed by 1 ml of 1.11 M ferric chloride. The flask was filled to volume with 0.046 M ferric chloride solution and the colour measured on a Zeiss DMR21 spectrophotometer at 500 nm. A standard curve, prepared by

Table 6: Total volatile esters values and equivalent labrusca/non-labrusca codes for Family 8021 (1986 to 1988)

Seedling Number	Total volatile esters (ppm)					
	1986	Code[a]	1987	Code	1988	Code
1	139	L	63	L	38	L
2	6	NL	55	L	2	NL
3	-	-	1	NL	4	NL
4	1	NL	3	NL	3	NL
5	89	L	24	L	59	L
6	-	L	59	L	61	L
8	91	L	56	L	55	L
10	1	L	-	-	4	NL
11	56	L	55	L	79	L
12	120	L	-	-	26	L
13	168	L	15	L	30	L
14	4	NL	1	NL	1	NL
15	183	L	147	L	261	L
17	-	-	56	L	93	L
18	105	L	100	L	58	L
19	1	NL	1	NL	-	-
20	118	L	28	L	100	L
21	108	L	54	L	51	L
23	174	L	82	L	154	L
24	44	L	17	L	25	L
25	1	NL	0	NL	1	NL
27	204	L	95	L	187	L
28	1	NL	1	NL	3	NL
29	2	NL	2	NL	2	NL
30	83	L	40	L	-	-
31	-	-	-	-	3	NL
32	54	L	16	L	128	L
33	165	L	59	L	238	L
34	51	L	12	NL	66	L
35	35	L	15	L	22	L
38	63	L	18	L	52	L
39	175	L	42	L	79	L
40	80	L	72	L	81	L
42	88	L	28	L	35	L
43	-	-	-	-	1	NL
44	-	-	0	NL	33	L
45	-	-	4	NL	23	L
46	184	L	36	L	170	L
47	-	-	0	NL	5	NL
48	56	L	28	L	52	L
50	1	NL	0	NL	2	NL
54	-	-	-	-	73	L

[a] L = labrusca, NL = non-labrusca flavour character

using various concentrations of ethyl acetate, was used to determine the concentration of the TVE in the sample.

Statistical analysis

Chi square analyses were performed on the observed ratios of labrusca/non-labrusca flavour components as they were identified by the MA and TVE analyses.

Results

The seedling families 8020 and 8021 (selfed populations of V. 72181 and V. 72182, respectively) displayed transgressive segregation in both MA and TVE distribution (Tables 3-6). All four distributions were highly skewed to the lower values of MA and TVE. To simplify the data, all MA and TVE values were coded as to labrusca or non-labrusca in flavour contribution, according to the threshold values in Table 2, and presented in Tables 3-6. These threshold values were also used by REYNOLDS *et al.* (1982).

The ratios postulated by the authors using genotypic assumptions of REYNOLDS *et al.* (1982) were tested and are presented in Tables 7-10.

Table 7: Chi square tests for MA segregation of Family 8020 (1986 to 1988)

Year	Predicted[a]		Observed	Statistic		
				X^2	P value	Significance[b]
1986	L/NL	3:1	18:9	0.50	$0.50<p<0.30$	ns
1987	L/NL	3:1	24:8	0.031	$0.90<p<0.70$	ns
1988	L/NL	3:1	25:10	0.08	$0.90<p<0.70$	ns
1986	L/NL	9:7	18:9	0.80	$0.50<p<0.30$	ns
1987	L/NL	9:7	24:8	3.84	$p = 0.05$	**
1988	L/NL	9:7	25:10	2.67	$0.10<p<0.05$	*

[a] L/NL = labrusca/non-labrusca flavour character

[b] significance levels for rejection: p=0.10=*, p=0.05=**, p=0.001=***

Table 8: Chi square tests for MA segregation of Family 8021 (1986 to 1988)

Year	Predicted[a]		Observed	Statistic		
				X^2	P value	Significance[b]
1986	L/NL	3:1	26:7	0.06	$0.90<p<0.70$	ns
1987	L/NL	3:1	28:9	0.013	$0.95<p<0.90$	ns
1988	L/NL	3:1	33:7	0.833	$0.50<p<0.30$	ns
1986	L/NL	9:7	26:7	5.87	$0.05<p<0.01$	**
1987	L/NL	9:7	28:9	4.92	$0.05<p<0.01$	**
1988	L/NL	9:7	33:7	10.15	$0.01<p<0.001$	***

[a] L/NL = labrusca/non-labrusca flavour character

[b] significance levels for rejection: 0=0.10=*, p=0.05=**, p=0.001=***

Discussion

The 3 : 1 segregation ratio for both MA and TVE in both families 8020 and 8021 had the most acceptable Chi square values. This would mean that the V. 72181 and V. 72182 genotype for both MA and TVE must be segregating for only one heterozygous locus each. For MA, if that locus is F, then the ff recessive must allow for some production of MA because no seedlings measured in family 8020 or 8021 had a zero level for MA with any consistency over the three harvest seasons. For it to be a locus other than F would preclude the grandparent Le Général and the great grandparent De Chaunac from having such low levels of MA. Perhaps the REYNOLDS hypothesis (REYNOLDS 1980) of an alternate MA synthesis pathway exists under the ff recessive condition as well as under the aa and/or mm recessive conditions.

The TVE inheritance pattern for both families 8020 and 8021 follows REYNOLDS' hypothesis (REYNOLDS 1980) since there was no condition completely precluding the synthesis of TVE.

Table 9: Chi square tests for TVE segregation of Family 8020 (1986 to 1988)

Year	Predicted[a]		Observed	Statistic		
				X^2	P value	Significance[b]
1986	L/NL	3:1	18:8	0.20	0.70<p<0.50	ns
1987	L/NL	3:1	24:8	0.0	p = 1	ns
1988	L/NL	3:1	27:8	0.013	0.90<p<0.70	ns
1986	L/NL	9:7	18:8	1.32	0.30<p<0.20	ns
1987	L/NL	9:7	24:8	3.84	p = 0.05	**
1988	L/NL	9:7	27:8	5.36	0.05<p<0.01	**

[a] L/NL = labrusca/non-labrusca flavour character

[b] significance levels for rejection: p=0.10=*, p=0.05=**, p=0.001=***

Table 10: Chi square tests for TVE segregation of Family 8021 (1986 to 1988)

Year	Predicted[a]		Observed	Statistic		
				X^2	P value	Significance[b]
1986	L/NL	3:1	24:9	0.009	0.95<p<0.90	ns
1987	L/NL	3:1	25:12	0.73	0.50<p<0.30	ns
1988	L/NL	3:1	28:12	0.31	0.70<p<0.50	ns
1986	L/NL	9:7	24:9	2.96	0.10<p<0.05	*
1987	L/NL	9:7	25:12	1.51	0.30<p<0.20	ns
1988	L/NL	9:7	28:12	2.54	0.20<p<0.10	ns

[a] L/NL = labrusca/non-labrusca flavour character

[b] significance levels for rejection: p=0.10=*, p=0.05=**, p=0.001=***

Acknowledgements

The assistance of R.B. PALABAY with the analytical determinations is gratefully acknowledged.

Literature cited

CASIMIR, D. J.; MOYER, J. C.; MATTICK, L. R.; 1976: Fluorometric determination of methyl anthranilate in Concord grape juice. J. Assoc. Offic. Analyt. Chem. **59**, 269-272.

FISHER, K. H.; 1981: Breeding for wine and table grapes adapted for Ontario conditions. Ann. Rpt. Hort. Res. Inst. Ont. 18-20.

FULEKI, T.; 1982: The Vineland Grape Flavour Index - a new objective method for the accelerated screening of grape seedlings on the basis of flavour character. Vitis **21**, 111-120.

NELSON, R. R.; ACREE, T. T.; LEE, C. Y.; Butts, R. M.; 1977: Methyl anthranilate as an aroma constituent of American wine. J. Food Sci. **42**, 57-59.

REYNOLDS, A. G.; 1980: The Inheritance of Methyl Anthranilate and Total Volatile Esters in *Vitis* spp. M. Sc. Thesis, Univ. of Guelph, Guelph, Ontario, Canada.

--- ; FULEKI, T.; EVANS, W. D.; 1982: Inheritance of methyl anthranilate and total volatile esters in *Vitis* spp. Amer. J. Enol. Viticult. **33**, 14-19.

THOMPSON, A. R.; 1950: A colourimetric method for determining esters. Austral. J. Sci. Res. 3 **A**, 128-135.

Breeding grapevines for tropical environments

J. V. Possingham, P. R. Clingeleffer and G. H. Kerridge

CSIRO Division of Horticulture, GPO Box 350, Adelaide, South Australia 5001

Summary: Grapevines are increasingly grown in the latitudes between the Tropics of Cancer and Capricorn. In many cases environments modified by elevation are utilized to create temperate growing conditions. The majority of tropical grapes are consumed fresh but some are dried (India) and others are made into wine (Brazil, Venezuela). Currently most plantings are of pure *Vitis vinifera* varieties. Early ripening, low acid cultivars such as Cardinal, Perlette, Ribier and Thompson Seedless which have a relatively short cycle between budburst and harvest are commonly used, and pruning is timed to ensure maturation before the onset of heavy tropical rains. Other *V. vinifera* varieties used in the tropics such as Muscat Hamburg, Teneron, Anab-e-Shahi, and Italia have bunch and skin characteristics that give them some resistance to rain damage.

There are a number of grapevine varieties that are hybrids between *V. vinifera* and other *Vitis* species which are currently grown in the tropics. These have some degree of resistance to fungal diseases and include Isabella, Kyoho, Delaware, Himrod, Campbell Early (*V. labrusca* hybrids), the Criolla hybrids (*V. caribaea* hybrids and Villard blanc (a complex French hybrid based on American species).

There is considerable scope to increase the resistance of grapes to the main fungal diseases encountered in the tropics such as downy and powdery mildew, anthracnose and bunch rots by using a range of *Vitis* species as parents. These hybrids should be based on species that do not give strong 'foxy' flavours and could involve complex French hybrids, *V. rotundifolia* and also Asian species such as *V. amurensis* and *V. armata*. CSIRO Merbein has a small hybridisation program aimed at developing new varieties for tropical environments.

Key words: tropics, Australia, Asia, America, Africa, ecology, physiology, cultivation, variety of vine, rootstock, pest, disease, resistance, breeding, genetic engineering.

Introduction

Grape production in the latitudes between the Tropics of Cancer and Capricorn is increasing with the development and expansion of industries in South-East Asia (Australia, South China, India, Indonesia, Philippines, Thailand, Taiwan), Central and South America (Brazil, Colombia, Ecuador, Mexico, Venezuela, West Indies) and Africa (Kenya, Nigeria, Zimbabwe). The majority of tropical grapes are used fresh but some are dried (India) and others made into wine (Brazil, Venezuela). In general the fruit produced is low in sugar and acid, of low quality and used for local consumption. However some countries are now exporting table grapes (e. g. India, Thailand, Venezuela). The wide diversity of environments (climate and soil), varieties, production techniques and major disease and pest problems provides a substantial challenge to breeders. A recent worldwide survey was conducted by CSIRO Division of Horticulture, as part of its commitments to a tropical viticulture working group of the International Society of Horticultural Science, to provide detailed documentation on the many diverse approaches to tropical viticulture and supplement existing limited information. It will provide a more rational basis for approaches to tropical grapevine breeding.

The Division maintains an interest in tropical viticulture because table grape production has developed in tropical regions in northern Australia to provide early ripening fruit, ahead of the temperate zones. The Australian situation is unique because commercial developments, although in their infancy, have occurred in a diverse range of environments, including the three main situations identified in the survey, i. e. in both the wet and dry tropics and at higher elevations to create temperate growing conditions. Material from the CSIRO table grape varietal evaluation and breeding program (Possingham and Clingeleffer 1986) is evaluated in the Australian environments and has been widely distributed to many of the countries producing tropical grapes.

This paper aims to provide an overview of current practices in tropical viticulture, both in Australia and overseas, identify the major problems requiring attention by breeders and discuss relevant aspects of the Division's breeding program.

1. Tropical grape growing in Australia

Commercial plantings of table grapes for the production of early ripening fruit are located in both the dry and wet tropical regions of Australia.

Dry tropics

Plantings in the dry tropics are located in central Australia in the Northern Territory near Tea Tree, lat. 22 °S, in sandy-loam soils and irrigated by trickle irrigation from subterranean basins. The total rainfall of around 270 mm falls mainly in the summer, December-March. Average maximum daily temperatures range from 20 °C in June, July to 36 °C in summer, December-February.

Because frosts occur in winter, a result of continental influences on climate, the vines go dormant in winter. They are pruned in June-July and managed similar to those in more temperate regions on sloping T-trellis (Clingeleffer 1985) to produce only one crop per year. Winter chilling is insufficient to fully break the dormancy and hydrogen cyanamide (Dormex) at a rate of 2-3 % is used to both advance maturity (McColl 1986) and promote uniform budburst. Varieties grown for early ripening in November are Beauty Seedless, Perlette, Fresno Seedless (Possingham *et al.* 1989), Flame Seedless and Cardinal.

Sultana is also grown but due to later ripening in December may be damaged by monsoonal rains. All varieties except the less fruitful Sultana are spur pruned. Gibberellic acid (GA) is used for bunch elongation, berry thinning and to enhance berry size of the seedless varieties but the response is lower than in more temperate regions, possibly due to the higher temperatures. Fungal diseases have not been a problem, although control measures are required for oidium *(Uncinula necator).* The major pest is the giant termite, *Mastotermes dariwiniensis* which can completely destroy vines.

CSIRO Division of Horticulture in collaboration with the Department of Primary Industry in the Northern Territory have established rootstock trials with about 20 genotypes of common nematode and phylloxera resistant rootstocks to evaluate those most suited to the very hot, dry arid environments.

Wet tropics

Commercial plantings in the wet tropics extend from as far north as Kununurra, lat. 16 °S in western Australia and Mareeba, 17 °S, Townsville, 15 °S, Charters Towers 20 °S and Rockhampton, 23 °S. Rainfall at all locations follows a distinct seasonal, wet-dry pattern with annual totals ranging from 665 mm in Charters Towers to 810 mm in Rockhampton. Soil types, even for one location may vary from sands to heavy clays.

Highland production

The high elevations at Mareeba (840 m) and Charters Towers (600 m) in Queensland provide temperature conditions with winter frosts. The growth pattern of the vines is similar to that described for the dry tropics and the vines are managed as temperate plantings. Muscat Hamburg is the dominant variety followed by Cardinal. Italia, Muscat Gordo blanco, Ribier and the disease resistant Isabella, a *V. labrusca* hybrid are also grown at Mareeba and Royal Ascot in Charters Towers.

Lowland production

Kununurra is the hottest and driest of these locations with daily maximum temperature exceeding 30 °C in all seasons and relative humidities of less than 50 % for the drier part of the year. Plantings are mainly experimental and include Flame Seedless, the Indian variety Anab-e-Shahi, the disease resistant species complexes Carolina Blackrose and Muscat St. Vallier and the Central American selection, Criolla negra.

Plantings at Rockhampton are dominated by Muscat Hamburg and Cardinal with some Italia and Early Muscat. In this locality, 23 °S on the tropic of Capricorn, frosts occur in winter and the vines are managed similar to in more temperate regions. By contrast, significant plantings at Townsville are in a frost free zone at low altitude. 730 mm of the annual rainfall of 873 mm falls between October and March, daily maximum temperatures, except for the peak of summer, range between 25 and 30 °C and the relative humidity between 50 and 75 % in the drier periods. These conditions produce continuous, rapid and vigorous shoot growth and the vines, which lack a period of winter chilling, remain evergreen without a period of dormancy. Twice yearly pruning techniques based on the Indian system of renewal pruning are used (Clingeleffer 1987). Longer fruiting canes of 6-8 nodes are retained in May-June to produce a crop in September-November and then pruned to short, unfruitful spurs for the development of renewal wood. The main varieties grown are Cardinal and Muscat Hamburg, but promising results have also been achieved with Sultana and Marroo Seedless, a large black berried variety, bred by CSIRO (Clingeleffer and Possingham 1988). Flame Seedless has not been productive. The vines are trained on sloping T-trellis and the pruning staggered so that the Cardinal, which has proved to be sensitive to rain, crops in September and Muscat Hamburg, which is rain tolerant, is harvested last in November. Hydrogen cyanamide is used to advance maturity and promote uniform budburst. The main fungal problems are anthracnose or black spot *(Elsinoe ampelina)*, downy mildew *(Plasmopara viticola)*, oidium or powdery mildew *(Uncinula necator)* and various bunch rots, in particular *Botrytis cinerea*. The major pests are mealy bug *(Pseudococcus adonidum)* and bunch mites *(Brevipalpus lewisi)*. Although nematodes have not been a problem, rootstocks, including the nematode resistant Ramsey *(V. champinii)* and the more common phylloxera resistant rootstocks, selected in Europe for wetter conditions e. g. SO 4 and 5 BB *(V. berlandieri x V. riparia)* are currently being evaluated in collaboration with CSIRO. Plantings on Ramsey have been excessively vigorous and difficult to manage.

2. Tropical grape growing around the world

Environment

Commercial grape production has expanded in the tropics in an extremely diverse range of environments. In South-East Asian countries (i. e. Indonesia, Philippines, Taiwan and Thailand) the main areas of production are frost free zones, at sea level in the wet monsoonal tropics which feature constant high temperatures around 30 °C, high relative humidity, greater than 75 %, and an annual rainfall of around 1,500 mm. Slightly drier, less humid conditions are found at elevated sites (600-900 m) in the main centres of production in South India near Bangalore (917 mm annual rainfall) and Maharashtra State in Central India, west of Bombay (700 mm annual rainfall). Elevated sites in Central America (Ecuador, Venezuela, Columbia) also have been selected to moderate climatic influences, the extreme case being in Ecuador where grapes are grown both at sea level in a frost free, warm arid environment and at 2,348 m in wetter (1,049 mm annual rainfall), humid but cooler conditions with an extended period of frost. The most arid tropical grape area is found in Mexico with an annual rainfall of less than 45 mm and extensive periods of frost.

Physiology

Production in the frost free, wetter regions provides a marked contrast to warm temperature viticulture. The constant high temperatures, high relative humidities and high rainfall (600-1,500 mm) produce rapid and vigorous shoot growth and amplify fungal and disease problems. The vines lack a period of winter chilling and remain evergreen without a period of dormancy. A number of crops may be produced in one year as the rapid rate of growth gives a very short growth cycle (e. g. Cardinal in the Philippines and Venezuela can produce 3 crops p. a.). It is not uncommon for vineyards to have vines at all stages of growth with production on a continual basis throughout the year. Time of pruning and pruning techniques are adjusted in some countries to minimise production during high rainfall periods (i. e. monsoonal wet seasons) when it is difficult to control pest and diseases and also to meet market demands. Tropical grape vines exhibit a high degree of apical dominance (CHADHA 1984) attributed to the lack of winter chilling considered necessary in warm temperate climates for high and uniform budburst. Special pruning techniques (see below) are required to overcome this problem. Commercial attempts to control apical dominance by leaf removal prior to pruning or restricting irrigation after harvest to induce dormancy have been unsuccessful. The use of hydrogen cyanamide to promote a more uniform budburst is currently being tested.

Production constraints include a short vine life (e. g. 7-10 years in Thailand), low bud fruitfulness and poor maturity (i. e. lignification) of replacement wood. The short vine life is thought to be due to the long term depletion of carbohydrates caused by the rapid and continuous shoot growth and production of more than one crop p. a. (OLMO 1970). Depletion of nutritional resources, nematodes and copper toxicity from fungicides are also given as reasons for the short vine life. Low bud fruitfulness can be attributed to excessive shade associated with the overhead pergola trellis system and the rapid and excessive shoot growth (CHADHA 1984). The poor maturation of replacement wood may also be associated with low carbohydrate levels (OLMO 1970), excessive shade, continuous growth and cropping, the lack of a dormant period and pruning soon after crop removal.

Management

Tropical vineyards, with few exceptions are low density plantings trained on overhead pergolas. This system contributes to excess vegetative growth, poor light interception, poor cane quality and poor colour development in berries (CHADHA 1984). To achieve acceptable levels of production, high bud numbers are retained on cane pruned vines. Cane pruning is used because basal nodes are unfruitful. Retention of high bud numbers gives sufficient fruitful shoots and overcomes problems associated with apical dominance and non-uniform bud burst as it is usual for only the terminal nodes to burst.

In most situations, the canes are about 6-10 buds in length. In India they are selected from renewal spurs or water shoots on head pruned vines (OLMO 1970), in Venezuela from established cordons (ROJNIC *et al.* 1972), and in Thailand from the terminal shoots of last seasons fruiting canes. In the Philippine island of Cebu the vines are planted in rows and trained on wide T-trellis with a central cordon along the rows. Canes from 2-bud renewal spurs are placed in a horizontal position at right angles to the direction of the row on both sides of the cordon, similar to the swing-arm trellis proposed for Sultana (CLINGELEFFER and MAY 1981).

The time of pruning is adjusted to stagger the supply, meet market demands and to avoid unfavourable climatic conditions during shoot and fruit development (CHADHA 1984). In India, renewal pruning, where the vines are pruned to unfruitful short spurs, is used to avoid cropping during the wet season (OLMO 1970). This practice also favours the maintenance of the vine shape and improves 'cane quality'.

To improve fruit set, the shoots may be tipped just prior to, or at flowering. Standard bunch trimming and thinning (i. e. to one bunch per shoot) is used to improve berry size and bunch

appearance. Leaf and lateral removal to develop an open canopy is also routinely used. Judicious use of supplementary irrigation and fertilizer, both inorganic and organic, is used to control the vigour on infertile soils. In Thailand the vines are planted in high mounds. The ditches between these mounds are used for the supply of supplementary irrigation and for drainage (PUNSRI and SUKUMALANDANA 1977).

Varieties

Contrary to popular opinion most plantings are of pure *V. vinifera* varieties. Some have bunch and skin characteristics that give them resistance to rain damage (e. g. Muscat Hamburg, Teneron, Anab-e-Shahi, Italia). Early ripening, low acid cultivars which have a relatively short cycle between budburst and harvest are commonly used to produce a number of crops per year or to ensure maturation before the onset of heavy tropical rains (e. g. Cardinal, Ribier, Perlette, Thompson Seedless, syn. Sultana). Hybrids between *V. vinifera* and other *Vitis* species are also grown. These have some degree of resistance to fungal disease and include *V. labrusca* hybrids (American hybrids), complex species hybrids (French hybrids) and *V. caribaea* hybrids (Criolla hybrids).

The main varieties grown in South-East Asia are Ribier (2-3 crops p. a.) in Indonesia, Cardinal (3 crops p. a.) in the Philippines and White Malaga (Teneron, 2 crops p. a.) and Cardinal (3 crops p. a.) in Thailand. The main varieties which produce 2 crops p. a., grown in Taiwan, are the *V. labrusca* hybrids Kyoho, Golden Muscat and Niagara and Cardinal, Black Queen and Italia. In South China Kyoho and Campbell Early, both *V. labrusca* hybrids are grown.

A diverse range of varieties producing 2 crops p. a. are grown in Bangalore, India. The most important are Sultana, Anab-e-Shahi and the *V. labrusca* hybrid Isabella (syn. Bangalore Blue) with smaller plantings of Perlette, Muscat Hamburg, Beauty Seedless, the local Indian selections Bhokri, Cheema Sahebi, Kali Sahebi and the *V. labrusca* hybrid, Himrod. In the more northern Indian regions only 1 crop p. a. is produced. Sultana, used for both table and drying purposes, is an important variety but Anab-e-Shahi, Ribier and Cheema Sahibi are also grown.

Varieties grown in the wetter frost free, low altitudes of Venezuela are Cardinal (3 crops p. a.), Italia and Isabella (both 2 crops p. a.) while at the more temperate, elevated sites common table grapes are grown, i. e. Ribier, Italia, Cardinal, Waltham Cross, Muscat Hamburg, Golden Champion, Emperor and Gros Colman (ROJNIC *et al.* 1972). The common varieties grown in Colombia are Ribier, Gros Colman, Italia and Isabella. Varieties grown at low altitudes to produce 2 crops p. a. in Ecuador are the *V. labrusca* hybrids Isabella and Niagara while Ribier is the most important variety grown at elevated sites. Varieties grown in Mexico are the standard table grape varieties from California.

Rootstocks

Rootstocks are not widely used in the tropics although nematodes are widely reported to affect productivity. Phylloxera is also reported in Mexico and Ecuador. CHADHA (1984) states that rootstocks will be necessary in India to overcome nematode problems, saline soils, low vine vigour and fruitfulness. In Thailand 1613 (Solonis x Othello) is used to some extent. The reported use of rootstocks in Ecuador includes local Criolla hybrids *(V. caribaea)*, Rupestris du Lot *(V. rupestris)* for phylloxera and SO4 *(V. berlandieri x V. riparia)* and Richter *(V. berlandieri x V. rupestris)* for nematodes. Similarly, in Venezuela Criolla negra, 1103 Paulsen *(V. berlandieri x V. rupestris)* and 5 BB *(V. berlandieri x V. riparia)* are used for nematodes.

Pests and diseases

Copious quantities of fungicides are required for production of grapes in tropical environments. The *V. vinifera* varieties are susceptible to a large range of fungal diseases, a situation exacerbated by the tropical climate which favours microbial development. The fungal

diseases anthracnose or black spot *(Elsinoe ampelina)*, downy mildew *(Plasmopara viticola)*, powdery mildew *(Uncinula necator)* and bunch rots, in particular *Botrytis cinerea* and miscellaneous secondary infections of damaged fruit are the major pests. Dead arm is reported as a problem in a number of countries although it is unclear whether it is *Phomopsis viticola* or dying arm *(Eutypa armeniacae)*. Unspecified rusts are reported as a problem in Asian countries.

Insect pests thrive under tropical conditions. The most serious problems are mealy bug (*Pseudococcus* spp.) and a range of mites including the bunch mites (*Brevipalpus* spp.) and *Eriophyes* spp. A diverse range of unspecified pests are also reported as problems. These include fruit piercing and sucking flies, moths, wasps, leaf hoppers and leaf skeletonizers, possibly thrips and aphids, stemboring beetles and American onion worm.

3. Breeding for tropical environments

Varietal information in the preceding sections indicates that tropical grape production has a narrow genetic base, limited to a narrow range of *V. vinifera* varieties and a few hybrid varieties grown for disease resistance. These include the *V. labrusca* hybrids Isabella, Delaware, Campbell Early, Kyoho, the complex French hybrids of American species, Villard blanc and Niagara and the *V. caribaea*, Criolla hybrids. More effort in vine breeding and varietal evaluation is required to fully exploit grapevine variability and develop varieties more suited to tropical environments. While the major benefits are likely to accrue from improved disease resistance, other areas of vine improvement must not be overlooked.

Physiological problems of poor budburst and apical dominance may be reduced by selection of varieties with a low chilling requirement, similar to stone fruit, or indirectly by the selection of varieties which are fruitful in basal nodes and suited to spur pruning. Selection of varieties which are more fruitful and develop larger bunches under tropical conditions which favour rapid shoot growth may improve productivity. The very high yields that are achieved with Anab-e-Shahi in India indicate that productivity should not be a limiting factor in tropical grape production. Fruit quality may be improved by selection of varieties with higher levels of acid and which mature to adequate sugar levels and have good fruit colour.

Priority should also be given to the development and selection of rootstocks for tropical conditions as they have the potential to regulate excessive vine vigour, reduce problems associated with soil type and soil born pests and improve vine longevity. For sandier soil types, in particular in drier regions, the nematode resistant rootstocks currently used for table grape production in Australia (Possingham *et al.* 1989) should be included in such a breeding and selection program, e. g. Ramsey *(V. champini)* or the lower vigour Teleki 5 A *(V. berlandieri x V. riparia)* and Schwarzmann *(V. rupestris x V. riparia)*. The drought tolerant types, e. g. 110 R and 1103 P (both *V. berlandieri x V. rupestris*) warrant study. Phylloxera resistant rootstocks selected for wetter areas in Europe, i. e. SO 4 and 5 BB (both *V. berlandieri x V. riparia*) should be included. The new selections of *V. rotundifolia* rootstocks developed by H. P. Olmo, University of California, Davis, which have some resistance to *Xiphinema index* nematode, have some potential to reduce fanleaf problems where it occurs and should also be tested. CSIRO has a rootstock breeding program which aims to develop multi-species complexes suited to a range of soil types incorporating genes for nematode resistance from *V. champini*, phylloxera resistance from *V. berlandieri*, *V. rupestris* and *V. riparia*, drought resistance, *V. rupestris* and tolerance of wetter conditions, *V. riparia*. Other species distributed in more tropical regions have also been included, i. e. *V. aestivalis*, *V. cinerea*, *V. caribaea*, *V. longii* and *V. rotundifolia*.

The major fungal disease problems, anthracnose, oidium, downy mildew and bunch rots found in the tropics have their origins in the American continent. Although there are varying degrees of susceptibility between various varieties of *V. vinifera*, which originate from the Central Asian/European regions, there appears to be little opportunity to breed disease resistant varieties without broadening the genetic base. Vine breeders at the turn of the century, both in Europe and the USA, who aimed to develop direct producers that were tolerant of phylloxera were also able to

incorporate genes for disease resistance from various American species. These selections are commonly referred to as American hybrids, i. e. hyrids involving mainly *V. vinifera* and *V. labrusca* or French hybrids based on many American species, e. g. Villard blanc is an 8 species complex. Although some of these hybrids or their progeny are currently grown for disease resistance in the tropics for table grapes (e. g. Isabella, Kyoho, Campbell Early) or wine production (e. g. Villard blanc) their fruit characteristics are less than desirable as *V. labrusca* hybrids generally have a 'foxy' flavour. There is considerable scope to increase the resistance of grapes to diseases encountered in the tropics and to reduce the heavy reliance on chemical protection. Breeding programs now include routine screening for disease resistance by dual culture *in vitro* e. g. for downy mildew (Stein *et al.* 1985; Barlass *et al.* 1986) or oidium (Stein *et al.* 1985). Detailed information can now be accessed by breeders to identify species and species combinations likely to be resistant to the various diseases. For example Doster and Schnathorst (1985) were able to show that cultivars of *V. labrusca* and hybrids between *V. vinifera* and *V. rupestris* were resistant to oidium. Most *V. vinifera* cultivars tested were not resistant. We also know that *V. labrusca* cultivars grown in the tropics are more resistant to anthracnose than *V. vinifera*. The Chinese have been particularly successful in the development of quality table grape varieties from hybrids of *V. labrusca* and *V. vinifera* (i. e. Fortuna 12, 14, 17, 18 and Phoenix 51). In the CSIRO breeding program, genes for resistance to downy mildew have been incorporated from complex hybrids. For example, Marroo Seedless, a cross of Carolina Blackrose and Ruby Seedless is resistant to downy mildew, even under wet tropical conditions in Townsville, a characteristic derived from its disease resistant breeding line which dates back to Villard blanc. Marroo Seedless is, however, susceptible to oidium. There is further potential to involve in breeding the Muscadine grape, *V. rotundifolia*, reported to be resistant to most diseases and also Asian species such as *V. amurensis* and *V. armata*.

There have been a number of new varieties recently released from breeding programs in the USA, reported to have disease resistant and acceptable fruit quality when grown in warm, humid environments in Florida, Arkansas and New York State. These varieties warrant testing in their own right and as parents in the tropics as they are resistant to anthracnose, oidium and powdery mildew and various bunch rots. They include the black *V. labrusca*, *V. vinifera* seedless selections, Reliance Seedless (Moore 1983), Mars Seedless (Moore 1985), and Einset Seedless (Reisch *et al.* 1986), very complex species hybrids involving *V. labrusca*, French hybrids and *V. aestivalis*, a native of Florida, Suwannee and Conquistador (Mortensen 1983), Orlando Seedless (Mortensen and Gray 1987) and Remaily Seedless (Pool *et al.* 1981).

CSIRO Merbein has a hybridization program aimed at developing new varieties for tropical environments. A diverse range of genotypes with disease resistance, e. g. *V. rotundifolia* (DRX55), complex species hybrids such as Chambourcin, Muscat St. Vallier, Villard blanc, Aurelia, Carolina Blackrose, Lady Patricia, Illinois 271-1, S14664, SV 12-309, SV 12-303 and *V. labrusca* hybrids such as Isabella, Glenora, Mantey and Suffolk Red have been used in crosses with *V. vinifera* table grapes e. g. Muscat Hamburg. In addition to the named variety Marroo Seedless (Clingeleffer and Possingham 1988), a cross of Carolina Blackrose and Ruby Seedless, promising selections being evaluated include progeny from DRX55 x Aurelia, Carolina Blackrose x Flame Seedless, Kishmishi (a misnamed large, red berried seeded variety) x Lady Patricia, Bicane x Villard blanc, Muscat Hamburg x Lady Patricia, Blackrose x Illinois 271-1. In some cases 2nd and 3rd generation crosses have produced very complex and involved breeding lines. As well *in-ovulo* embryo culture from seedless x seedless genotypes (Barlass *et al.* 1988) has been used to develop new breeding lines from material in the CSIRO germplasm collection or pollen imported from the USA.

4. Plant transformation

In addition to the conventional breeding strategies for tropical grapevine improvement, the emerging technologies of direct plant transformation must also be considered. The technology is

already established for many herbaceous annuals and will doubtless be applied to woody perennials. Research within the CSIRO Division of Horticulture has been used to develop techniques for grapevine transformation and an experimental gene has been successfully introduced. The task lies ahead to introduce genes of economic importance. Of particular interest in relation to grapevine transformation is protection from fungal disease. Currently attention is being given to the response of plants to pathogenic infections. Many plants respond by synthesizing pathogenic response proteins (PR) which include hydrolytic enzymes to defend against pathogenic attack. Two such enzymes are chitinase which degrades chitin, the major cell wall component of oidium and β-glucanase which degrades β-glucans, constituents of cell walls of downy mildew hyphae.

In contrast to conventional breeding, where progeny are highly heterozygous and include varietal characteristics from both parents, a better approach might be to provide a selected variety with a gene for chitinase or β-glucanase or both, by plant transformation methodology. The aim would be to lyse the hyphae of invading fungal diseases. This would allow the conventional breeder to concentrate on the many pleiotropic (multigenic) gene characters which are not suited to these new methodologies, i. e. yield, flavours etc.

References

BARLASS, M.; MILLER, R. M.; ANTCLIFF, A. J.; 1986: Development of methods for screening grapevines for resistance to infection by downy mildew. I. Dual culture *in vitro*. Amer. J. Enol. Viticult. **27**, 61-66.

-- -- ; RAMMING, D. W.; DAVIS, H. P.; 1988: In-ovulo embryo culture: a breeding technique to rescue seedless x seedless table grape crosses. Austral. Grapegrower Winemaker, No. 272, 123-125.

CHADHA, K. L.; 1984: Grape research in India - priorities and suggested approach for the future. Indian J. Hort. **41**, 145-159.

CLINGELEFFER, P. R.; 1985: Specialised trellis design for table grape production. Proc. The Production, Promotion and Marketing of Western Australian Table Grapes, September, 1985, 68-71. Rural and Allied Industries Council, Perth, W. A.

-- -- ; 1987: Production of grapes in tropical areas. Proc. 2nd Natl. Table Grape Tech. Conf., July 1986, 46-49. Stanthorpe, Qld.

-- -- ; MAY, P.; 1981: The swing-arm trellis for Sultana grapevine management. S. Afr. J. Enol. Viticult. **2**, 37-44.

-- -- ; POSSINGHAM, J. V.; 1988: Marroo Seedless: A new table grape variety. Agricult. Sci. (Austral.) 1 (2), 18-19.

DOSTER, M. A.; SCHNATHORST, W. C.; 1985: Comparative susceptibility of various grapevine cultivars to the powdery mildew fungus, *Uncinula necator*. Amer. J. Enol. Viticult. **36**, 101-104.

MCCOLL, C. R.; 1986: Cyanamide advances the maturity of table grapes in central Australia. Austral. J. Exp. Agricult. **26**, 505-509.

MOORE, J. N.; 1983: 'Reliance' Seedless Grape. HortScience **18**, 963-964.

-- -- ; 1985: 'Mars' Seedless Grape. HortScience **20**, 313.

MORTENSEN, J. A.; 1983: 'Suwannee' and 'Conquistador' grapes. HortScience **18**, 767-769.

-- -- ; GRAY, D. J.; 1987: 'Orlando Seedless' grape. HortScience **22**, 327-328.

OLMO, H. P.; 1970: Report to the Government of India. FAO/UNDP Report No. TA 2825. FAO, Rome.

POOL, R. M.; REMAILY, G. W.; REISCH, B. I.; WATSON, J. P.; KIMBALL, J. H.; 1981: 'Remaily Seedless' grape. HortScience **16**, 232-233.

POSSINGHAM, J. V.; CLINGELEFFER, P. R.; 1986: Selection and breeding of table grapes for Australia. Proc. 19th Intern. Viticultural and Oenological Congr. 1986, Santiago Chile, 808-819.

-- -- ; -- -- ; KERRIDGE, G. H.; 1989: Developments in the Australian table grape industry. Proc. 20th Intern. Viticultural and Oenological Congr. (in press).

PUNSRI, P.; SUKUMALANDANA, C.; 1977: Grape growing in Thailand. In: CHADHA, K. L.; RANDHAWA, G. S.; PAL, R. N. (Eds.): Viticulture in Tropics, 24-31. Horticultural Society of India, Bangalore.

REISCH, B. I.; REMAILY, G. W.; POOL, R. M.; WATSON, J. P.; 1986: 'Einset Seedless' grape. HortScience **21**, 155-156.

ROJNIC, I.; ROMERO, T.; SALAS, A.; 1972: Manual Practico para el Cultivo de la Vid en Estado Zulia. Fondo de Desarrollo Fruiticola - Fondefru. Caracas, Venezuela.

STEIN, U.; HEINTZ, C.; BLAICH, R.; 1985: The in-vitro testing of grapevine cultivars for oidium and plasmopara resistance. Z. Pflanzenkrankh. Pflanzensch. **92**, 355-359.

Study of phenotypic variation by analysing data gathered together by O.I.V. on new varieties of table grapes

R. WAGNER

INRA Station de Recherches Viticoles, B.P. 13, F-34750 Villeneuve-lès-Maguelonne, France

Summary: If we consider progenies of seedless, muscat or early cultivar (SME cv.), they may be prone to show some differences in their distribution for phenologic or cultural characteristics, when compared to more common varieties (control cv.). Data gathered together by O.I.V. on 190 new table grape cultivars originating from 37 different breeding stations was used to test this hypothesis. Only the most objective characteristics (for instance: berry weight) were chosen, so as to reduce observer's influence on cv. descriptions. Differences between SME and control cvs could be shown in mean, variance and/or distribution analysis for some characteristics. In comparison, no significant change appeared for the same features between black versus white cvs. These provisional results have to be confirmed by experimental studies, but the tendencies shown may still be of interest in breeding work.

Key words: table grape, variety of vine, variation, ecology, phenotype, seedlessness, muscat flavour, early maturity, biometry, analysis, breeding.

Introduction

Breeding is only possible if some variation exists in the biological material to be improved. To maintain variability, every breeder is concerned about genetic resources.

If we are interested in seedless, muscat or early cvs, the question is: is the variability potential of progenies of these cvs equal to that of progenies from more common cvs? The problem is particularly important with seedlessness. Since most of these cvs are related to Sultanina, these progenies may be more inbred than other, non-seedless progenies. This situation may also be found, but to a lesser degree, with characters such as muscat or earliness. It is well known that inbreeding implies a risk of loss of variability, but also the possibility of reduced fitness.

The study published by the Office International de la Vigne et du Vin on new varieties of table grapes (WAGNER and TRUEL 1988) gave us the opportunity to see if differences can be detected, at least for some characters, among these groups of cvs.

Materials and methods

Plant material

Data used in this study were collected from all known grape breeders. O.I.V. asked them to fill out two types of forms:

- one for each new variety registered: information on cultural characters,
- one for the location where these varieties had been bred: information on climatic and training system variables.

Characters selected for the study

Only the most objective characteristics described were chosen to reduce observer bias on cvs descriptions.

Experimental design

37 stations belonging to 19 countries, situated in very different climatic areas (ranging from Canada and USSR to Israel) gave information on 190 cvs. As every station often contributed to

Table 1: Regression between cultural variables (x1-x6) and climatic variables: average temperature and water-supply for the 72 to 139 cvs observed. t values for null hypothesis and significance

independant variables / dependant variables	temperature	water supply
x1 = time of bud bursting (OIV d.n.)	2.23 *	0.49 NS
x2 = time of fruit maturity (OIV d.n.)	3.42 ***	0.67 NS
x3 = cluster weight (OIV d.n.)	5.71 ***	0.32 NS
x4 = berry weight (OIV d.n.)	5.26 ***	0.27 NS
x5 = number of seeds per berry	1.64 NS	1.45 NS
x6 = weight of 100 seeds (g)	0.59 NS	2.36 *

*,**,***,NS : significant at the 5%, 1%, or 0.1% levels, respectively.

OIV d.n. = OIV descriptor numbers for the different variables.

every group of cvs, differences due to soil, climate or training systems may be minimal when making comparisons between groups of cvs. This hypothesis can be only valid if enough stations are considered. As climatic variables were available for most stations, a control of the validity of this hypothesis was possible: if the assumption made is true, then temperature and water availability averages from relevant groups of cvs are expected not to be significant. If they are, then differences among groups may originate both from cvs differences and unbalanced location distribution of these cvs. In the latter case it is not easy to determine which factor is the most important.

Soil differences could not be controlled. Also considered was whether growth regulators and ringing were used; all places (and cvs) submitted to such cultural practices were discarded from the study. For this reason, seedless cvs could not generally be analysed, or when they were, this condition could not be applied, so that the results must be considered with this restriction.

Table 2: Comparison between seedless and seeded cvs, muscat and neutral cvs, early and non-early cvs, black and white cvs. The only characters listed in the table are those which are significantly different for their means, their variances or their distributions

statistics	comparision between :			
	SDL/SDD cvs	MUSCAT/ NEUTRAL cvs	EARLY/NON- EARLY cvs	BLACK/ WHITE cv
mean	-	cluster wght	-	-
	berry wght	berry wght	berry wght	-
variance	-	cluster wght	-	-
	-	berry wght	-	-
	-	-	nb s./b.	-
distri-	-	cluster wght	-	-
bution	berry wght	berry wght	berry wght	-

cluster wght = cluster weight (x3)

berry wght = berry weight (x4)

nb s./b. = number of seeds per berry (x5)

As black versus white cvs showed no differences with either climatic variables or with cultural characters, only random differences were used to evaluate differences which were found among other groups of cvs with a completely nested design (random effect model).

Results

1. Test of the hypothesis: no differences in climate variables between groups of cvs

Temperature and water supply showed no differences between:

- black and white cvs,
- early versus non early cvs.

Between muscat and neutral cvs, no differences were found for water supply, but a highly significant difference appeared for temperature:

- the average temperature for locations where muscat cvs were bred and observed was 12.04 °C,
- to compare to: 13.48 °C for neutral cvs.

The same result was found for

- seedless, 14.97 °C and
- seeded cvs, 12.95 °C,

which was not unexpected, as seedless cvs are mostly grown and bred in hot countries.

Table 3: Means of different variables for black and white cultivars. Only seeded cvs have been considered

variables	WHITE cvs	BLACK cvs	F value
x1 = time of bud bursting (OIV d.n.)	4.84	4.63	0.58 NS
x2 = time of fruit maturity (OIV d.n.)	4.52	3.84	3.10 NS
x3 = cluster weight (OIV d.n.)	4.62	4.39	0.91 NS
x4 = berry weight (OIV d.n.)	5.44	5.31	0.30 NS
x5 = number of seeds per berry	2.14	2.17	0.07 NS
x6 = weight of 100 seeds (g)	4.60	4.31	1.34 NS

Significance levels, see table 1.

OIV d.n. = OIV descriptor numbers for the different variables.

2. Regression between cultural and climatic variable (Table 1)

In general, only regression with temperature gave significant positive coefficients, with the exception of x5 and x6. All cultural variables were independant of water supply, only x6 (weight of 100 seeds) was positively correlated with water supply.

3. Comparisons made for cultural variables between

- seedless (SDL) and seeded (SDD) cvs,
- muscat and neutral cvs (among only SDD cvs),
- early and non-early maturing cvs (among only SDD cvs),
- black and white cvs (among only SDD).

In general, only cluster weight and/or berry weight are found significantly different for their means (or variances, or distributions) between the groups of cvs compared (Table 2).

Black and white cvs have similar means (Table 3), variances and distributions for all the six characters considered.

Table 4: Means of different variables for early and non-early cvs. Only seeded cvs have been considered

variables	EARLY cvs	NON-EARLY cvs	F value
x3 = cluster weight (OIV d.n.)	4.29	4.57	1.03 NS
x4 = berry weight (OIV d.n.)	4.70	5.63	14.66 **
x5 = number of seeds per berry	2.10	2.18	0.47 NS
x6 = weight of 100 seeds (g)	4.58	4.36	0.66 NS

Significance levels, see table 1.

OIV d.n. = OIV descriptor numbers for the different variables.

Early cvs have smaller berries than more later ones (Table 4) and their seed number per berry seems to be less variable:

s5=3.15, standard deviation for early cvs,

to compare with

s5 = 5.57 for non-early cvs.

Between muscat and neutral cvs two characters are different: cluster weight and berry weight. In both comparisons neutral cvs show greater means (Table 5) and variances.

Only mean and distribution in berry weight are different between SDD/SDL cvs. Mean berry weight found for SDL cvs is

$\overline{x4} = 3.94$

to compare with

$\overline{x4} = 5.37$, for SDD cv.
(F value = 22.76 ***).

4. Correlations between cultural variables

Table 6 gives simultaneously three different correlation matrices; each of the first three lines corresponds to the first line of a correlation matrix, respectively for:

- muscat cvs
- black-and-neutral cvs
- white-and-neutral cvs.

As before, only SDD cvs were considered. These three groups of cvs gave significant correlation coefficients between x1 and x2, x1 and x4, x3 and x4.

Table 5: Means of different variables for muscat and neutral cultivars. Only seeded cvs have been considered

variables	MUSCAT cvs	NEUTRAL cvs	F value
x1 = time of bud bursting (OIV d.n.)	4.47	4.89	2.35 NS
x2 = time of fruit maturity (OIV d.n.)	4.04	4.18	0.14 NS
x3 = cluster weight (OIV d.n.)	4.14	4.72	6.05 **
x4 = berry weight (OIV d.n.)	5.05	5.58	5.09 *
x5 = number of seeds per berry	2.03	2.22	2.92 NS
x6 = weight of 100 seeds (g)	4.49	4.39	0.14 NS

Significance levels, see table 1.

OIV d.n. = OIV descriptor numbers for the different variables.

- Correlation between x1 and x2: This relation holds also within seedless (SDL) cvs. The value of the correlation coefficient is high:
  $r = +0.62$ ($P > 0.001$),
  when calculated on all seeded (SDD) cvs.
- Correlation between x1 and x4: Early bud burst is correlated with small berry weight. The value of the correlation coefficient is lower than the former:
  $r = +0.48$ ($P > 0.001$),
  when calculated for all SDD cvs.
- Correlation between x3 and x4: Cluster weight and berry weight are highly correlated:
  $r = +0.64$ ($P > 0.001$),
  when calculated on all SDD cvs.
- As the other correlation coefficients of these matrices are generally just at the edge of significance ($P = 0.05$), it seems safer, provisionnally, to doubt their reality.

Table 6: Correlation matrices for six characteristics (x1-x6) and three groups of seeded varieties: muscat cvs (MUS.cvs), black-and-neutral cvs (BL.cvs), white-and-neutral cvs (WH.cvs). The names of cvs groups are put at the place where significant correlation coefficients should figure . — = non-significant correlation coefficients

variables	x1	x2	x3	x4	x5	x6
variables						
x1 = time of	1.00	MUS.cvs	-	MUS.cvs	-	-
bud bursting	1.00	BL.cvs	-	BL.cvs	-	-
(OIV d.n.)	1.00	WH.cvs	WH.cvs	WH.cvs	-	WH.cvs
x2 = time of		1.00	-	-	-	MUS.vcs
fruit maturity		1.00	-	-	-	-
(OIV d.n.)		1.00	WH.cvs	WH.cvs	-	-
x3 = cluster-			1.00	MUS.cvs	-	-
weight			1.00	BL.cvs	-	-
(OIV d.n.)			1.00	WH.cvs	-	-
x4 = berry-				1.00	-	-
weight				1.00	-	BL.cvs
(OIV d.n.)				1.00	-	-
x5 = number of					1.00	-
seeds per berry					1.00	-
					1.00	-
x6 = weight						1.00
of 100 seeds						1.00
(g)						1.00

OIV d.n. = OIV descriptor numbers for the different variables.

Discussion and conclusion

There are not many studies on grape phenotypic variability. Generally, data considered is taken either from ampelographic collections (Huglin 1958; Benin *et al.* 1985) or from seedling collections during the process of grape breeding (Fanizza 1979; Lefort and Bronner 1981; Calo *et al.* 1987). In both cases, the plant material considered is situated at the same place. In this study, data were collected from all over the table grape growing area. These results may obviously appear as more general. In fact, they must be considered only as heuristic results. They may give some interesting indications, if the hypothesis of good balance of climatic variables can be accepted. This must be kept in mind in the following discussion.

1. Regression between cultural and climatologic variables

x1, x2, x3 and x4 (earliness of bud burst and berry maturity, cluster weight and berry weight) are positively correlated with average air temperatures. This means that hot areas are prone to breed later cvs, with bigger bunches and larger berries. But it is unexpected that cluster and berry weight are not related with water supply, as x6 (weight of 100 seeds) is.

2. Seeded versus seedless cvs

The big difference found for berry weight (x4 = 3.94 and x4 = 5.37) was expected. It includes not only varietal factors, but also climatic (SDL cvs are bred in hotter climates) and cultural factors (ringing, gibberellic acid treatments not applied on SDD cvs). It must be emphasized that x4 is the only character which gave a significant difference between SDL and SDD cvs, as the other cultural characters (earliness, cluster weight) showed similar means, in spite of climatic and cultural factors capable of causing divergences.

3. Muscat versus neutral cvs

Differences were found for cluster weight (x3) and berry weight (x4). In fact, these results can also be explained by the highly significant positive regressions between x3, x4 and average temperature of the place of breeding: it seems that new muscat cvs are more frequently registered in cooler areas than neutral cvs. In consequence they have, on average, smaller clusters and smaller berries.

4. Early versus non-early cvs

Neither climatic nor cultural factors differ between these two groups, so differences found (for berry weight) may really be attributed to earliness divergence.

5. Correlation between cultural variables

The preceding result is confirmed in these correlations: x4 (berrx weight) is correlated with x1 (time of bud burst), and also with time of fruit maturity (only in white-and-neutral cvs).

The other remarkable fact is the strong correlation between:

- x1 and x2 (earliness of bud burst and of fruit maturity),
- x3 and x4 (cluster weight and berry weight).

In conclusion, it appears that few characters show differences for mean, variance and/or distribution between the groups of cvs considered. Of the six variables considered, only cluster weight and berry weight gave significant results for SDL/SDD, muscat/neutral and early/non-early cvs, but not for black versus white cvs. Differences not related with climatic or cultural factors were only observed between early and non-early cvs for berry weight.

References

BENIN, M.; GASQUEZ, J.; BESSIS, R.; LABROCHE, C.; 1985: Tests de laboratoire pour l'évaluation de la variabilité enzymatique et morphologique chez la vigne. Coll. Moët-Hennessy Amélioration de la Vigne et Culture *in vitro*, Paris, 31.

CALO, A.; COSTACURTA, A.; CANCELLIER, S.; 1987: Variabilità del rapporto glucosio-fruttosio nelle bacche, in discendenza da incrocio di viti. Riv. Viticolt. Enol. **40**, 91-102.

FANIZZA, G.; 1979: Genotypic and phenotypic variation and correlations in wine-grape varieties (*Vitis vinifera*) grown in Apulia (Italy). Genet. Agr. **33**, 37-44.

HUGLIN, P.; 1958: Recherches sur les Bourgeons de la Vigne: Initiation Florale et Développement Végétatif. Thèse, Univ. de Strasbourg.

LEFORT, P. L.; BRONNER, A.; 1981: Modalités, contraintes et efficacité de la sélection sur descendances de plein-frères chez la vigne. Agronomie **1**, 667-678.

SANDHU, A. S.; JAWANDA, J. S.; UPPAL, D. K.; 1985: Phenotypic variability in respect of berry quality in intercultivar crosses of grapes. Indian J. Agricult. Res. **42**, 213-217.

WAGNER, R.; TRUEL, P.; 1988: Nouvelles Variétés de Raisins de Table et de Raisins Secs. Ouvrage édité par l'Office International de la Vigne et du Vin, Paris.

Potassium partitioning between leaves and clusters: Role of rootstock

O. Failla [1]), A. Scienza [1,2]), G. Stringari [2]), M. Falcetti [2])

[1]) Istituto di Coltivazioni Arboree, Università degli Studi di Milano, Via Celoria, 2, I-20133 Milano, Italy

[2]) Istituto Agrario Provinciale, Via E. Mach, 1, I-38010 San Michele all'Adige, Trento, Italy

Summary: Different scion/rootstock combinations in grapevine (*Vitis vinifera*/*Vitis* spp.) were tested for nutritional properties and juice composition. Chardonnay and Cabernet Sauvignon each grafted on 22 rootstock varieties (10 new crosses and 12 already used in viticulture) were grown in outdoor pots containing very poor nutritional substrate.

Crop load had a strong effect on juice composition and potassium nutrition. Results indicate that under our experimental conditions rootstock can have an effect on potassium partitioning between leaves and cluster: 9 out of 22 rootstocks that we tried were able to improve leaf potassium content without inducing a significant increase in juice potassium content.

Key words: potassium, nutrition, translocation, rootstock, scion, leaf, bunch, growth, yield, must quality, biometry.

Introduction

Excessive or deficient mineral content in fruits can impair fruit quality in many horticultural crops. Frequently, optimal fruit mineral nutrition does not correspond with optimal leaf mineral nutrition, but optimal leaf mineral nutrition is important for high yield and high plant efficiency (Faust 1980). Thus, regulation of mineral partitioning between leaves and fruits is an important factor in regulating plant productivity and crop quality.

In grapevines, high yield can be obtained with high leaf potassium nutrition (Champagnol 1988), but frequently high leaf potassium nutrition corresponds to excessive potassium accumulation and high pH values in berry juice, and particularly to insufficient wine quality (Mattick *et al.* 1972; Hale 1977; Munyon and Nagel 1977; Boulton 1980; Morris and Cawthon 1982; Morris *et al.* 1980, 1983, 1987; Champagnol 1986; Ryser *et al.* 1989).

Potassium partitioning between leaves and clusters depends on grape variety, crop load of the vines, harvest date, potassium and water soil availability. Data of Morris *et al.* (1987) indicate that in Arkansas Gewurztraminer had higher potassium petiole content and lower potassium juice concentration than Cynthiana, and that the application of potassium fertilizer to Gewurztraminer did not cause an increase in potassium in petioles or juice, whereas fertilization caused an increase in petiole potassium with De Chaunac and an increase in both petiole and juice potassium with Cabernet Sauvignon.

Data from Hepner and Bravdo (1985) indicate that in Carignan and Cabernet Sauvignon a high crop load was able to reduce potassium levels in leaves and juice and that in Cabernet S. high soil water availability increased the leaf and juice potassium, but that both the effects were more consistent in leaves than in juice.

Morris and Cawthon (1982) were able to indicate an effect of crop load and irrigation on juice potassium but not on petiole potassium with Concord. Similarly, Freeman and Kliewer (1983) noted a more consistent effect of irrigation on juice potassium than on petiole potassium.

Morris *et al.* (1983) in a pot trial showed that with Concord, when the potassium supply ranged from 0 to 12 g/plant, the leaf potassium content passed from 1.25 to 3.5 % dw and the juice potassium concentration raised only from 2.7 to 3.3 g/l.

Table 1: Rootstock varieties and new crosses tested for nutritional behaviour grafted with Chardonnay and Cabernet Sauvignon

Rootstock	Parents
Used varieties	
KOBER 5BB (K5BB)	Vitis Berlandieri x V. riparia
3309 C	V. riparia x V. rupestris
1103 P	V. Berlandieri x V. rupestris
420 A	V. Berlandieri x V. riparia
110 R	V. Berlandieri x V. rupestris
140 Ru	V. Berlandieri x V. rupestris
41 B	V. vinifera (cv. Chasselas) x V. Berlandieri
SO4	V. Berlandieri x V. riparia
GOLIA	Castel 15.612 x Vitis rupestris du Lot
SCHWARZMANN	Vitis riparia x V. rupestris
FERCAL	BCI (V. Berlandieri x V. vinif. cv. Colombard) N. 1 x 333 EM (V. vinifera cv. Cabernet S. x V. Berlandieri)
New crosses	
USMI 1	V. Berlandieri self pollinated
USMI 2	V. Berlandieri self pollinated
USMI 3	V. Berlandieri x V. riparia
USMI 4	V. riparia x V. rupestris
USMI 5	V. Berlandieri x V. vinifera
USMI 6	V. Berlandieri self pollinated
USMI 7	V. riparia x V. rupestris
USMI 8	V. Berlandieri x V. riparia
USMI 9	V. Berlandieri x V. riparia
USMI 10	V. riparia x V. rupestris

Delas *et al.* (1989) indicated that potassium fertilization had greater effect on petiole potassium than on juice potassium and that at low potassium availability the petiole potassium content decreased with greater extent than juice potassium.

Also, rootstocks seem to be effective on potassium partitioning. Delas *et al.* (1989) found that the correlation coefficients between petiole and juice potassium content were affected by rootstock. They also showed some different behaviours of Cabernet S. grafted on different rootstocks: Riparia at the same petiole potassium levels as SO4 and Fercal induced lower juice potassium concentration.

Our hypothesis is that rootstock could have a significant role in potassium partitioning between leaves and clusters. It should assure an adequate leaf potassium content for adequate productivity (source activity, bud differentiation and assimilate translocation) but should not favour high potassium accumulation in grape juice.

Different physiological patterns are possible to explain the rootstock effect on potassium partitioning: e. g. rates in potassium uptake during the vegetative season, intensity and persistency of shoot apex growth that can compete with cluster for potassium, and regulation of leaf senescence and thus of potassium translocation from leaves to clusters.

Data from screening of new rootstocks for nutritional efficiency verify the possible role of rootstock in potassium partitioning between leaves and clusters.

Materials and method

Different scion/rootstock combinations in grapevine (*Vitis vinifera/Vitis* spp.) were tested for nutritional properties and juice composition and characteristics in 1988 at the experimental farm of the University of Milano in Montanaso Lombardo (Po Valley).

4-year-old vines were grown in outdoor pots (30 l) containing very poor nutritional substrate, constituted of 50 % peat (in volume), with the following characteristics: pH in water 7.6; total Ca carbonate (De Astis method) 8.2 %; soluble Ca carbonate (Drouineau-Galet method) 0.50 %; total nitrogen (Kjeldhal method) 0.46 %; available P (Olsen method) traces; exchangeable K ($AcNH_4$ method) 30 μg/g in K_2O. Pots were kept at field capacity during the entire season by means of trickle irrigation, water loss was replaced daily. To measure the mineral efficiency under these poor soil nutrition conditions, every pot was only slightly fertilized. Each pot was supplied with nitrogen (0.28 g as NH_4NO_3), potassium (0.75 g as K_2SO_4), phosphorus (2.4 g as $Ca(H_2PO_4)_2$), magnesium (0.5 g as $MgSO_4 \cdot 7\,H_2O$) and micronutrients (Mn, Cu, Zn, B, Mo and Fe).

Two cultivars, Chardonnay and Cabernet Sauvignon, each grafted on 22 rootstocks, were chosen. 10 new crosses (USMI series) from the Milano Pomology Institute were compared to rootstocks already used in viticulture (Table 1). Vines were arranged in one randomized block with two replications. To standardize the plants at the beginning of the season, 4 shoots with 2 flower clusters each per vine were retained. Flowering was the 1st week of June in Chardonnay and the 2nd in Cabernet S., while veraison was the 1st and the 2nd week of August, respectively.

When ripe (Chardonnay 30th August, Cabernet S. 4th October), all the grape clusters were collected and weighed. The juice obtained with a manual wine press was analysed for soluble solids (refractometric method), titratable acidity (with NaOH N/10, end pH 8.2), pH, malic acid (enzymatic method), tartaric acid (colorimetric method) and potassium (spectrophotometric method). A sample of basal (4-6 node) and apical (last 3-4 fully expanded) leaves were collected. Potassium was determined by plasma emission spectrometer in the leaf samples. The total shoot length was also measured.

Relations between nutrition and juice characteristics were analysed by correlation. The effects of rootstock and scion on nutrition and juice characteristics were tested by variance and covariance analyses. Differences between the average values were verified by Tukey (1956) test ($P = 0.05$).

Results

The scion/rootstock interaction was never significant, therefore results will be presented and discussed only as scion and rootstock main effects.

Relationships between crop load and juice composition (Table 2)

Both in Chardonnay and Cabernet S. grape yield per vine was negatively related with soluble solids, pH and K content of juice, whereas tartaric acid and titratable acidity were negatively associated with crop load.

Relationships between crop load and potassium leaf content (Table 3)

Crop load decreased the potassium content both in basal and apical leaves.

Table 2: Regression coefficients of the relations between vine crop load and juice composition. CH = Chardonnay, CS = Cabernet Sauvignon . NS: non-significant, *, **, ***: P ≤ 0.05, 0.01, 0.001

JUICE COMP.	CV	SOLUBLE SOLIDS	pH	TITRAT. ACIDITY	MALIC ACID	TARTARIC ACID	K
CROP	CH	-0.58***	-0.57***	0.34**	NS	0.48***	-0.42***
LOAD	CS	-0.44***	-0.57***	0.31**	0.35***	0.30**	-0.51***

Table 3: Regression coefficients of the relations between vine crop load and leaf potassium content at harvest time. CH = Chardonnay, CS = Cabernet Sauvignon. NS: non-significant, *, **, ***: P ≤ 0.05, 0.01, 0.001

LEAF K	CV	BASAL LEAVES	APICAL LEAVES
CROP	CH	-0.45***	-0.31***
LOAD	CS	-0.31***	-0.29**

Table 4: Regression coefficients of the relations between juice K and juice composition. CH = Chardonnay, CS = Cabernet Sauvignon. NS: non-significant, *, **, ***: P ≤ 0.05, 0.01, 0.001

JUICE COMP.	CV	SOLUBLE SOLIDS	pH	TITRAT. ACIDITY	MALIC ACID	TARTARIC ACID
JUICE	CH	0.46***	0.48***	NS	NS	NS
K	CS	0.42***	0.22*	-0.24*	NS	-0.22*

Relationships between juice potassium content and juice composition (Table 4)

Both in Chardonnay and Cabernet S. potassium content of the juice correlated with its soluble solids concentration and with its pH levels.

Relationships between leaf potassium content and juice composition (Table 5)

Potassium content of leaves correlated with potassium in the juice in both the cultivars. The other juice characteristics correlated with leaf potassium only in Chardonnay, where soluble solids, pH and malic acid values were positively associated with leaf potassium.

Table 5: Regression coefficients of the relations between leaf K content and juice composition. CH = Chardonnay, CS = Cabernet Sauvignon. NS: non-significant, *, **, ***: P ≤ 0.05, 0.01, 0.001

JUICE COMP.	CV	SOLUBLE SOLIDS	pH	TITRAT. ACIDITY	MALIC ACID	TARTARIC ACID	K
BASAL	CH	0.32*	0.43***	NS	0.34**	-0.23*	0.58***
LEAF K	CS	NS	NS	NS	NS	NS	NS
APICAL	CH	0.27*	0.22*	NS	0.35**	NS	0.52*
LEAF K	CS	NS	NS	NS	NS	NS	NS

Table 6: Scion effect on juice composition; means adjusted by crop load effect. Means followed by the same letter are not different (P = 0.05)

	SOLUBLE SOLIDS g/l	pH	TITRAT. ACIDITY meq/l	MALIC ACID meq/l	TARTARIC ACID meq/l	K meq/l
CHARDONNAY	155a	3.06a	108a	50a	85a	15.8a
CABERNET S.	169b	3.02b	121b	72b	84a	18.1b

Table 7: Rootstock effect on juice composition evaluated by variance and covariance analyses with vine crop load as covariate variable. F = Fisher's F, P = Fisher's F probability

		SOLUBLE SOLIDS	pH	TITRAT. ACIDITY	MALIC ACID	TARTARIC ACID	K
VARIANCE	F	1.945	1.905	2.011	1.661	1.370	2.391
ANALYSIS	P	**0.022**	**0.026**	**0.017**	0.063	0.169	**0.040**
COVARIANCE	F	1.394	1.575	1.797	1.666	1.072	1.905
ANALYSIS	P	0.157	0.086	**0.039**	0.062	0.399	**0.026**

Scion and rootstock effects on vine vigour and crop load (Fig. 1)

Total shoot length did not correlate with yield per vine. Average number of shoots per vine was 3.8. It was not influenced by scion and rootstock due to experimental standardization of the plants. Total shoot length was slightly controlled by rootstock, Golia induced a high shoot growth, while USMI 7 was characterized for inducing low shoot growth in the scion.

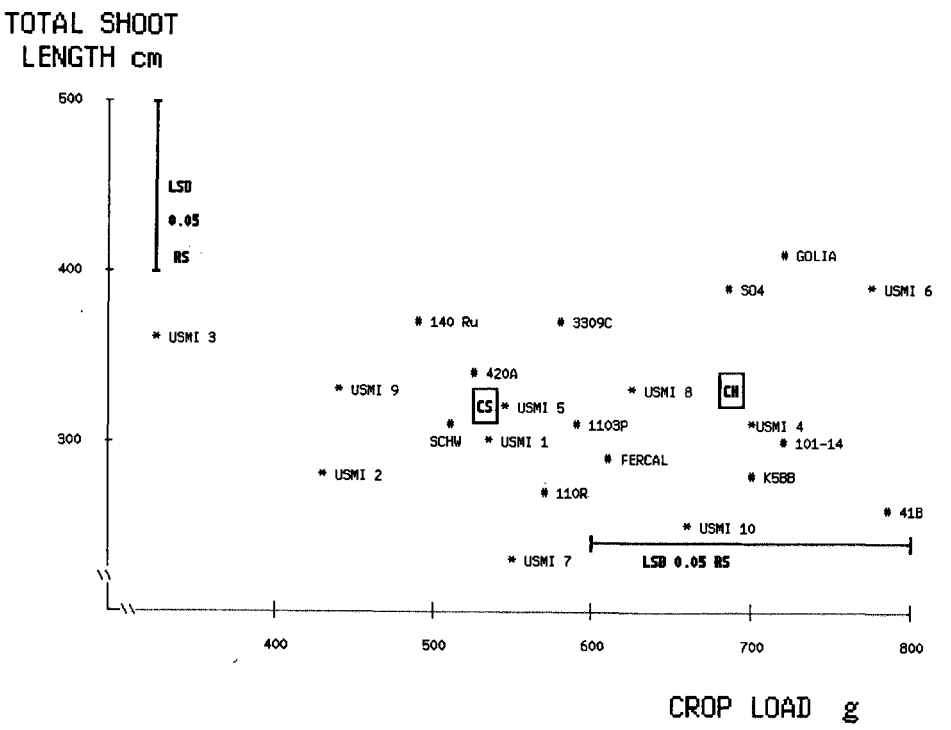


Fig. 1: Scion and rootstock effect on vine vigour and crop load.

In spite of the initial plant standardization, the number of grape clusters per vine was influenced both by scion and rootstock, whereas average weight of grape cluster was influenced only by scion. Consequently, crop load per vine was controlled both by scion and rootstock. Chardonnay had a higher grape production than Cabernet S.; USMI 6 and 41 B induced high grape yield; USMI 2 , USMI 3 and USMI 9 induced low grape yield.

Scion and rootstock effect on juice characteristics (Tables 6 and 7)

Due to the correlation between juice characteristics and crop load, scion and rootstock effects on juice composition were studied by variance and covariance analyses.

Table 8: Scion effect on leaf potassium content at harvest time; means adjusted by crop load effect. Means followed by the same letter are not different (P = 0.05). CH = Chardonnay, CS = Cabernet Sauvignon

LEAVES	CH	CS
BASAL	0.41a	0.32b
APICAL	0.40a	0.36b

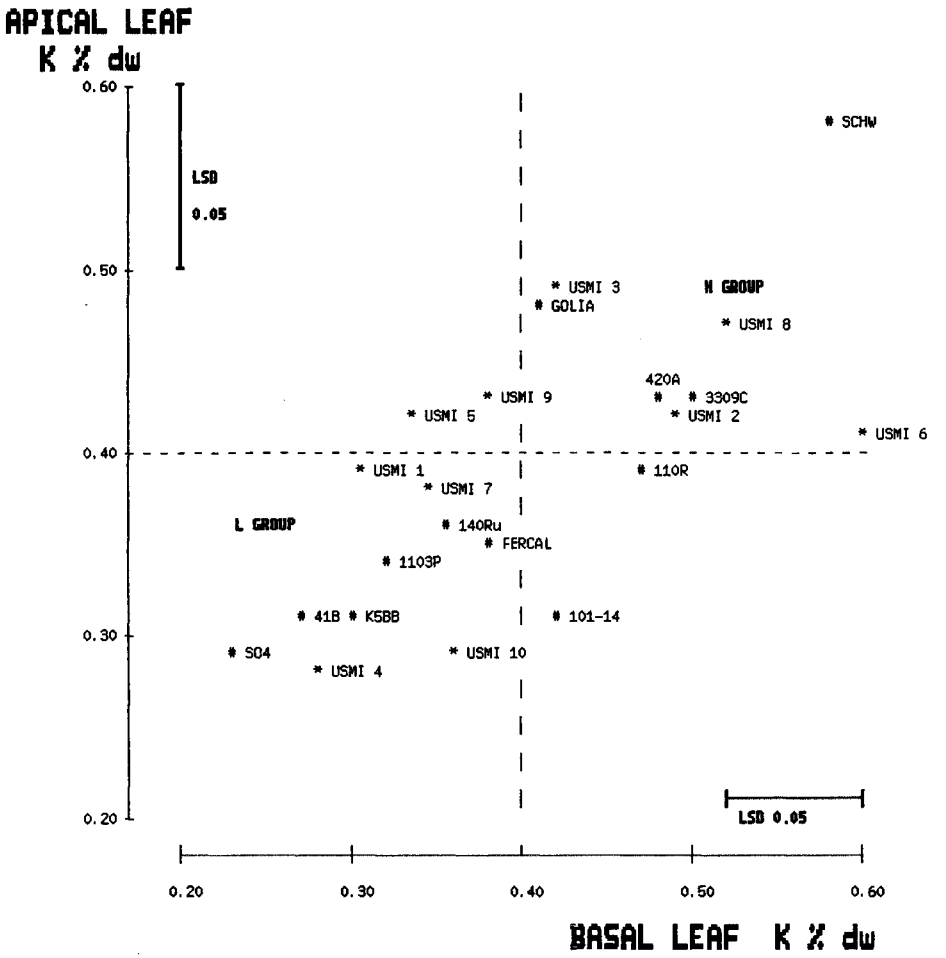


Fig. 2: Scion and rootstock effect on basal and apical leaf potassium content. Means adjusted by the crop load effect.

Scion varieties were different in juice characteristics with the exception of tartaric acid content.

Rootstock had an effect on soluble solids and pH but these effects were related with the different crop load induced by the rootstock itself, in fact its effect was still not significant when evaluated with covariance analysis with crop load as covariate variable.

Titratable acidity and potassium content of the juice were affected by rootstock. This effect was reduced, but still significant when adjusted by crop load. Malic and tartaric acid contents of the juice were not affected by rootstock.

Scion and rootstock effect on potassium nutrition in leaves (Table 8, Fig. 2)

Both scion and rootstock had an effect on leaf potassium content.

Cabernet S. had a lower leaf potassium content than Chardonnay.

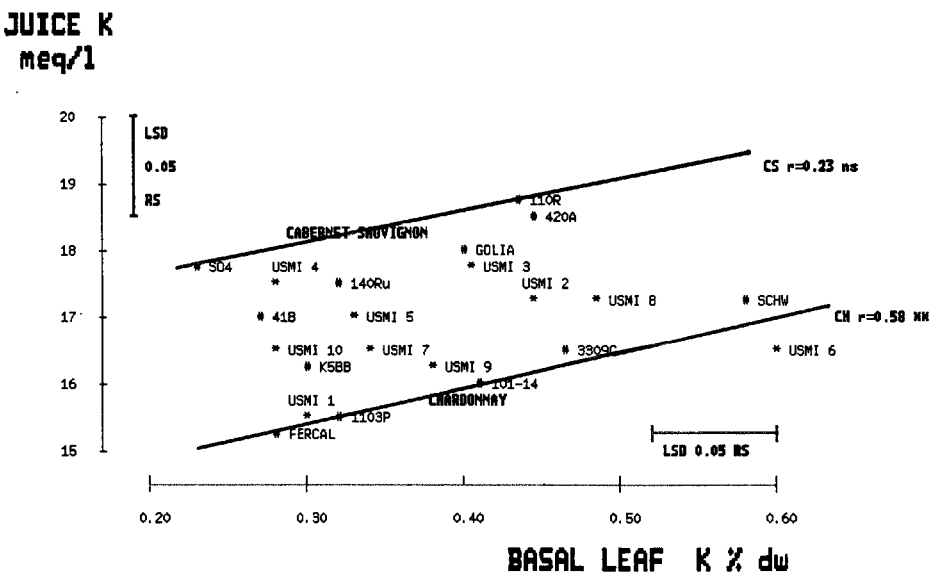


Fig. 3: Scion and rootstock effect on potassium partitioning between basal leaves and berry juice. Means adjusted by crop load effect.

Two rootstock groups were detected: the first group (L group) with potassium level below approximately 0.4 % dw both in basal and apical leaves; the second group (H group) with potassium level above approximately 0.4 % dw both in basal and apical leaves.

Scion and rootstock effect on potassium partitioning between leaves and berry juice (Fig. 3)

Cabernet S. had higher juice potassium and lower leaf potassium content than Chardonnay.

Rootstock had a greater effect on potassium leaf content in respect of potassium juice content. With the exception of USMI 1, Fercal and 1103 P which induced a juice potassium content lower than 16 meq/l, all the other rootstocks induced an average juice potassium content ranging from 16 to 18.5 meq/l with 'high' or 'low' leaf potassium content.

Discussion

Under our experimental conditions, potassium nutrition and juice characteristics were strongly related to crop load of the vines. it reduced sugar and potassium accumulation in the berry, increased their acidity and reduced leaf potassium content. For this reason, result interpretation has to take in consideration the crop load effect.

In Chardonnay the similar effect of crop load on potassium content of leaves and juice, and on soluble solids in juice, determined the correlation among these parameters. Therefore, the positive relation between potassium nutrition and sugar content of juice does not seem due to a greater leaf efficiency but to a different 'source-sink' relation.

In Cabernet S. the lack of relation of leaf potassium with soluble solids and potassium in juice seems to be related to the late leaf sampling date. At sampling time leaves were senescent due to nutrient shortage and were not at a proper nutritional index.

When adjusted by crop load effect, the scion effect was significant on juice composition (Table 6). The differences in juice composition between the two cultivars seem to be related with their different length and period of the ripening phase. In fact, the higher soluble solid and potassium content of Cabernet S. could be related with its longer ripening period, and the lower malic acid content of Chardonnay with the higher temperature that occurred during its ripening.

Similarly, the scion effect on potassium leaf content seems to be related with the different sampling dates and the different states of leaf senescence. Cabernet S. leaves were sampled 1 month after Chardonnay leaves.

For what concerns the rootstock effect when it was adjusted by the crop load effect, it was only significant on vigour, titratable acidity and potassium content of the juice, and on leaf potassium levels.

The small effect of the rootstock on plant vigour seems to be related to the pot condition. The pot volume limiting root growth also limited shoot growth.

The great effect of rootstock on vine crop load has to be related to the young age of the vines, during which rootstock has generally a higher effect on flower initiation.

Rootstocks had an effect on potassium partitioning between leaves and berry juice, indeed they induced similar juice potassium contents, with different levels of leaf potassium.

Rootstocks could be divided into two groups in relation to their capability of controlling leaf potassium nutrition. The first group (H group) was able to assure higher leaf potassium nutrition, in the second group (L group) leaf potassium nutrition was low.

These results confirm the variability among rootstocks in potassium nutrition and suggest that, at least in poor soil conditions, an adequate rootstock can raise the leaf potassium nutrition without increasing the juice potassium content.

From our data, choice of rootstock seems to be a mild method in regulation of potassium partitioning within the vine.

Literature cited

BOULTON, R.; 1980: The relationship between total acidity, titratable acidity and pH in wine. Amer. J. Enol. Viticult. **31**, 76-80.

CHAMPAGNOL, F.; 1986: L'acidité des moûts et des vins. 2° partie. Facteurs physiologiques et agronomiques de variation. Progr. Agric. Vitic. **103**, 361-374.

– – ; 1988: Rôle du potassium dans la physiologie de la vigne. Progr. Agric. Vitic. **105**, 431-435.

DELAS, J.; MOLOT, C.; SOYAR, J. P.; 1989: Fertilisation minérale de la vigne et teneurs en potassium des baies, des moûts et des vins. 4° Symp. Intern. Enologie, Bordeaux, 15-17 juin 1989.

FAUST, M.; 1980: Modern concepts in fruit nutrition. In: ATKINSON, D.; JACKSON, J. E.; SHARPLES, R. O.; WALLES, W. M. (Eds.): Mineral Nutrition of Fruit Trees, 11-16. Butterworths, Sevenoaks.

FREEMAN, B. M.; KLIEWER, W. M.; 1983: Effect of irrigation, crop level and potassium fertilization on Carignane vines. II. Grape and wine quality. Amer. J. Enol. Viticult. **34**, 197-207.

HALE, C. R.; 1977: Relation between potassium and the malate and tartrate contents of grape berries. Vitis **16**, 9-19.

HEPNER, Y.; BRAVDO, B.; 1985: Effect of crop level and drip irrigation scheduling on the potassium status of Cabernet Sauvignon and Carignane vines and its influence on must and wine composition and quality. Amer J. Enol. Viticult. **26**, 140-147.

MATTICK, L. R.; SHAULIS, N. J.; MOYER, J. C.; 1972: The effect of potassium fertilization on the acid content of 'Concord' grape juice. Amer. J. Enol. Viticult. **23**, 26-30.

MORRIS, J. R.; CAWTHON, D. L.; 1982: Effect of irrigation, fruit load, and potassium fertilization on yield, quality, and petiole analysis of Concord (*Vitis labrusca* L.) grapes. Amer. J. Enol. Viticult. **33**, 145-148.

– – ; – – ; FLEMING, J. W.; 1980: Effect of high rates of potassium fertilization on raw product quality and changes in pH and acidity during storage of 'Concord' grape juice. Amer. J. Enol. Viticult. **31**, 323-328.

– – ; SIMS, C. A.; CAWTHON, D. L.; 1983: Effects of excessive potassium levels on pH, acidity, and color of fresh and stored grape juice. Amer. J. Enol. Viticult. **34**, 35-39.

– – ; – – ; STRIEGLER, R. K.; CACKLER, S. D.; DONLEU, R. A.; 1987: Effects of cultivar, maturity, cluster thinning, and excessive potassium fertilization on yield and quality of Arkansas wine grapes. Amer. J. Enol. Viticult. **38**, 260-264.

MUNYON, J. R.; NAGEL, C. W.; 1977: Comparison of methods of deacidification of musts and wines. Amer. J. Enol. Viticult. **28**, 79-89.

RYSER, J. P.; AERNY, J.; MURISIER, F.; 1989: Foliar diagnosis as a tool in achieving quality. In: Methods of K-Research in Plants, 249-257. International Potash Institute, Worblaufen-Bern, Switzerland.

TUKEY, J. W.; 1956: The problem of multiple competition. In: SNEDECOR, G. W. (Ed.): Statistical Methods, 251-254. Iowa State University Press, Ames, IA, USA.

On the new very early table varieties obtained by crossing different varieties of grapevine

L. Avramov, D. Tadijanovic, K. Pavlovic, M. Jovanovic and N. Gasic

Faculty of Agriculture, Nemanjina 6, YU-11081 Zemun, SFR Yugoslavia

Summary:

1. In order to obtain new table grape varieties which ripen before or at the same time as variety Pearl of Csaba, we performed many intervarietal crossings at our experimental station, using the following parents: Pearl of Csaba, Chasselas Bouvier, Muscat Ottonel, Queen of Vineyard, Cardinal, Dattier de Beyrouth, Ribier.
2. The Government Commission acknowledged five very early varieties, three of which have also better agrobiological and technological characteristics than the variety Pearl of Csaba.
3. The new variety Demir Door ripens on average 6 d, the new variety Early of Belgrade 3-5 d earlier, and the new variety Grochanka ripens at the same time or 1-3 d later as the control variety Pearl of Csaba.
4. Due to multiple regression analysis, biological characteristics as yield, cluster and berry weights and sugar content strongly depend on meteorological conditions (temperature, rainfall and solar radiation); this is valid for all the new varieties and the variety Pearl of Csaba at all locations investigated.
5. Analysis of variance (level of 0.05 and 0.01) shows that compared to the control variety we obtained with new varieties significantly greater yields, very significant large cluster and berry weights and very significantly better uvological characteristics of berry. The average yields for all investigated locations were increased in comparison to the control variety Pearl of Csaba: Demir Door +3.004 kg/ha, Early of Belgrade +6.259 kg/ha, Grochanka +7.065 kg/ha.
6. Organoleptic characteristics are much improved with all new varieties which is illustrated by increased indexes.
7. All investigated varieties are acknowledged genotypes of *Vitis vinifera* L., and all of these new varieties exhibited better biological and technological parameters than Pearl of Csaba.

Key words: table grape, variety of vine, early ripening, ecology, Yugoslavia, crossing, growth, yield, berry, must quality, sensory rating, physical properties, disease resistance, cold resistance.

Introduction

After acknowledgement of some very early varieties and after testing their behaviour under different agroecological conditions (Avramov *et al.* 1978, 1982, 1988; Avramov 1987, 1988; Pavlovic 1983; Korac 1989), we can discuss now the advantages of these varieties which ripen before or at the same time as variety Pearl of Csaba.

Materials and methods

Crossing was performed using the varieties Pearl of Csaba, Chasselas Bouvier, Muscat Ottonel, Queen of Vineyard, Cardinal, Dattier de Beyrouth, Ribier etc. Crossing and propagation were done at the Fruit-Grape Experimental Station 'Radmilovatz'of the Faculty of Agriculture, Zemun.

The evaluation of the selected genotypes was carried out by the Commission for Variety Acknowledgement under production conditions similar to those of some ampelographic collections in our and foreign countries.Special attention was paid to changes of varietal characteristics on dependence of mesoclimatic conditions taking into account locations and years.

We used the following statistical methods: trend analysis, multiple regression analysis and analysis of variance.

In this paper we will show the data only for three most successful varieties which ripen before or at the same time as Pearl of Csaba.

Results

From the results obtained during the production of grapes in different agroecological areas of our country, the varieties Demir Door, Early of Belgrade and Grochanka were the most successful and economically most interesting ones. Control variety was Pearl of Csaba.

Variety Demir Door (Table 1)

This variety resulted from the crossing Muscat Ottonel x Queen of Vineyard.

The most important morphological and physiological characteristics are: vigorous growth, flower physiologically hermaphroditic, medium-sized cluster, large berries, yellow-green berry colour, semi-firm berry texture, muscat-like taste. The time of ripening is on average 6 d earlier than Pearl of Csaba.

Compared with the control variety, Demir Door is more resistant to *Botrytis cinerea*, to oidium and to low winter temperatures.

Analysis of the data given in Table 1 shows that compared to Pearl of Csaba the variety Demir Door exhibited significant quantitative differences which under index points reach the next limits: average yield (kg/ha) 134 IP; average cluster weight 110 IP; average berry weight 152 IP; average cluster length 123 IP; average sugar content 105 IP; berry skin crush resistance 208 IP; organoleptic characteristics value 138 IP.

Table 1 shows that we have obtained a new genotype which in comparison with the variety Pearl of Csaba exhibits better agrobiological and technological characteristics, including very early ripening, on average 6 d before Pearl of Csaba.

The analysis of variance (0.05-0.01 level) showed that compared with control variety Demir Door exhibited significantly greater yields, very significantly larger average cluster and berry weights, a very significant increase in berry skin crush resistance and also very significant increase of the resistance of berry to separation from pedicel. Yield, cluster weight, berry weight and sugar content showed very high correlations with temperature, rainfall and solar radiation.

Variety Early of Belgrade (Table 2)

This variety was obtained from a crossing of the varieties Chasselas Bouvier x Dattier de Beyrouth.

Most important morphological and physiological characteristics are: vigorous growth, flower functionally hermaphroditic, large cluster, large berries, yellow-green skin, berry texture semi-firm, taste very pleasant. Ripening occurs 3-5 d earlier than with Pearl of Csaba.

Compared with Pearl of Csaba, Early of Belgrade is much more resistant to diseases, to drought and to low winter temperatures.

Multiple regression analysis indicates that yield is poorly correlated with temperature, rainfall and solar radiation. However, cluster weight, sugar content and acid content are strongly correlated with these meteorological factors.

The data in Table 2 show that the variety Early of Belgrade exhibits significant differences in comparison to Pearl of Csaba and the values in index points reach the next limit: average yield 168 IP; average cluster weight 287 IP; average berry weight 125 IP; average cluster length 177 IP; average sugar content 125 IP; berry skin crush resistance 163 IP; resistance of berry to separation from pedicel 121 IP; organoleptic characteristics value 145 IP.

Table 2 also shows that we have obtained with variety Early of Belgrade a new genotype which in comparison with the variety Pearl of Csaba exhibits better agrobiological and technological characteristics including very early ripening.

The analysis of variance (0.05-0.01 level) showed that compared to Pearl of Csaba Early of Belgrade exhibits significantly very greater yields, cluster and berry weights and sugar content.

Table 1: Average values of major characters for varieties Pearl of Csaba and Demi Door investigated under different ecological conditions

Characters	Pearl of Csaba (Control)	Demir Door (New variety)	Difference	Index of increase (Points)
1. Yield (kg/ha)	8,687	11,691	3,004	134,50
2. Cluster weight (g)	131,80	144,80	13,00	110,00
3. Cluster length (cm)	8,50	10,50	2,00	123,00
4. Berry weight (g)	2,21	3,36	1,15	152,00
5. Sugar content (%)	16,80	17,60	0,80	105,00
6. Total acid content (g/l)	6,60	6,70	0,10	101,00
7. Berry skin crush resistance (g/cm^2)	489,00	1022,60	533,60	208,00
8. Resistance of berry to separation from pedicel (g)	375,40	715,40	340,00	190,00
9. Organoleptic characteristics value (points 1 - 10)	6,30	8,70	2,40	138,00
10. Time of ripening	24.07.	18.07.	6 days	---

Table 2: Average values of major characters for varieties Pearl of Csaba and Early of Belgrade investigated under different ecological conditions

Characters	Pearl of Csaba (Control)	Early of Belgrade (New variety)	Difference	Index of increase (Points)
1. Yield (kg/ha)	9.206	15.456	6.259	168,00
2. Cluster weight (g)	137,90	396,20	258,30	287,00
3. Cluster length (cm)	8,50	15,10	6,60	177,00
4. Bery weight (g)	2,08	2,55	0,47	122,00
5. Sugar content (%)	14,00	17,50	3,50	125,00
6. Total acid content (g/l)	7,20	7,40	0,20	102,00
7. Berry skin crush resistance (g/cm^2)	324,80	531,70	206,90	163,00
8. Resistance of berry to separation from pedicel (g)	463,70	980,80	517,10	211,00
9. Organoleptic characteristics value (points 1 - 10)	5,96	8,66	2,70	145,00
10. Time of ripening	3.08.	29.07.	5 days	---

Table 3: Average values of major characters for varieties Pearl of Csaba and Grochanka investigated under different ecological conditions

Characters	Pearl of Csaba (Control)	Groschanka (New variety)	Difference	Index of increase (Points)
1. Yield (kg/ha)	9,206	16,271	7,065	176,00
2. Cluster weight (g)	137,90	302,10	164,20	219,00
3. Cluster length (cm)	8,50	16,72	8,22	196,00
4 Berry weight (g)	2,08	3,03	0,99	145,00
5. Sugar content (%)	14,00	18,10	4,10	129,00
6. Total acid content (g/l)	7,20	6,70	0,50	0,93
7. Berry skin crush resistance (g/cm^2)	324,80	567,30	242,50	174,00
8. Resistance of berry to separation from pedicel (g)	463,70	644,60	180,90	139,00
9. Organoleptic characteristics value (points 1 - 10)	5,96	8,16	2,20	137,00
10. Time of ripening	3.08.	5.08.	2 days	---

Variety Grochanka (Table 3)

The variety Grochanka resulted from the crossing Pearl of Csaba x Dattier de Beyrouth.

The most important morphological and physiological characteristics are: vigorous growth, flower functionally hermaphroditic, medium-sized cluster, large berry, firm berry texture, very pleasant wine taste. Variety Grochanka ripens at the same time or 1-3 d later than variety Pearl of Csaba.

The variety is more resistant to diseases and to low winter temperatures than the control.

Multiple regression analysis shows that yield was poorly correlated with temperature, rainfall and solar radiation. The cluster weight was strongly correlated and the sugar and acid contents were very strongly correlated with these factors.

The data in Table 3 show that in comparison with Pearl of Csaba the new variety Grochanka exhibits significant differences and the values in index points reach the next limit: average yield 176 IP; average cluster weight 219 IP; average berry weight 145 IP; average cluster length 196 IP; average sugar content 129 IP; berry skin crush resistance 174 IP; resistance of berry to separation from pedicel 139 IP; organoleptic characteristics value 137 IP.

Analysis of variance (0.05-0.01 level) showed that in comparison to variety Pearl of Csaba the variety Grochanka has very significantly greater yield, cluster weight, sugar content and a very significant increase in berry skin crush resistance.

Table 3 shows that we have obtained with variety Grochanka also a new genotype which in comparison with Pearl of Csaba exhibits better agrobiological and technological characteristics including very early ripening.

Discussion

The results obtained show that from crossings where one of parents or both were very early ripening we attained F_1 seedlings which had characteristics of earlyer maturation than the parents.

From many populations and after a large number of crossings we obtained many interesting seedlings with very early maturation. After acknowledgement of the varieties, in our praxis the best results exhibited the three varieties Demir Door, Early of Belgrade and Grochanka.

The fundamental idea was to create varieties which mature earlier or at the same time as variety Pearl of Csaba. Our varieties obtained exhibited under different ecological conditions very early maturation, namely:

- The variety Demir Door ripens on average 6 d before Pearl of Csaba.
- The variety Early of Belgrade ripens 3-5 d before Pearl of Csaba.
- The variety Grochanka ripens at the same time or 1-3 d after Pearl of Csaba.

From the genetical point of view we can assume that the donators for the early maturation were the varieties Pearl of Csaba, Chasselas Bouvier and Queen of Vineyard.

Our results also show that new varieties have significantly or very significantly better agrobiological and technological characteristics than Pearl of Csaba taking into account wide geographical areas. The data given in Tables 1, 2 and 3 demonstrate greater yields, larger cluster and berry weights, higher sugar contents, better uvological characteristics of berry and greater resistance to cryptogamic diseases and to low winter temperature.

Coefficients of multiple regression analysis show that the new varieties exhibited low, strong and very strong dependence on mesoclimatic conditions of location and of years of the investigations.

The new very early ripening varieties do not have very large berries as varieties Cardinal, Dattier de Beyrouth, Ribier, etc. Future crossings to improve berry size are necessary to meet the requirements of our own and of foreign markets.

Literature

AVRAMOV, L.; 1987: Posebno vinogradarstvo. Beograd.

– – ; 1988; Savremeno gajenje vinove loze. Nolit, Beograd.

– – ; LOVIC, R.; TADIJANOVIC, Dj.; JURCEVIC, A.; JOVANOVIC, M.; RUZEVIC, M.; 1982: Contribution à l'étude des caractères agrobiologiques de quelques nouveaux cultivars yougoslaves de la vigne. Symp. Intern. Raisin de Table et Raisin Sec, Heraclion, 193-200.

– – ; STANOJEVIC, S.; TODOROVIC, N.; TADIJANOVIC, Dj.; JURCEVIC, A.; JOVANOVIC, M.; 1978: L'influence de quelques facteurs météorologiques dans différentes localités sur le rendement et certains caractères physicochimiques des raisins nouveaux de table en Yougoslavie. 1er Symp. Ecologie de la Vigne, Constanta, 287-294.

– – ; ZUNJC, D.; GASIC, N.; SUBOTICKI, M.; 1988: Prilog poznavanju rodnosti i nekih uvoloskih karakteristika novih jugoslovenskih veoma ranih stonih sorti stvorenih u Radmilovcu. Jugoslovensko Vinogradarstvo i Vinarstvo (3-4).

INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES; 1983: Grape Descriptors. IBPGR Secretariat, Rome.

KORAC, N.; 1989: Ampelographic investigations of table grapevine varieties in the Fruska Gora viticulture area. Doctoral dissertation, Novi Sad.

PAVLOVIC, K.; 1983: Ispoljavanje nekih agrobioloskih i tehnoloskih svojstava novih jugoslovenskih stonih sorti vinove loze u zavisnost od mezoklimatskih uslova lokaliteta. Mrs. dissertation, Zemun.

Investigations on the photosynthetic productivity of some newly selected grapevine varieties

TODORKA SLAVCHEVA

Institute of Viticulture and Enology, 5800 Pleven, Bulgaria

Summary: Investigations were carried out on the photosynthetic productivity of 5 wine varieties which had been selected at the Institute of Viticulture and Enology: Storgoziya, Nikopolski mavrud, Pomoriiski biser, Dunavska gamza and Dunavski lazur. The first of them was grafted on Chasselas x Berlandieri 41 B rootstock, each of the others was on three rootstocks: Chasselas x Berlandieri 41 B, Berlandieri x Riparia SO 4, and (Berlandieri x Riparia Kober 5 BB) x (Rupestris Martin) III-102 D.

For all varieties, the leaf dry matter per unit leaf area was greatest when they were grafted on III-102 rootstock. During the vegetation period, the specific leaf weight increased, but the rate of dry matter accumulation in leaves was not constant and gradually decreased with time.

The content of pigments and the leaf area per vine were greatest on SO 4 rootstock. On this rootstock, the highest yield was obtained for all cultivars mainly at the expense of the grape cluster weight. The thickness of the trunk under the graft-union, though, was smallest and an irregular increase of the diameters of the two components, rootstock and scion, occurred.

Key words: rootstock, scion, variety of vine, grafting, photosynthesis, chlorophyll, yield, production, phenology, statistics, Bulgaria.

Introduction

In our previous investigations (SLAVCHEVA 1981, 1988; STOEV and SLAVTCHEVA 1982) the effect of the most important ecological factors – light, temperature, carbon dioxide, soil humidity – and of some agricultural factors – training systems, planting density – on the photosynthetic rate of grapevine was shown. Our efforts have recently been directed to investigating physiological and biochemical peculiarities of grapevine plant in relation to variety and also choice of rootstock. This is aimed at facilitating selection of perspective grapevine varieties with optimum parameters of leaf photosynthesis and with valuable agrobiological characteristics, as well as at utilizing the obtained knowledge for the progress of grapevine breeding. Some results in this field are presented in this paper.

Materials and methods

The experimental work was carried out at the Institute of Viticulture and Enology, Pleven. Five wine varieties bred at the institute were investigated: Storgoziya, Nikopolski mavrud, Pomoriiski biser, Dunavska gamza and Dunavski lazur. The first of these was grafted on Chasselas x Berlandieri 41 B rootstock, each of the other cultivars on three rootstocks: Chasselas x Berlandieri 41 B, Berlandieri x Riparia SO 4 and (Berlandieri x Riparia Kober 5 BB) x (Rupestris Martin) III-102 D[1], the latter having been developed at the Institute of Viticulture and Enology (DIMITROV 1977, author's certificate no. 23562). During the vegetation period, dry matter accumulation in leaves, pigment content, leaf area, grape yield, as well as other additional characteristics were determined.

Dry matter accumulated in leaves during the day was determined after the method of SACHS (POPOV *et al.* 1957), leaf samples were taken for this purpose at 8. a.m., 12 p.m. and 4 p.m. three times during the vegetation period in phenophases of flowering, onset of grape maturation

[1]) For brevity: 41 B, SO 4, III-102 .

(véraison), and maturation. Specific leaf (dry) weight was measured as mg/dm^2. Pigment content (chlorophyll a, chlorophyll b and carotenoids) was determined spectrophotometrically (Pochinok 1976) during the same phases as mg % (mg per 100 g dry weight). Leaf area per vine was calculated as m^2 (Carbonneau 1976; Slavcheva 1983, 1987). Grape yield was measured as kg per grapevine.

The data were statistically processed (Mudra 1958; Barov and Naidenova 1969).

Table 1: Specific leaf weight during the day (mg dry wt/dm^2)

Rootstocks		Cultivars				
		Nikopolski mavrud	Pomoriiski biser	Dunavska gamza	Dunavski lazur	Storgoziya
			Flowering			
41 B	I[1])	454.4	494.2	495.4	459.1	469.4
	II[1])	488.8	524.7	522.1	497.8	498.1
	III[1])	500.3	555.5	559.6	521.5	524.9
SO 4	I	451.8	493.0	502.3	467.4	
	II	488.2	526.5	543.3	492.6	
	III	503.1	549.2	566.6	520.6	
III-102	I	469.3	504.2	527.6	469.1	
	II	493.1	537.0	553.0	509.2	
	III	528.0	549.2	573.4	519.5	
			Onset of maturation			
41 B	I	675.9	727.4	717.1	684.5	627.9
	II	708.7	775.4	766.3	721.2	660.6
	III	722.4	767.6	781.4	733.0	665.8
SO 4	I	670.3	718.2	737.6	684.3	
	II	712.8	762.3	781.5	716.5	
	III	728.6	795.2	795.0	724.5	
III-102	I	697.5	757.4	747.8	687.9	
	II	743.2	791.1	790.5	728.2	
	III	746.2	806.0	812.5	738.6	
			Maturation			
41 B	I	675.4	796.0	763.3	700.2	656.8
	II	698.0	825.6	784.5	712.5	694.8
	III	711.4	830.9	801.5	712.9	694.6
SO 4	I	680.5	791.5	798.1	697.1	
	II	712.4	841.5	811.5	708.0	
	III	724.8	820.4	816.5	709.3	
III-102	I	726.6	841.5	811.6	740.9	
	II	766.1	901.5	833.5	768.7	
	III	757.1	868.1	828.2	748.3	

[1]) First (8 a.m.), second (12 p.m.), third (4 p.m.) samples in the day-time.

Results and discussion

In Table 1 data are shown on specific leaf weight at 8 a.m., 12 p.m. and 4 p.m. during flowering, onset of maturation and maturation. The specific weight of leaves increased during the day as a result of their photosynthetic activity irrespective of variety and rootstock. This allowed to use summarized equations for characterization of the general regularities. The dry matter increment of leaves during the day can be described by linear regressions as follows:

Rootstock	Flowering	Onset of maturation	Maturation
41 B	$y = 408.6 + 8.06x$	$y = 629.6 + 8.30x$	$y = 695.7 + 4.46x$
SO 4	$y = 415.1 + 7.74x$	$y = 634.6 + 8.21x$	$y = 717.3 + 3.39x$
III-102	$y = 435.3 + 6.95x$	$y = 657.6 + 7.83x$	$y = 763.7 + 2.88x$

In the equations y is the specific leaf weight, i. e. the leaf dry weight per unit leaf area (mg/dm^2), and x the time of day (h between 8 a.m. and 4 p.m.). During flowering the t-test for the three rootstocks was $t_{exp.} > t_{tab.}$, during onset of maturation this was relevant to 41 B and SO 4 rootstocks, during grape maturation $t_{exp.} < t_{tab.}$. The rate of dry matter accumulation in leaves during the day, i. e. the change in leaf dry weight per unit leaf area per unit time, was highest on 41 B rootstock, and lowest on III-102. The specific leaf weight, in contrast, was greatest on III-102 and lowest on 41 B (significant differences). Furthermore, the dry matter accumulation rate in leaves during the day depended also on the phase; it was highest during onset of maturation and lowest during maturation.

Changes in specific leaf weight during the day can be described by a non-linear regression, polynomial of second degree:

Rootstock	Flowering	Onset of maturation	Maturation
41 B	$y = 324.6 + 22.75x - 0.60x^2$	$y = 464.9 + 37.77x - 1.24x^2$	$y = 612.2 + 18.53x - 0.56x^2$
SO 4	$y = 307.1 + 26.62x - 0.78x^2$	$y = 500.2 + 31.43x - 0.95x^2$	$y = 624.2 + 19.10x - 0.63x^2$
III-102	$y = 311.2 + 28.64x - 0.89x^2$	$y = 456.6 + 42.54x - 1.42x^2$	$y = 435.8 + 58.28x - 2.22x^2$

The meaning of y and x is the same as above.

After differentiating the above-mentioned equations the following was obtained:

Rootstock	Flowering	Onset of maturation	Maturation
41 B	$\frac{dy}{dx} = 22.75 - 1.20x$	$\frac{dy}{dx} = 37.77 - 2.48x$	$\frac{dy}{dx} = 18.53 - 1.12x$
SO 4	$\frac{dy}{dx} = 26.62 - 1.56x$	$\frac{dy}{dx} = 31.43 - 1.90x$	$\frac{dy}{dx} = 19.10 - 1.26x$
III-102	$\frac{dy}{dx} = 28.64 - 1.78x$	$\frac{dy}{dx} = 42.54 - 2.84x$	$\frac{dy}{dx} = 58.28 - 4.44x$

In the equations dy/dx is the rate of dry matter accumulation in leaves during the day ($mg/dm^2 \cdot h$) and x as formerly the time of day between 8 a.m. and 4 p.m. (h). This dry matter accumulation rate depended on the rate of net assimilates production and the rate of their translocation to the other organs. It decreased linearly with time from the morning hours (8 a.m.) towards the afternoon hours (4 p.m.), which was probably due to overcharging the photosynthetic apparatus with assimilates, causing a depression of photosynthesis. The coefficient before x is a measure of the reduction of dry matter accumulation rate in leaves per unit time.

Statistic data processing was complicated by the use of a non-linear regression to describe the change in specific leaf weight during the day. Verification by an F-test did not give better results, so we could satisfy ourselves with a linear regression. Experimental data, however, are better described by a non-linear regression and, provided a sufficiently large number of replicates is available, it is preferable.

Data examination for each variety showed that the already mentioned tendencies remain with little exceptions, so that the use of generalized data proved to be adequate. (Under the aspect of varieties the data will be examined in more detail in another paper.) Highest values of specific leaf weight were obtained for cvs Pomoriiski biser and Dunavska gamza, and lower values were obtained for Dunavski lazur, Nikopolski mavrud and Storgoziya. The differences between the two groups of cultivars were significant ($F_{exp.} > F_{0.001}$). Specific leaf weight is a typical characteristic of variety and rootstock.

In Table 1 it is also seen that the specific leaf weight increased during the vegetation period. Its change could be described by a non-linear regression, as follows:

41 B	$y = 475.1 + 4.870x - 0.0207x^2$	$(F_{exp.} = 54.94 > F_{0.001} = 27.00)$
SO 4	$y = 477.9 + 5.047x - 0.0223x^2$	$(F_{exp.} = 44.66 > F_{0.001} = 27.00)$
III-102	$y = 486.3 + 5.012x - 0.0199x^2$	$(F_{exp.} = 49.12 > F_{0.001} = 27.00)$

In the equations y is the specific leaf weight (mean value from the three measurements during the day) as mg/dm^2, and x the time in days (d) after the earliest date of measuring (in this case during flowering).

When we differentiated the equations, the first derivative of y with respect to x was considered as rate of dry matter accumulation in leaves during the vegetation period. This looked like this:

41 B	$\frac{dy}{dx} = 4.870 - 0.0414x$
SO 4	$\frac{dy}{dx} = 5.047 - 0.0446x$
III-102	$\frac{dy}{dx} = 5.012 - 0.0398x$

In the equations $^{dy}/_{dx}$ is the gain in leaf dry matter, calculated per unit leaf area per unit time (mg/dm^2 • d) and x the time in days (d) after florescence. The rate of dry matter accumulation decreased uniformly in the course of time, most slowly on III-102 and most rapidly on SO 4 rootstock. Considered for each cultivar, the results are analogous; the value of $^{dy}/_{dx}$ (average for the vegetation period) was smallest on SO 4 and it was highest on III-102 except for cv. Dunavska gamza, where in general the differences were small.

This fact could be related to the translocation of assimilates toward clusters and other sinks. According to Vasev (1976), interrupted or retarded assimilate flow causes an increase in leaf dry weight and reduces water content and plastid pigment amount. Export of photosynthates from leaves is controlled by the needs of the individual sinks (Stoev and Ivantchev 1977; Orth 1983; Eibach and Alleweldt 1985). The cluster becomes the principal sink for photosynthates produced in the middle third of the shoot after fruit-set during its most intensive growth (Balcar and Hernandez 1988) and particularly at onset of maturation (Stoev and Ivantchev 1977).

From Table 2 it is seen that for all cultivars on SO 4 rootstock a higher yield was obtained mainly at the expense of cluster weight, i. e. reciprocally to the rate of dry matter accumulation in leaves. This shows the correctness of such an approach.

Leaf area correlates to yield ($r = +0.799 > r_{0.01} = 0.684$). For all varieties the leaf area per vine was greatest on SO 4 rootstock, mainly at the expense of the mean area per leaf (Table 3).

Table 2: Grape yield (kg/vine)

Rootstocks	Cultivars				
	Nikopolski mavrud	Pomoriiski biser	Dunavska gamza	Dunavski lazur	Storgoziya
41 B	4.619	5.600	5.844	5.540	7.530
SO 4	6.520	7.304	6.717	6.495	
III-102	5.403	4.864	4.404	5.056	

Table 3: Leaf area (m^2/vine)

Rootstocks	Cultivars				
	Nikopolski mavrud	Pomoriiski biser	Dunavska gamza	Dunavski lazur	Storgoziya
41 B	9.9	9.6	6.8	8.2	13.1
SO 4	12.0	12.2	7.0	12.6	
III-102	10.2	6.3	6.3	5.3	

Table 4: Thickness of the trunk (mm) (A) above and (B) under the graft-union

Rootstocks	Cultivars				
	Nikopolski mavrud	Pomoriiski biser	Dunavska gamza	Dunavski lazur	Storgoziya
			A		
41 B	32.5[1])	32.0	28.5	29.9	32.4
SO 4	34.3	33.6	29.1	31.0	
III-102	32.6	33.6	29.1	31.2	
			B		
41 B	32.7	35.6	33.2	33.3	35.9
SO 4	28.4	28.9	25.7	28.2	
III-102	34.7	35.1	31.1	34.7	

[1]) The data are mean values from 3 years.

As to thickness of the trunk (which is a consumer as well) under the graft-union, it was smallest when the cultivars were grafted on SO 4, probably owing to rival interrelations, i. e. the assimilates

Table 5: Pigment contents in the leaves (mg/100 g dry wt)

Rootstocks	Cultivars				
	Nikopolski mavrud	Pomoriïski biser	Dunavska gamza	Dunavski lazur	Storgoziya
			Chlorophyll a		
41 B	415[1])	406	382	406	497
SO 4	477	439	383	435	
III-102	434	408	369	391	
			Chlorophyll b		
41 B	138	130	114	128	159
SO 4	141	154	108	126	
III-102	124	119	107	116	
			Carotenoids		
41 B	209	204	191	198	238
SO 4	239	216	198	213	
III-102	229	218	194	195	

[1]) The data are mean values from the three measurements in phenophases of flowering, onset of grape maturation, and maturation.

were distributed predominantly in the overground part and besides an irregular thickening of both components, rootstock and scion, occurred (Table 4). In contrast, on III-102 rootstock, probably owing to the presence of a smaller consumer (cluster) a greater portion of dry matter was accumulated in the leaves and subsequently in the perennial parts, which corresponds to some results obtained by DIMITROV (1984, unpublished data).

The contents of chlorophyll a, chlorophyll b and carotenoids in leaves are shown in Table 5. The data concerning rootstock III-102 are from 2 years. The highest pigment content was characteristic of cv. Nikopolski mavrud, followed by Pomoriïski biser, the lowest of cv. Dunavska gamza; on 41 B rootstock, the pigment content was greatest for cv. Storgoziya. Cv. Pomoriïski biser was not significantly different ($F_{exp.} < F_{0.05}$) from Nikopolski mavrud in regards to pigment contents, but the differences were significant for Dunavski lazur and Dunavska gamza ($F_{exp.} > F_{tab.}$). For all varieties the total content of pigments was highest on SO 4 rootstock, mainly at the expense of chlorophyll a and the carotenoids.

Pigment contents continuously decreased during the vegetation period. This could be expressed for chlorophyll a by a linear regression, as follows:

41 B	$y = 530 - 1.840x$	($t_{exp.} = 6.523 > t_{0.001} = 5.408$)
SO 4	$y = 515 - 1.158x$	($t_{exp.} = 7.163 > t_{0.001} = 5.408$)
III-102	$y = 571 - 2.302x$	($t_{exp.} = 7.328 > t_{0.01} = 4.604$)

In the equations y is the content of chlorophyll a (mg/100 g dry weight) and x the time (d) after florescence.

Analogous equations were obtained for chlorophyll b:

41 B	$y = 163 - 0.509x$	($t_{exp.} = 5.052 > t_{0.01} = 3.499$)
SO 4	$y = 156 - 0.325x$	($t_{exp.} = 2.287 < t_{0.05} = 2.365$)
III-102	$y = 164 - 0.634x$	($t_{exp.} = 2.309 < t_{0.05} = 2.776$)

where y is the content of chlorophyll b and x as above.

For the carotenoids:

41 B	$y = 248 - 0.685x$	$(t_{exp.} = 3.686 > t_{0.01} = 3.499)$
SO 4	$y = 251 - 0.491x$	$(t_{exp.} = 2.775 > t_{0.05} = 2.365)$
III-102	$y = 273 - 0.871x$	$(t_{exp.} = 5.496 > t_{0.01} = 4.604)$

And, finally, jointly for chlorophylls + carotenoids:

41 B	$y = 926 - 2.868x$	$(t_{exp.} = 5.788 > t_{0.001} = 5.408)$
SO 4	$y = 921 - 1.973x$	$(t_{exp.} = 6.479 > t_{0.001} = 5.408)$
III-102	$y = 1002 - 3.794x$	$(t_{exp.} = 7.503 > t_{0.01} = 4.604)$

Therefore, with all the three rootstocks pigment contents in leaves decreased during the vegetation period. This change was not observed for chlorophyll b (SO 4 and III-102 rootstocks).

It should be noted that during flowering pigment contents in the leaves of the cultivars, grafted on III-102 rootstock, was higher compared to the other two rootstocks, but at the end of the maturation phase it was already lower. Obviously on III-102 rootstock conditions were created for faster shoot maturation, which is one of the premises for its better frost resistance (SLAVCHEVA, unpublished data; POPOV 1988).

A negative correlation ($r > r_{0.001}$) existed between the specific leaf weight and the pigment contents in leaves, both characteristics depended on the time (date), i. e. they changed during the vegetation period.

In conclusion, differences were established in the investigated physiological and other characteristics in relation with variety and rootstock. The obtained knowledge can be utilized in grapevine breeding.

Acknowledgements

The valuable technical assistance of Mrs. A. GADEVSKA and Mr. T. TRIFONOV is gratefully acknowledged.

References

BALCAR, I.; HERNANDEZ, I.; 1988: Translocation of photosynthate in grapevine shoots during the growth period. Vitis **27**, 13-20.

BAROV, V.; NAIDENOVA, P.; 1969: Statistical Methods for Field and Vegetation Experiments [Bulg.]. Zemizdat, Sofiya.

CARBONNEAU, A.; 1976: Principes et méthodes de mesure de la surface foliaire. Essai de caractérisation des types de feuilles dans le genre *Vitis*. Ann. Amélior. Plantes **26**, 327-343.

EIBACH, R.; ALLEWELDT, G.; 1985: Einfluß der Wasserversorgung auf Wachstum, Gaswechsel und Substanzproduktion traubentragender Reben. III. Die Substanzproduktion. Vitis **24**, 183-198.

MUDRA, A.; 1958: Statistische Methoden für Landwirtschaftsversuche. Paul Parey Verlag, Berlin, Hamburg.

ORTH, C.; 1983: Einfluß der Wasserversorgung auf die Assimilattranslokation bei Reben. Diss., Univ. Hohenheim. [Ref.: Vitis **23**, 1 C 3].

POCHINOK, KH. N.; 1976: Methods of Biochemical Analysis of Plants [Russ.]. Naukova Dumka, Kiev.

POPOV, K.; 1988: Investigation on the influence of rootstock III-102 D on the vegetative and reproductive growth of grapevine cv. Merlot [Bulg.]. Lozar. Vinar. **37** (3), 20-22.

-- -- ; DOCHEVA-POPOVA, R.; GEORGIEV, G. KH.; GUSHTEROV, G. K.; 1957: A Practical Handbook of Plant Physiology [Bulg.]. Nauka i Izkustvo, Sofiya.

SLAVCHEVA, T.; 1981: Influence of Some Ecological Factors of Grapevine Photosynthesis [Bulg.]. Thesis, Inst. Viticulture and Enology, Pleven.

-- -- ; 1983: Theoretical curves for determining the leaf area in some grapevine varieties [Bulg.]. Grad. Lozar. Nauka **20** (3), 89-97.

-- -- ; 1987: The effects of planting density and plant formation on the leaf area of cv. Dimiat grapevines [Bulg.]. Rasteniev dni Nauki **24** (12), 92-98.

-- -- ; 1988: The productivity of grapevine (*V. vinifera* L.) and the methods of cultivation. Intern. Symp. Plant Mineral Nutrition and Photosynthesis, Varna, 4.-10.10.1987, Vol. II, 310-314. Bulgarian Academy of Sciences, Sofiya.

STOEV, K. D.; IVANTCHEV, V.; 1977: Données nouvelles sur le problème de la translocation descendante et ascendante des produits de la photosynthèse de la vigne. Vitis **16**, 253-262.

-- -- ; SLAVTCHEVA, T.; 1982: La photosynthèse nette chez la vigne (*V. vinifera* L.) et les facteurs écologiques. Connaiss. Vigne Vin **16**, 171-185.

VASEV, V.; 1974: Influence of the flow of assimilates on the structure of the leaves as photosynthetic apparatus [Bulg.]. In: Plant Physiology, Vol. **2**, 121-133. Georgi Dimitrov Agricult. Acad. Sofiya.

Grape breeding in China

LUO FANGMEI and ZHANG FENGQIN

Beijing Botanical Garden, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Summary: The paper introduces the achievements of grape breeding in China since 1950. So far, around 50 new varieties and many new lines of grape have been produced. Some new varieties are cold resistant, disease resistant with high yield and are suitable for making wine. Some are early maturing with large, seedless berries. Some are suitable for the climate of high humidity and for cultivation in rainy areas of southern China. Some bear large clusters and berries of beautiful appearance and are of high yield and quality. Some are suitable for canning and making juice.

Key words: breeding, variety of vine, Vitis amurensis, China, wine grape, table grape, juice, yield, must quality, maturation, frost resistance, disease resistance, salt resistance, ecology.

Although viticulture has a history of over 2000 years in China, grape breeding was only started in the early 1950s. So far, the best method for breeding new varieties of grape is cross-breeding. Therefore interspecific hybridization and intervarietal hybridization are carried out widely at different institutions and colleges all over China. Around 50 new varieties and many new lines of grape have been produced through selection, evaluation and cultivation experiments in the past 40 years and remarkable success has been achieved.

In 1951, Jilin Institute of Pomology produced Gongniang No. 1 (Muscat Hamburg x *Vitis amurensis*) and Gongniang No. 2 (*V. amurensis* x Muscat Hamburg).

Gongniang No. 1 is a highly cold resistant variety which can overwinter safely without being buried for protection, even in places where the lowest temperature reaches -22 °C. It is also a disease resistant variety with high yield and good quality. Tonghua Winery of Jilin Province won a prize in the National Wine Competition 1984, for its 'Princess Red Wine' made from grapes of Gongniang No. 1. This variety is cultivated in large areas in Jilin, Heilongjiang, Liaoning, Gansu Provinces and the Inner Mongolia Autonomous Region.

The grape variety Heishan was produced by Xingcheng Institute of Pomology, Chinese Academy of Agricultural Sciences by crossing Black Hamburg and *V. amurensis*, and Shanmeigui was produced by crossing Muscat Hamburg and *V. amurensis* in 1952.

Beichun, Beimei and Beihong were produced by crossing Muscat Hamburg and *V. amurensis* at the Beijing Botanical Garden, Institute of Botany, Chinese Academy of Sciences in 1954. Beichun, in particular, is a disease resistant variety with high yield, cold resistance and high sugar content in fruits, suitable for making red wine. It can overwinter safely without being buried for protection in northern China. It is also disease resistant, tolerant to humid condition and very adaptable to rainy areas of southern China. It is distributed everywhere in China.

Besides these, several table grape varieties were bred by the Beijing Botanical Garden in 1960 through hybridization between varieties. These include Jingzaojing (Queen of the Vineyards x Thompson Seedless) and Jingkejing (French Blue x Black Monukka), which are seedless and early maturing varieties, Jingyu (Italia x Queen of the Vineyards), which is an early maturing variety with large berries, and Jingfeng (Queen of the Vineyards x Black Monukka), which is a late maturing variety with high yield. Among these, Jingzaojing is outstanding, producing large and handsome clusters with berries bigger than those of Thompson Seedless. The berries are oval, yellowish-green, with thin skin, crisp pulp, and of high quality, weighing on average 2.6 g. It is also suitable for making raisins and for canning. It is cultivated in large areas in Xinjiang, Gansu and Inner Mongolia of China.

In 1956, Hongshan Horticulture Farm, Pingdu County in Shandong Province produced the new table grape varieties Zexiang, Zeyu and Zefeng by crossing Muscat Hamburg and Longyan. The variety Zexiang is disease resistant and has high yield. Clusters are medium and conical; berries are medium, elliptical or oval, weighing on average 5.6 g each, yellowish-green, with strong muscat flavor similar to Muscat Hamburg, and of good quality. It has been cultivated widely in Shandong Province.

In 1957, breeding work for wine varieties was started in the Shandong Institute of Wine Grape. The main target was breeding varieties which are good for making high quality red or white wine and suitable for cultivation in the area of middle and lower reaches of the Yellow River. At the same time, breeding for table grape varieties and varieties for canning and juice-making was also carried out at this Institute. Varieties produced include Quanbai (Riesling x Verdot) and Quanyu (Riesling x Muscat Hamburg), suitable for making dry white wine, Meiyu and Meinong, suitable for making dry red wine, Meichun, suitable for making red wine and Hongzhilu, suitable for making red wine and for pigment. The parents of the last four varieties are Merlot and Verdot. In addition, the quality variety Baotuhong which is cold resistant, disease resistant and suitable for making red wine was produced at this Institute by crossing Sweet Water and *V. amurensis* in 1964. These varieties all have high yield and the wines made from them are of good quality. They are favoured by growers and are widely distributed.

Table grape varieties, Shandong Zaohong, Zaohuang, Quanlongzhu and Honglianzi were bred at the same Institute by crossing Muscat Hamburg and Queen of the Vineyards in 1963, Cuihong and Hongxiangjiao were bred by crossing Muscat Hamburg and Triumph in 1964. Shangdong Zaohong is a good variety producing medium clusters, with medium berries weighing on average 4-5 g each. It is a disease resistant variety with high yield and the berries ripen in early July in Jinan, Shandong Province. It is an early maturing variety of good quality, the colour, flavor and taste of its berries are just like those of Muscat Hamburg. It is a favorite of people in large areas and is being disseminated.

In 1960, The Institute of Saline-Alkali Soil Utilization in Liaoning Province produced Zifeng (Black Hamburg x Niagara), which has the distinguished feature of tolerating saline-alkali conditions and can grow normally in a soil with 0.22 % salinity.

A F_1 hybrid progeny produced from Muscat Hamburg x *V. amurensis* was obtained in 1961 by Agronomy College in Xiongyue of Liaoning Province. A new cold resistant variety Xiongyuebai was bred in 1967 with Longyan as the maternal parent, and the best hybrid progeny of Muscat Hamburg x *V. amurensis* as the male parent. It has been evaluated as a fine variety suitable for making high quality white wine through selection, cultivation trials and experience with wine making for several years. It is presently very popular.

In 1962, Zhengzhou Institute of Pomology, Chinese Academy of Agricultural Sciences bred Zhengzhou Zaohong (Muscat Hamburg x Pearl of Csaba), and Heijianiang (Seibel No. 2 x Carignane), the latter shows strong disease resistance, uniform maturity and produces ruby-coloured high-quality wine. These two varieties are now popular in the area of the former course of the Yellow River.

In 1963, Zaomeigui was produced by the Department of Horticulture, Northwest Agricultural University by crossing Muscat Hamburg and Pearl of Csaba. It is an early maturing variety, with grapes harvested in early July in Baoji of Shaanxi Province. Its yield is higher than that of Pearl of Csaba. The berries are purple-red, with strong muscat flavor and the quality is also higher than that of Pearl of Csaba.

In 1963, Baimeikang and Zimeikang were bred by the Department of Horticulture, Jiangxi Agricultural University by crossing Muscat Hamburg and Campbell Early, Meiye was bred by crossing Muscat Hamburg and *V. flexuosa*, and Meiyehei by crossing Meiye and Black Hamburg. These varieties are adapted to climates of high humidity.

In order to solve the problem of insufficient supply of grapes with rich pigments to the wine making industry, two new good varieties, Yan No. 73 and Yan No. 74 (Alicante Bouschet x Muscat Hamburg), which bear dark-colour berries, were bred by Yantai Wine-making Company in 1966. These two varieties played a positive role in adjusting wine colour and improving wine quality.

In 1973, the Institute of Forestry and Pomology, Beijing Academy of Agricultural Sciences started to breed table grape varieties. The target was to produce early-maturing or seedless varieties. Zaomanao, Cuiyu and Yanhong were bred by crossing Muscat Hamburg and Jingzaojing; Zizhenzhu was bred by crossing Muscat Hamburg and Pearl of Csaba. Among them, Zaomanao has high yield, large clusters and very beautiful, medium-sized berries, weighing 4.2 g each on average. The berry is oblong, purple-red, and of high quality, with thin skin, crisp pulp and sweet taste. These four varieties were already being grown on a trial basis in Beijing, Liaoning, Jilin, Ningxia, Gansu, Shandong, Henan and Hebei Provinces.

In 1973, the Institute of Pomology, Shanxi Academy of Agricultural Sciences produced a new table grape variety, Guibao, by crossing Ispissar and Muscat VIRa. The main characteristic of this variety is the large and uniform cluster with beautiful appearance. The berries are also large, purple-red, with crisp pulp and strong muscat flavor. It produces high yields of good quality. The berries do not drop or split and tolerate transportation. The variety is suitable for cultivation in Shanxi Province.

Different new grape varieties were produced by Dalian Institute of Agricultural Sciences in 1973 through hybridization between varieties. Some bear extra large berries of beautiful appearance, produce high yields and are of quality; some bear seedless, large berries, produce high yields and are of quality; some are suitable for making wine or for canning; some have strong cold resistance and disease resistance; such as Jifeng (Kyoho x Jixiang), Fenghuang No. 12 (Muscat of Alexandria x Flame Tokay and Pobeda), Fenghuang No. 51 (Muscat of Alexandria x Cardinal), Meigui Seedless (Muscat of Alexandria x Jingzaojing), etc. Among them, Fenghuang No. 51 is an early maturing variety, its clusters and berries are large, berries are of special shape, purple-red, with thin skin, thick pulp, and sour-sweet flavour. The berries do not split or fall off, tolerate transportation, and are of fine texture. In short it is a variety of high quality and high yield, which has been well received by a great number of cultivators. Its area of cultivation is expanding.

A number of institutions have engaged in grape breeding, they are: Institute of Pomology, Academy of Agricultural Sciences in Shanxi; Shandong Agricultural University; Shanxi Agricultural University; Jilin Institute of Special Local Products; Institute of Horticulture, Xinjiang Academy of Agricultural Sciences; Shanshan Research Center of Grape, Melon and Fruits Exploitation of Xinjiang; Institute of Horticulture, Shanghai Academy of Agricultural Sciences; Inner Mongolian Institute of Horticulture; Changli Institute of Pomology in Hebei Province; and others.

These institutions have not only bred new varieties, but also studied the genetics of parents and hybrid progenies, including the inheritance of cold resistance, flower types, phenological phases and economic characters of fruits of progenies of hybrids between different species. These studies and analyses provided guidelines for cross-breeding and parent selecting.

Chinese grape breeders agree that the native wild species of China, *V. amurensis*, is an excellent cold resistant parent for breeding cold resistant grape varieties. Some of the F_1 hybrids produced can overwinter safely without being buried for protection even at -29 °C. When repeated hybridization was performed between cultivars and F_1 hybrids, the cold resistance decreased, but the characters and quality of the fruits were improved.

V. amurensis is dioecious. In the 1950s the Beijing Botanical Garden hybridized hermaphrodite cultivars and male plants of *V. amurensis*. As the result of this hybridization, 50% male flowers appeared. Recently, a hermaphrodite-flowered form of *V. amurensis* was obtained in

China. Jilin Institute of Pomology has crossed the hermaphrodite form of *V. amurensis* with hermaphrodite cultivars, and the ratio of hermaphrodite flowers in the progeny was more than 70 %. When the female-flowered form of *V. amurensis* was crossed with the hermaphrodite cultivars, the ratio of hermaphrodite flowers in the progeny was 50 %. Therefore, the hermaphrodite form of *V. amurensis* is the best parent for breeding a cold resistant variety.

When *V. amurensis* was crossed with early maturing or late maturing varieties, the progeny tended to be mid-late or late maturing, neither early maturing nor extremely late maturing progeny appeared.

The cluster of *V. amurensis* is loose, berries are small, and the berries of *labrusca-vinifera* hybrid varieties have strawberry or foxy flavor. Since the fragrance of strawberry affects the quality of wine, *vinifera* varieties which are of high yield and could make good wine were selected as parents to be crossed with *V. amurensis* for breeding cold resistant wine varieties. No *labrusca-vinifera* hybrid variety was selected.

The inheritance tendency of wild characters of *V. amurensis* is strong. Cultivars which bear large and high-quality fruits should be selected to cross with *V. amurensis* for breeding cold resistant table grape varieties. After that, the excellent plants among the progeny should be selected for repeated hybridization.

Experiments have demonstrated that Pearl of Csaba and Queen of the Vineyards are good parents for breeding early maturing table grapes. Jingzaojing, Thompson Seedless, Black Monukka are good parents for breeding seedless grapes. Muscat Hamburg, Muscat of Alexandria, Black Hamburg and Flame Tokay are good parents for breeding table grape varieties. *Vinifera* varieties which are of high yield and good for making wines should be selected for breeding wine varieties.

These are the main achievements of grape breeding in China. Research is continuing in many Chinese institutions. Results of such research will be published in different pomological or horticultural journals. Any suggestion will be appreciated.

References (translated titles)

FAN, BANGWEN *et al.*; 1985: The biological characteristics of new grape cultivars, Baimeikang, Zimeikang, Meiye and Meiyehei. Acta Agriculturae Universitatis Jiangxiensis 4, 27-32.

FENG, YIBIAO *et al.*; 1981: Yan No. 73 and Yan No. 74 – new good cultivars of pigment grapes. Science and Technology of Grape 3, 6-8.

HE, NING *et al.*; 1981: Inheritance of characteristics of grape vines in breeding for cold resistance by interspecific hybridization. Acta Horticulturae Sinica 1; 1-8.

HE, PUCHAO *et al.*; 1980: Results of the test on grape cv. Zaomeigui on Baoji Horticultural Farm. Science and Technology of Grape 2, 8-10.

LI, JIANYI *et al.*; 1985: Zifeng, a new good cultivar of grape. Liaoning Fruits 2, 38-40.

LI, SHENGCHEN *et al.*; 1983: Thirty years of grape introduction and acclimatization. Collected Papers on Plant Introduction and Acclimatization 3, 11-17.

LI, YIYUAN *et al.*; 1987: Breeding for new cultivars of table grape. China Fruits 2, 11-12.

LI, YIYUAN *et al.*; 1987: Zaomanao, Zizhenzhu, Cuiyu and Yanhong – new grape varieties. Acta Agriculturae Boreali-Sinica 2 (3), 90-98.

LI, XIANJUN *et al.*; 1985: Characteristics and cultivation technique of grape cv. Zifeng. Viticulture and Enology 3, 12-14.

OUYANG, SHOURU *et al.*; 1989: Experiment of selecting and breeding table grape cv. Guibao. Shanxi Fruits 1, 2-5.

SHANDONG EXPERIMENTAL STATION OF VITICULTURE; 1978: New cultivars of grape. Science and Technology of Grape 1, 8-9.

SHANDONG EXPERIMENTAL STATION OF VITICULTURE; 1979: An introduction of new grape cultivars for wine-making. Science and Technology of Grape 3, 4-6.

SHANDONG INSTITUTE OF WINE GRAPES; 1985: New grape cultivars for wine-making. Viticulture and Enology 4, 1-3.

SHAO, JIYUAN *et al.*; 1981: The description of three new cultivars of grape. Shandong Fruits 2, 8-11.

WU, DELING *et al.*; 1981: Tendency of characteristics inheritance of grapes in breeding for cold resistance. Science and Technology of Grape 3, 1-5.

WU, JINGJING; 1988: Some promising lines of grape vines. Shanxi Fruits 3, 27-28.

WU, JINGJING; 1989: Report on the breeding of Fenghuang No. 51, a new cultivar of early grape with large berries. North China Fruits 1, 22-26.

ZHANG WEICHANG; 1987: Experiment report on breeding grape cv. Xiongyuebai. Viticulture and Enology 4, 4-9.

Section 3: Resistance/tolerance to pests and diseases

Grape selection for resistance to biotic and abiotic environmental factors

N. I. Guzun, P. N. Nedov, V. T. Usatov, I. A. Kostrikin and L. F. Meleshko

The Moldavian Research Institute for Viticulture and Enology 'Virul', 59, Virul Street, Kishinev, 277034, USSR

Summary: Most of the viticultural regions of the USSR are located under conditions of limiting biotic and abiotic factors, with frosts, drought, fungal diseases, phylloxera, mites, grape berry moths and some others being of primary importance. The main breeding organizations have been creating for more than 40 years new table and wine cultivars with complex resistance according to long-term programs. These cultivars are own-rooted and capable of wintering in outdoor culture with a limited amount of spray treatments, if any.

In crossing, Amur grape and its hybrids, cultivars Seibel and Seyve Villard and some others are used as donors of resistance. Using biophysical and cytoembryological methods, gametes are treated with physical and chemical mutagenic factors in order to increase the variability range of F_1 seedlings, aiming at higher efficiency of selection. The process of selection is accelerated if seedlings are grown hydroponically. Analysis of the F_1 hybrid population determines the nature of the inheritance of valuable agricultural characters and the selection of pairs. The *in vitro* method is used when seedlings are grown from non-vital seeds, callus embryoids and in accelerated propagation of valuable genotypes providing virus and bacteria elimination.

More than 50 cultivars with complex resistance have been bred during 35 years. More than 10 of them have been recommended for culture (Moldova, Lyana, Vostorg, Sukholimanski biely, Pervenets Magaracha, and others), while the remainder are being tested in different viticultural regions of the Soviet Union.

Key words: genetics, breeding, selection, analysis, resistance, cold, drought, virus, bacteria, fungus, pest, Vitis, variety of vine, USSR.

Once in 10-12 years grapevines in the vineyards of the Black Sea regions are damaged by frosts and dangerous winters, leading to production losses in commercial plantings. During years with rainy summer outbreaks of diseases (downy mildew, oidium, anthracnose, grey rot) and pests (spider mites, grape berry moths etc.) are observed, which reduce yield quantity and quality. Under these conditions, classical varieties do not always produce stable yields. New varieties with improved resistance to limiting factors of biotic and abiotic nature are necessary.

In the scientific centres dealing with viticulture (Kishinev, Odessa, Yalta, Novocherkassk, Erevan), more than 30 years of basic research and long-term grape breeding programmes have performed to meet requirements of modern industrial viticulture. A variety model is elaborated for each region. The requirements for new varieties depend on the consumer's needs and taste, the industry's plan and the cultivation methods to be used. Quality characters combined with characters of resistance to low temperatures, dangerous fungal diseases and pests, necessary for each region, are accounted for in the 'variety model' and the selection procedure. Selection criteria include: for table varieties – appearance, taste qualities, transportability, ability to long-term storage, short vegetation period; for wine varieties – high sugar content, short vegetation period, high juice yields, suitability for mechanized harvest, colour quality and stability of wines, combined with quality characters peculiar to varieties which are already recommended.

The next step of the programme is the identification of parent material for breeding new varieties. Successful selection of parent pairs provides with a high variability of characters, plasticity of hybrid progeny and results in elite selections. It has been determined that crosses involving the species *V. cordifolia, V. longii, V. cinerea, V. monticola, V. berlandieri, V. riparia, V. rupestris, V. amurensis* result in varieties which are bearers of complex characters of resistance. *V. vinifera* varieties of the Black Sea basin group are used as donors of physiological factors of high adaptation to growing conditions. Some varieties from Georgia have higher resistance to downy mildew, grey rot and phylloxera (E.I. Chamagua, V.P. Gotserydze). Varieties of the West European group are donors of high quality. Varieties of the eastern group are grown for transportable table grape

varieties suitable for storage. R. Seibel's and Seyve Villard's interspecific hybrids give the possibility of advancing the creation of complex resistant varieties (frost, fungal diseases, root phylloxera, etc.).

Analysis of our investigations demonstrated that the method of introgressive selection, application of interspecific hybridization, using physical and chemical mutagenic factors increase the variability spectrum in the F_1 generation. The following rules are used in hybridization:

1) Varieties obtained as a result of the latest selections are used as donors of resistance.

2) Selection of parent pairs is directed to growing new varieties for their use in special purpose (early table grape, champagne trend, cognac etc.).

3) Analysis of the F_1 generation (elements of genetics by economic valuable characters) is carried out using accelerated methods of growing seedlings in conditions of closed hydroponics (NPO 'Vierul'). On this basis, the combining ability of parent pairs is determined, allowing more effective selection.

4) Seedling (elite) selection is carried out on the basis of careful evaluation of plants in regards to quality (micro-vinification, organoleptic evaluation), resistance to winter frost, fungal diseases and phylloxera.

5) Production testing of candidates in varieties under different climatic conditions, transferring the best of them to state variety test.

The heritability of malvidin-diglucoside content shall be especially mentioned. Scientific work carried out in Novocherkassk and Kishinev on this problem showed that heredity of oak and colouring properties in the berries of F_1 hybrids occurred with clear division into classes, which was evidence of discrete character heritability. Malvidine content is inherited independently. In some combinations, elite selections are obtained, which have no trace of malvidine. Variability amplitude of the given group of chemical combinations shows the polygenic character of inheritance. When crossing *V. vinifera* and *V. amurensis*, seedlings both with and without diglucosides are obtained in the first generation. It may be supposed that it depended from a 'dose effect', introduced by the European component and the possibility of partial or complete regression of a 'wild type' genes effect from the Amur grape.

In the past 25-30 years more than 500,000 seedlings have been obtained at the selectional centres of the USSR. 80 of these genotypes have been proposed to state for advanced testing and 25 varieties have been registered until 1989.

Grapevine resistance to pests and diseases

In 'Plant Immunity to Parasitic Diseases' by N. I. Vavilov (1935) it was noted that the most effective control method against pests and diseases of agricultural plants is breeding and introduction of resistant varieties into production. The basic method of selecting agricultural plants for resistance in general and in grapevine especially includes determination of susceptibility of initial breeding material (species, present interspecific hybrids and cultured varieties) for use of resistant genotypes as donor parents.

For providing the resistance value of collection species, complex interspecific hybrids and *Vitis* varieties as well as progeny forms received from different crossing combinations it appeared possible to carry out investigations in the next basic ways:

1) Investigations on the nature of resistance (factors causing non-susceptibility) of grapevine to main harmful organisms.

2) Exposure of correlated connections between resistance factors and outer manifestation of resistance under field conditions.

3) Elaboration of methods for forming artificial and natural infectious background for carrying out resistance evaluation of parent components and hybrid progeny and creation of reliable score scales for determining the degree of resistance of species, varieties and selection elites of grapevine to pathogenic organisms and pests.

4) Investigations of resistance genetics exposing regularity of inheritance of characters of resistance and quality for their using in selection for resistance in future.

Above-mentioned investigations have been carried out and are being continued by scientific workers of Plant Protection Department, immunologists of Moldavian Institute of Viticulture NPO 'Vierul' and Phytopathology Department of Kishinev Agricultural Institute (D.D. VERDEREVSKY, K.A. VOITOVICH, I.N. NAIDYONOVA, P.N. NEDOV, A.P. GULER, G.E. VESMINSH, L.F. SUPOSTAT, E.A. KYABURU, O.S. REBEZA, PEREPELYTSA, and others). Investigations on grape selection are continued by scientific workers of Selection Department of Moldavian Research Institute of Viticulture and Enology NPO 'Vierul' (M.S. JURAVEL, G.M. KARADJY, N.I. GUZUN, M.V. TSYPKO, G.M. BORZYKOVA, F.A. OLARI, G.A. SAVIN, A.D. POPOV, I.P. GAVRILOV, and others).

Investigations on resistance of grapevines to pathogenic organisms and pests during the last 10 years yielded the following principle results:

1) The presence of well expressed resistance of *Vitis* varieties to pathogens of main grapevine fungal diseases has been determined. These included: downy mildew (*Plasmopara viticola* BERL. et DE JONI), oidium (*Uncinula necator* BURR, *Oidium tuckeri* BERK.), grey rot (*Botrytis cinerea* PERS.), anthracnose (*Gloeosporium ampelophagum* SACC.), and brenner (*Pseudopeziza tracheiphila* MÜLL. THURG.). No resistance (at degree needed for selection) to spot necrosis pathogen (*Racodiella vitis* STERENB., *Mollisia vitis*) was discovered. All grapevine species and varieties presented in ampelographic collection are affected almost to an equal degree by this disease under conditions favourable for pathogen development.

Chronic diseases of bacterial and viral etiology occupy a special place. As to bacterial canker (*Agrobacterium tumefaciens* CONN.), it was determined that there are certain different qualities of resistance to this disease among *Vitis* species, leading to tumor formation. However, all species, interspecific hybrids and European grapevine varieties may be potential infection bearers. And analogy to harmful virus diseases of grapevine was noted. As to chronic diseases of viral and bacterial origin, phytosanitary selection methods may be applied for obtaining healthy clones to be used in breeding of virus and bacterium free plantings. Virus free and bacterial canker tested clones are widely planted and intensively bred.

2) Anatomical-morphological, physical-biochemical and antimicrobial factors responsible for non-susceptibility of grapevine to fungal diseases have been exposed. The principal ones are: activity of oxidation-reduction processes, identity of readyly soluble protein fractions of plant tissue and parasites, as well as some antimicrobial tissue properties.

3) A method of resistance evaluation using 5-score scales of resistance to fungal diseases was developed and applied to determine the degree of resistance of approximately 2500 species, interspecific hybrids and European grapevine varieties. Several species, interspecific hybrids and a few varieties which were distinguished by resistance to fungal diseases are used successfully as donors in resistance selection.

4) Factors resulting in grapevine resistance to the principal pests were studied; these are: root and grape phylloxera (*Dactylosphaera vitifolii* SHIMER), red spider mite (*Panonychus ulmi* KOCH), grape berry moths (*Lobesia botrana* DEN. et SCHIFF. and *Eupoecilia ambiguella* HB.). It was determined that the character and intensity of rot process played a principal role in destruction of roots after phylloxera damage. We came to the conclusion that different (by virulence) grape phylloxera races are absent. Different damage degree of the same species is connected to the physiological-biochemical state of the plant but not to the presence of races differing in aggressiveness. Morphological leaf features and some biochemical factors, referring 'preferance or non-preferance' of tissues for feeding are the main factors of resistance to red spider mite. As to phylloxera (root and grape) and red spider, well evident resistance differentiation was shown. Investigations on the resistance to grape berry moths led to the conclusion that within the genus *Vitis* there are insignificant differences in susceptibility. It was also noted that an important factor in

comparable resistance of grapevine varieties to grape berry moths are aromatic constituents, which are contained in the flowers and act as attractants to these pests.

5) Laboratory, vegetative and field evaluation methods for selection of genotypes resistant to fungal diseases and to pests were developed; in this case 5-score scales were used for mathematical processing of the obtained data. The results showed that in the F_1 generation resulting from different crossing combinations of interspecific hybrids and high quality European grape varieties, resistances vary within wide limits and are inherited polygenic dominantly independently (in many cases) from each other exposing heterosis and transgression.

In general it may be concluded that there are no absolute genetic limits for obtaining varieties recombinants having complex field resistance to abiotic and biotic factors in combination with fruit quality similar to that of European varieties. Selection practice of resistant varieties which is carried out in different regions of the USSR and countries of Council for Mutual Economic Assistance (CMEA) testify to this. As a result of resistance breeding, the following varieties are being cultivated or undergoing advanced testing: table grape varieties: Moldova, Juravel's Jubilee, Kodryanka, Frumoasa alba, Suruchensky bely, Vierul-59, Vostorg, Agat donskoy, Lanka, Tayr, Muscat odessky; wine varieties: Vioryka, Muscat de Yaloveny, Plai, Negru de Yaloveny, Golubok, Rubin tairovsky, Karin, Adysy, Pervenets Magaracha, Antey, etc. These varieties may be cultivated using one to two pesticide sprayings, or without chemical protection, are resistant to low temperatures down to -26 °C, regenerate fruiting shoots after severe winters, have stable annual yields equal in quality to those of regionalized varieties, some of them are tolerant to root phylloxera and may be grown in own-rooted culture.

In the USSR, work in grapevine selection is done in close collaboration with scientists of Bulgaria, Hungary, Romania, Czechoslovakia. An active exchange of selection material and new varieties takes place.

Resistance to transmission of grapevine fanleaf virus by *Xiphinema index* in some *Vitis* species and hybrids

G. STAUDT and H.-H. KASSEMEYER

Staatliches Weinbauinstitut Freiburg, Merzhauser Str. 119, D-7800 Freiburg, F. R. Germany

Summary: Many vineyards in Germany are infested by nematodes which can transmit virus diseases. Breeding of rootstocks resistant to nematode feeding and virus transmission is an important way to control these virus diseases.

A method has been developed for testing breeding stocks for these characteristics with regard to grapevine fanleaf virus (GFV) and its vector *Xiphinema index*.

The plants to be tested were first grown together in a single pot with both GFV-infected plants of *Vitis* cv. Siegfried and plants of the same cultivar which were virus negative according to an ELISA test: 2 months after planting, the pots were inoculated with about 50 nematodes. In a second experiment, single healthy plants of different hybrids and species were potted and inoculated with about 200 nematodes carrying GFV. After 3-10 months, roots were inspected visually for swellings and galls and tested for the presence of GFV by ELISA.

In all cases, the previously healthy cv. Siegfried showed symptoms of feeding on the roots and these roots showed a positive reaction to the ELISA test. After 6 months, GFV could also be detected by ELISA in the basal parts of the stems.

The reaction of the test plants was dependent on their genotype. A high susceptibility to feeding by the nematodes and high percentage of transmission to GFV was displayed by American species and interspecific hybrid rootstocks. One of the *V. vinifera* x *V. rotundifolia* hybrids showed no visual symptoms of nematode feeding and no virus transmission 9 months after inoculation.

Key words: Vitis, variety of vine, root, fanleaf, virus, nematodes, Xiphinema index, vector, transmission, resistance, bioassay.

Introduction

Many vineyards in Germany are infested by nematodes which can transmit viruses. The most serious viral pathogens are:

grapevine fanleaf virus (GFV)
arabis mosaic virus (ArMV)
raspberry ringspot virus (RRV)
tomato blackring virus (ToBRV)
strawberry latent ringspot virus (SLRV)

These so-called nepoviruses are transmitted by different species of nematodes, of which *Xiphinema index* is perhaps the most important one due to its known ability to transmit GFV and to its worldwide distribution. It is widely accepted that ArMV is vectored by *X. diversicaudatum*, raspberry ringspot virus by *Longidorus macrosoma*, and tomato blackring virus by *L. attenuatus*.

For many years German grape growers were quite successful in controlling virus diseases transmitted by nematodes with pre-plant soil fumigation. However, in recent years efficient nematicides have become unavailable. Breeding rootstocks resistant to nematodes now appears to be the sole solution to the problem of nematode transmitted virus diseases.

As pointed out by KUNDE *et al.* (1968) and in accordance with ROHDE (1965), COOK and EVANS (1987) and MÜLLER (1989), resistance against nematodes can be defined as an interaction between the nematodes and grapevines which retards or prevents maturation and/or reproduction of the nematodes. Damage of feeding of the parasitic nematodes is not the problem in Germany, but rather virus transmission. Therefore, only an extreme of resistance, which could be called absolute or high resistance, is the type of resistance we are looking for. Some authors, for example HARRIS (1983), used the term immunity for this type of resistance. But, as immunity is widely used with describing antigen-antibody reactions, this term should be avoided in plant pathology.

The production of swellings and/or galls is a reaction of the host plant and therefore a question of sensitivity to the attack of the nematodes. Differences occur with respect to this characteristic between species and cultivars, but this reaction should not be regarded as a type of resistance. Plants which show no reaction or decline in yield are called tolerant. As pointed out earlier, we are not interested in tolerant plants because virus transmission is the paramount problem and this can only be solved satisfactorially by absolutely resistant rootstocks or such plants which do not allow virus transmission.

Any breeding project should begin with screening the collections for resistance. In regard to resistance against the dagger nematode *X. index*, this has already been done bgy various authors such as KUNDE *et al.* (1968), BOUBALS and PISTRE (1978), BOUQUET (1981) and COIRO *et al.* (1985). In regard to transmission of viruses the results are not always convincing. KUNDE *et al.* rated *V. arizonica* and *V. candicans*, in addition to other species, as resistant. According to the investigations of WEISCHER (1980) these are only tolerant. This has been confirmed by our recent investigations which showed that GFV could easily be transmitted to *V. arizonica* by *X. index*.

Materials and methods

Tests for nematode resistance with absolute resistance in view should only be conducted under controlled application of nematodes, as described by BOUBALS and PISTRE (1978) and BOUQUET (1981). The goal of our investigations was to develop a method with which plants could be screened for nematode resistance and/or virus transmission within a reasonable time.

All plants tested were *in vitro* propagated and therefore absolutely free of nematodes and GFV. In our initial experiments, a plant to be tested was grown together in a single pot with a GFV infected plant of cv. Siegfried, which is an interspecific hybrid very sensitive to nematode feeding and to which it is very easy to transmit GFV. Another plant of the cv. Siegfried, which was virus negative according to an ELISA test, was planted in the same pot. 2 months after planting, the pots were inoculated with 20 ml soil containing about 50 nematodes. Occurrence of nematode feeding and subsequent transmission of virus was monitored by the virus negative Siegfried plant.

In our further experiments the pots with single plants to be tested were inoculated directly with ca. 200 viruliferous (GFV) nematodes. After different lengths of time, roots were inspected visually for swellings and/or galls and tested for the presence of GFV by ELISA.

Results and discussion

Already 3 months after inoculation it was possible to make definite statements about the host reaction and virus transmission as well. This was, for example, the case for *V. rupestris, V. riparia* and the rootstocks Kober 5 BB and 125 AA. There were certain genotypes which needed further investigation or needed a longer exposure to nematode feeding to assure an accurate rating.

In the table results are summarized which were collected over a period of 3-10 months of exposure to nematodes. A high susceptibility to feeding by *X. index* and high percentage of transmission of GFV was displayed by:

the rootstocks:

cv. Kober 5 BB	*(V. riparia x V. berlandieri)*
cv. Kober 125 AA	*(V. riparia x V. berlandieri)*
cv. FR 419	a newly released cultivar with *V. cinerea* in its pedigree

the interspecific hybrids:

cv. Siegfried	
cv. FR 993-60	one of the most promising selections for wine production (STAUDT *et al.* 1984) and

Transmission of GFV by *Xiphinema index* to *Vitis* species and cultivars within 3-10 months

Species/cultivar	Number of plants tested	Number of GFV infected plants	% Infection	Symptoms of roots to nematode feeding
cv. Kober 125AA	26	26	100	+
cv. Kober 5BB	26	26	100	+
cv. FR 419	16	16	100	+
cv. Siegfried	120	95	79	++
cv. FR 993-60	53	45	85	++
V. arizonica	15	9	60	-
V. riparia gloire	24	22	92	+
V. rupestris	37	35	95	++
No. 030-51	110	64	58	+
cv. Riesling	26	9	35	++
No. 043-43	62	10	16	+
No. 039-16	60	0	0	-

the species:

V. arizonica
V. riparia
V. rupestris

No. 030-51 which is identical with *V. vinifera* #4 from the Middle East (WALKER *et al.* 1985).

There seems to be a reasonable resistance to virus transmission in cv. Riesling and this is being further investigated.

As expected, the two *V. vinifera* x *V. rotundifolia* hybrids obtained by OLMO (1954) (PATEL and OLMO, 1955; JELENKOVIC and OLMO 1968) showed the best resistance ratings. Only 10 out of the 62 plants tested of No. 043-43 showed virus transmission as a result of nematode feeding. This result, similar to that of cv. Riesling, but to a lesser extent, is as yet unexplained. It may be accounted for by a reduced attraction of nematodes by the roots, or to a reduced transmission or replication of the viruses.

The highly resistant No. 039-16 showed visually no symptoms of nematode feeding at all. Up to now, we do not know whether this really can be attributed to prevention of nematode feeding, virus transmission or virus replication.

Our investigations under way are in favor of the first explanation. According to recent results of WEISCHER (1988), the failure of virus transmission to *V. rotundifolia* by *X. index* may be attributed to a sensitivity reaction which prevents virus replication and/or virus distribution. This would mean that we can reckon with sources of resistance against nematode feeding and virus transmission in *V. rotundifolia* and their hybrids.

Both hybrids are already patented by the University of California Davis and recommended for planting (WALKER *et al.* 1989). Unfortunately, these hybrids cannot be used as rootstocks in Germany. Poor adaptation to our climatic conditions is the main handicap. But there are also some difficulties in using No. 039-16 in our breeding program. Cytological disharmonies in this hybrid, which may result from the different chromosome numbers of the parent species, lead to a serious reduction in fertility. Pollen fertility of No. 039-16 is below 1 % and the pollen grains, which seem to be functional, are giant pollen grains which may have originated by restitution during meiosis. As a consequence, they may have the doubled somatic chromosome number.

From the investigations conducted to date it can be concluded that resistance to nematode feeding and virus transmission is a rare characteristic and screening relevant species will be necessary to search for further sources of resistance.

Literature

BOUBALS, D.; PISTRE, R.; 1978: Résistance de certaines Vitacées et des porte-greffes usuels en viticulture au nématode *Xiphinema index* et à l'inoculation par le virus du court-noué (GFLV). Génétique et Amélioration de la Vigne, INRA, Paris, 199-207.

BOUQUET, A.; 1981: Resistance to grape fanleaf virus in Muscadine grape inoculated with *Xiphinema index*. Plant Dis. **65**, 791-793.

COIRO, M. I.; LAMBERTI, F.; BORGO, M.; EGGER, E.; 1985: Reproduction of *Xiphinema index* on different grapevine rootstocks. Phytopathol. Medit. **24**, 177-179.

COOK, R.; EVANS, K.; 1987: Resistance and tolerance. In: BROWN, R. H.; KERRY, B. R. (Eds.): Principles and Practice of Nematode Control in Crops. Academic Press.

HARRIS, A. R.; 1983: Resistance of some *Vitis* rootstocks to *Xiphinema index*. J. Nematol. **15**, 405-409.

JELENKOVIC, G.; OLMO, H. P.; 1968: Cytogenetics of *Vitis*. III. Partially fertile F_1 diploid hybrids between *V. vinifera* L. x *V. rotundifolia* MICHX. Vitis 7, 281-293.

KUNDE, R. M.; LIDER, L. A.; SCHMITT, R. V.; 1968: A test of *Vitis* resistance to *Xiphinema index*. Amer. J. Enol. Viticult. **19**, 30-36.

MÜLLER, J.; 1989: Zur Definition von Resistenz und anderer Fachbegriffe in der Nematologie. Nachr.bl. Dt. Pflschutzd. **41**, 137-139.

OLMO, H. P.; 1954: L'hybride *vinifera x rotundifolia* et sa valeur en obtention. Bull. OIV **27** (278), 68-75.

PATEL, G. I.; OLMO, H. P.; 1955: Cytogenetics of *Vitis*: I. The hybrid *V. vinifera x V. rotundifolia* MICHX. Amer. J. Bot. **42**, 141-159.

ROHDE, R. A.; 1965: The nature of resistance in plants to nematodes. In: Symposium on Nematology. Phytopathology **55**, 1159-1162.

STAUDT, G.; BAUER, O.; KOEPCHEN, W.; 1984: Resistenzzüchtung bei Reben. Dt. Weinbau **39**, 1538-1543.

WALKER, M. A.; MEREDITH, C. P.; GOHEEN, A. C.; 1985: Sources of resistance to grapevine fanleaf virus (GFV) in *Vitis* species. Vitis **24**, 218-228.

--- ; WOLPERT, J. A.; VILAS, E. P.; GOHEEN, L. A.; LIDER, A. C.; 1989: Resistant rootstocks may control fanleaf degeneration of grapevines. Calif. Agricult. **43**, 13-14.

WEISCHER, B.; 1980: The host-parasite relationships between the vector nematode, *Xiphinema index*, and some *Vitis* spp. Proc. 7th Meeting Intern. Counc. Virus Disease Grapevine, Niagara Falls, Canada, 139-146.

-- --; 1988: Angewandte Nematodenforschung - Herausforderung und Nutzung. Wiss. Z. Ernst-Moritz-Arndt-Univ., Greifswald, Math.-Nat.wiss. Reihe **37**, 47-51.

The genetics of resistance to grapevine fanleaf virus in *Vitis vinifera*

M. A. Walker and C. P. Meredith

Department of Viticulture and Enology, University of California, Davis, California 95616, USA

Summary: Two wild *Vitis vinifera* accessions from the Middle East previously found to be resistant to grapevine fanleaf virus (GFV) were selfed and also crossed to a GFV-susceptible female cultivar. Five seedling populations of 60 plants each were established. A micrografting procedure was developed for screening the seedlings whereby single-node seedling stem segments were cleft-grafted go GFV-infected stocks *in vitro*. After 8 weeks, scion tissue was scored phenotypically and assayed by ELISA to measure virus titer. Resistance to GFV appears to segregate as a recessive trait controlled by at least two genes.

Key words: fanleaf, GFV, resistance, genetics, crossing, selection, micrografting, serology, ELISA.

Introduction

Fanleaf degeneration, one of the most serious diseases affecting world viticulture, is a disease complex caused by grapevine fanleaf virus (GFV) and the feeding of the vector, *Xiphinema index*. The nematode-vectored nature of the disease was first discovered by Hewitt *et al.* (1958), and this discovery initiated efforts to control the disease. Fumigants and nematicides aimed at eradicating the vector were used at first, but they proved unsuccessful in California (Raski *et al.* 1983). A rootstock breeding program at the University of California, Davis began with a screen of *Vitis* species for *X. index* resistance (Kunde *et al.* 1968). These efforts resulted in the release of two rootstocks with field resistance to fanleaf degeneration (Lider and Goheen 1986), VR 039-16 (United States Patent # 6166) and VR 043-43 (United States Patent # 6319). Since the release of these rootstocks, GFV has been detected in scions on both of them (Walker *et al.* 1989). It appears that, although the two rootstocks have high levels of resistance to *X. index* feeding (Lider and Goheen 1986), chance nematode probing transmits GFV. The next step in the development of fanleaf degeneration-resistant rootstocks is to combine GFV resistance with *X. index* feeding resistance.

The search for GFV resistance began with a screen of the *Vitis* germplasm held at the University of California, Davis and resistance was found in Middle Eastern *V. vinifera* accessions (Walker *et al.* 1985). Resistant and susceptible plants identified in that study have been used to produce hybrid, selfed and open-pollinated seedling populations in an effort to characterize GFV resistance.

Materials and methods

The *V. vinifera* accessions used as parents are as shown in the table.

All of these accessions except Almeria were collected by H. P. Olmo in 1948 in the Middle East - 030-44 in Shirwandah, Iran and the siblings, 030-51 and 030-53, in Adhai, Afghanistan (H. P. Olmo, personal communication). Almeria is a pistillate cultivar and does not set fruit without external pollen (Olmo 1943). 030-53 was originally classified as pistillate, but had functional pollen and was used as a male parent in these crosses. 030-44 was classified as staminate, however it behaved as a hermaphrodite and produced seed each year. 030-51 was staminate and only set seed after chemical hermaphrodization following the techniques of Negi and Olmo (1966) and Srinivasan and Mullins (1979).

Accession number, vineyard location and GFV response of the parents used in the crosses for characterization of GFV resistance

Accession	Location	GFV response
cv. Almeria (UCD clone 1)	Tyree IV R7v22	susceptible
O30-53 (UCD number)	Armstrong M3v20	susceptible
O30-44 (" ")	" " M3v17	resistant
O30-51 (" ")	" " M3v18	resistant

The following seedling populations were produced:
(1) O30-44 open pollinated (resistant plant O. P.)
(2) O30-51 chemically hermaphrodized and selfed (resistant selfed)
(3) Almeria x O30-44 (susceptible x resistant)
(4) Almeria x O30-51 (susceptible x resistant)
(5) Almeria x O30-53 (susceptible x susceptible).

60 seedlings from each cross were randomly selected for each of the populations, except for the O30-51 selfed population, which consisted of all 51 plants produced.

The seedlings were inoculated with GFV by micrografting. Highly GFV-infected (ELISA values > 1.999 OD 405_{nm}) *V. vinifera* cv. Cabernet Sauvignon from a vineyard in the Napa Valley, California, was used as the inoculum source. Shoots were harvested from greenhouse-grown GFV-infected vines and brought into the laboratory for sterilization. Sterilized one-node stem segments (to be used as rootstocks) were trimmed to about 30 mm, their lateral buds removed, and placed in 25 x 150 mm culture tubes, containing 25 ml of rootstock medium, capped and sealed with parafilm. The rootstock medium consisted of 1/2 strength MS (Murashige and Skoog 1962) packaged salts (# 500-1117 EF, Gibco Laboratories, Grand Island, NY), 1/2 strength MS vitamins, no sucrose, 1 mg/l indole-3-acetic acid (# I-1250, Sigma Chemical Co., St. Louis, MO), 300 mg/l cefotaxime (Hoechst-Roussel Pharmaceuticals, Inc. Somerville, NJ), and 6 g/l Sigma plant tissue culture agar (# A-1296). Endophytic bacteria (*Pseudomonas* sp.) were present in the GFV inoculum plants and in the potted seedlings. The cefotaxime was added to control this bacterium and did not appear to have a deleterious effect on GFV spread or graft compatibility. The rootstocks were grown for 3-4 weeks at 27 °C in a growth chamber with 16 h daylength prior to micrografting. Contaminated cultures or those that had not initiated roots were discarded.

Sterilized single-node seedling stem pieces, approximately 30 mm long, were used as scions and the pre-rooted GFV-infected stem pieces as rootstocks. The rootstock and scion were placed on sterile filter paper in a sterile 125 mm glass petri dish. A 10-15 mm longitudinal cut was made at the apical end of the rootstock stem piece through the node towards the base. The basal end of the seedling stem piece was tapered with two slanting cuts and fitted into the rootstock piece with forceps. Care was taken to match the cambium layers of rootstock and scion on at least one side. The completed grafts were placed into 25 x 150 mm culture tubes, containing 25 ml of rootstock medium modified with the addition of 10 g/l sucrose and the omission of growth regulators, capped and wrapped with parafilm. Culture conditions for the micrografts were the same as for rootstock cultures. Four micrografts were made for each seedling.

Samples were collected after 8 weeks. The optimal sample included only scion shoot growth from the lateral bud. Any additional scion stem tissue needed to bring sample weights up to the minimum 100 mg was taken with care to avoid the graft union and union callus tissue to insure that rootstock tissue was not sampled.

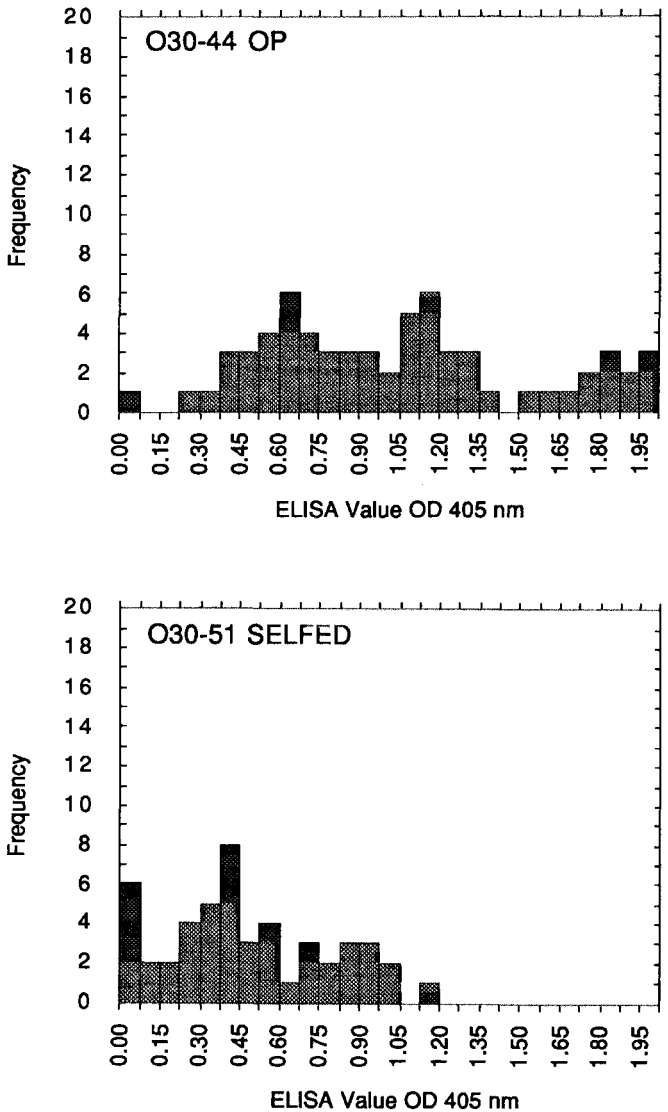
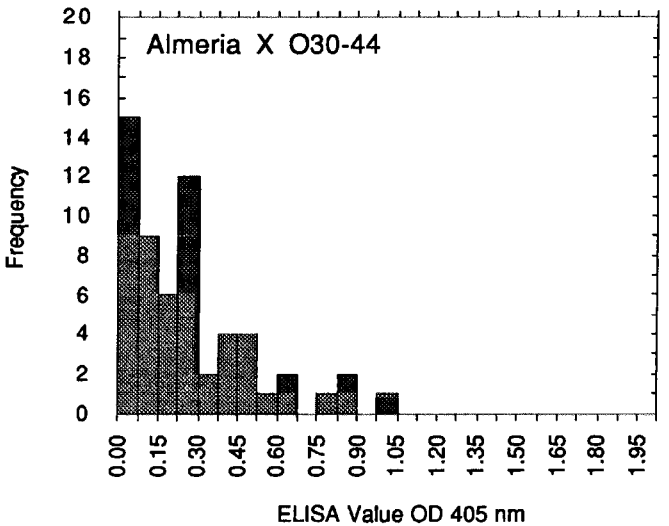


Fig. 1: The highest ELISA values obtained for each seedling within the different seedling populations.

Samples were placed in plastic scintillation vials and GFV extraction buffer was added at a 1/10 (w/v) dilution. Extraction buffer consisted of 0.1 M phosphate buffered saline, 2 % polyvinyl pyrrolidine-40, and 0.5 % Tween 20. The samples were collected, partially frozen to a slurry, then ground with a Brinkman Polytron homogenizer, PT10 probe (Brinkman Instruments, Inc. Westbury, NY), on number 6 setting for 20-25 s, and frozen at -20 °C until used. ELISA (enzyme-linked immunosorbent assay) was used to detect GFV in the samples following the procedures of CLARK and ADAMS (1977). ELISA reactions were read at 405 nm after a 1 h substrate incubation, and values below 0.075 OD 405_{nm} were considered resistant. The inoculated scions were given morphological ratings from 1 to 4 as follows:



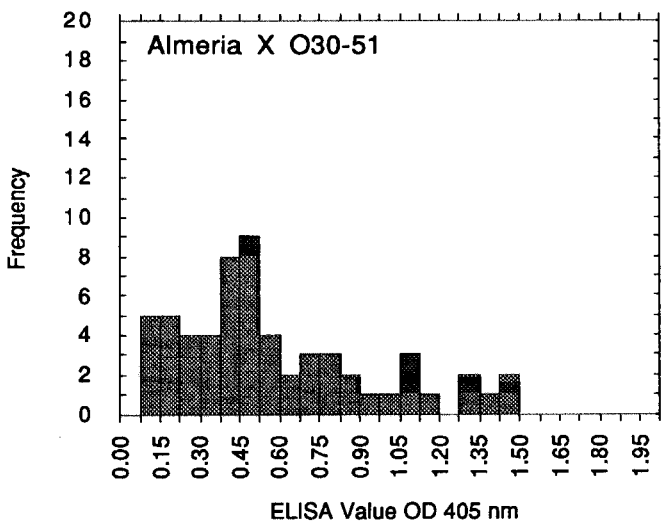


Fig. 1 (continued overleaf).

1 – normal growth
2 – internodes elongated; tall, but not as vigorous as 1; leaves smaller and often vitreous and misshapen
3 – internodes compressed; 3-5 shoots produced from a single lateral bud
4 – internodes greatly compressed with multiple shoots from the lateral bud, producing a moss-like mat of tissue.

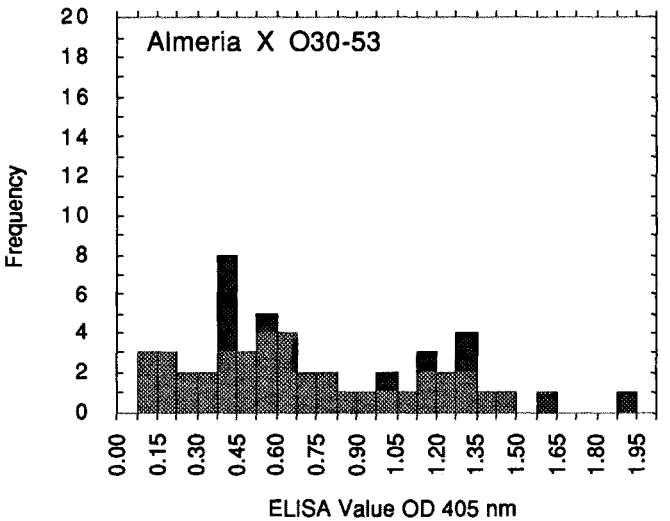


Fig. 1 (continued).

Results

There was a broad distribution of ELISA values within and among the seedlings in each population. In order to reduce some of this variability, the highest ELISA value obtained for each seedling was selected and histograms were constructed (Fig. 1). Histograms were also plotted for the morphological ratings corresponding to the high ELISA values (Fig. 2).

All levels of gene control for one gene and two unlinked genes were evaluated using ELISA and morphological ratings. Only the highest ELISA value obtained for each seedling was used. Morphological ratings of the replications within a seedling were relatively consistent and all of the values were used to evaluate gene models. ELISA values and morphological ratings were classified into various numbers of groups depending on which gene control model was being tested. Fig. 3 presents the four classes that might be expected if a parent heterozygous for two genes with dominance at both loci was selfed.

When ELISA and morphological data were considered as representing either resistant or susceptible classes, without intermediate groups, and seedlings within each population were classified on this basis, the O30-44 OP and the O30-51 selfed seedling populations appear to segregate as though GFV resistance is controlled by two unlinked recessive genes with duplicate dominant epistasis controlling susceptibility. Chi-square analysis supported this hypothesis. There were no good fits of the seedling data with any other gene control model.

Discussion

The ELISA frequency distributions did not appear to fit into discrete classes. This lack of definition could have been due to broad segregation for resistance in the progeny, inconsistencies in the micrografting procedure and ELISA evaluation, or environmentally induced variability in the seedling and stock pieces. Multigene segregation or segregation of an environmentally unstable trait may have been responsible for the broad distribution of ELISA values in each seedling population. Micrografting did produce variable results in many of the inoculated seedlings.

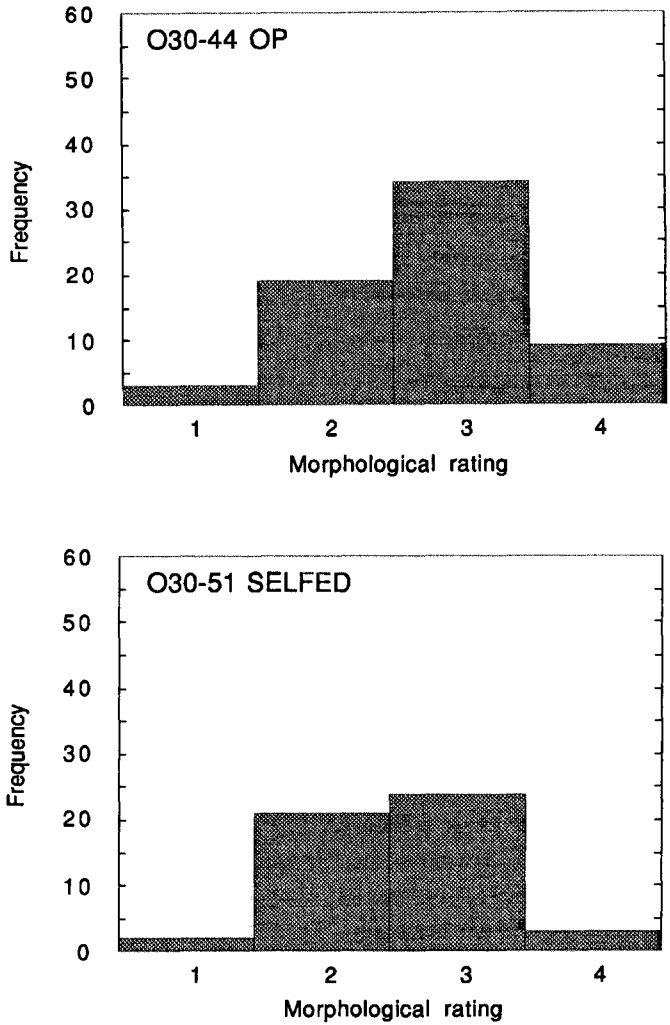


Fig. 2: Morphological rating corresponding to the highest ELISA value of each seedling within the different seedling populations. 1 = normal growth; 2 = internodes elongated, tall, but not as vigorous as 1, leaves small and often vitreous and misshapen; 3 = internodes compressed, 3-5 shoots produced from a single lateral bud; 4 = internodes greatly compressed with multiple shoots from the lateral bud, producing a moss-like mat of tissue.

(continued overleaf.)

Limiting the frequency distributions to each seedling's highest ELISA value and its corresponding phenotype (Figs. 1 and 2) was a means of reducing this variability, but these histograms may not represent the actual resistance reaction, and may impair a quantitative appraisal of resistance.

The stem pieces taken from both seedlings and stock plants for micrografting were used without regard to position on the shoot, or vigor of the seedlings. The physiological state of these donor plants may have contributed to the observed variability within and between the micrografts, both in terms of their stored carbohydrate reserves and their hormone levels.

Another possibility is that the continuous, quantitative nature of ELISA values was not amenable to detection of discrete classes. This last consideration may be important when

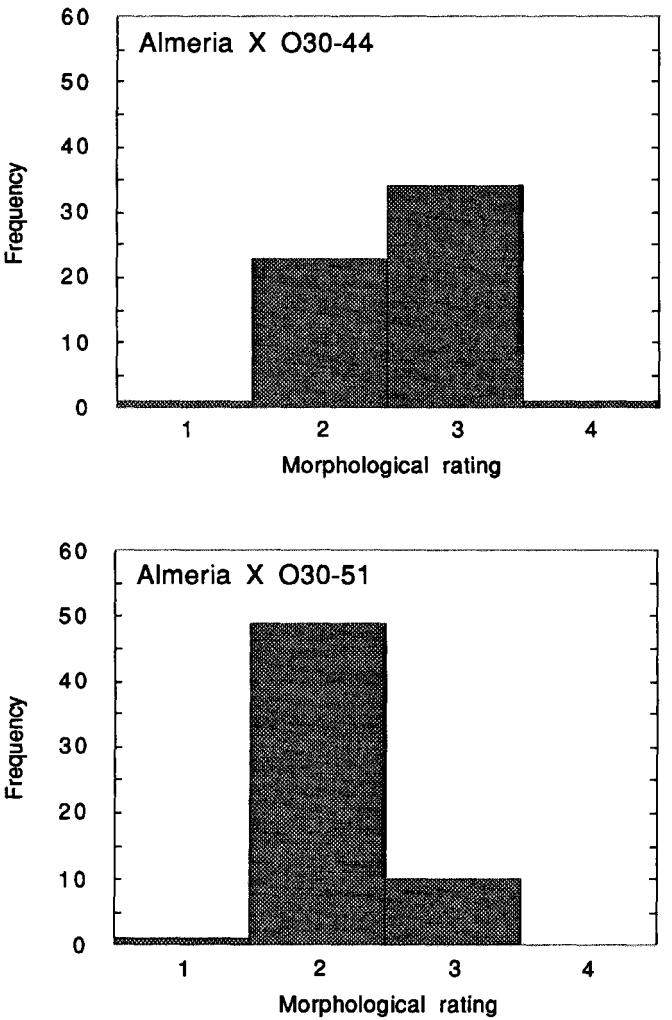


Fig. 2 (continued).

interpreting the results after the ELISA and phenotypic values were reduced to susceptible or resistant classes.

The O30-44 OP and the O30-51 selfed progeny ELISA values seemed to segregate widely, which may have been due to inconsistencies in the micrograft system and its ELISA evaluation, or to the heterozygosity of the parents. If the resistant parents, O30-44 and O30-51, are heterozygous for two unlinked resistance genes, then the susceptibility of Almeria needs to be questioned. If Almeria is considered homozygous susceptible, then crosses between it and the resistant parents should mirror the genotypes of the resistant parent's gametes, and give 1 : 1 : 1 : 1 ratios. These ratios were not detected; there are at least two possible explanations to account for this. Almeria is considered to be ancient Spanish cultivar, but it shares morphological features with Middle Eastern cultivars. It could have originated in the Middle East and been brought to Spain later. Given the hypothesized coevolution of GFV and *V. vinifera* in the Middle East (Hewitt 1970; Vuittenez

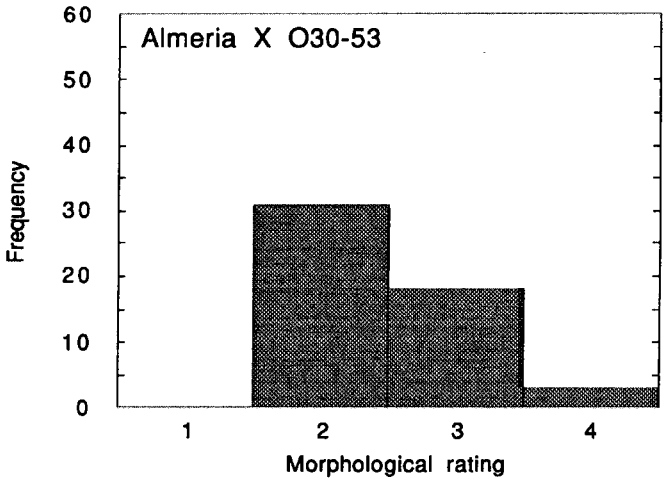


Fig. 2 (continued).

1970), Almeria may share resistance genes with the resistant parents. If resistance is a quantitative trait, then 'test crosses' to Almeria would not be easily resolvable whether it is homozygous or heterozygous.

Conclusions about the inheritance of resistance to GFV require a better understanding of parental reactions to GFV after micrografting and conclusive data to allow quantitative comparisons between parents and progeny. Parental micrograft data were weak and produced more questions than answers. Detection of a quantitative trait is dependent upon accurate parental appraisal, which is necessary for comparisons with progeny populations.

GFV was not detectable in 15 seedlings in the Almeria x O30-44 population and 4 in the O30-51 selfed population, but at the same time these seedlings had morphological ratings suggesting GFV infection. This occurrence was unusual and might be explained as a disease reaction. GFV could be localized at the graft union in these cases and prevented from spreading into scion tissues. This localization might alter the seedling's normal hormonal balance and cause morphological change in the absence of GFV. Seedlings responding in this manner should be reexamined, not only for virus, but also for unusual hormone levels in the scion.

2 seedlings in the O30-44 OP population, 87-7-29 and 87-15-14, and 1 in the Almeria x O30-51 population, 88-9-10, exhibited tolerance, that is, GFV was detectable in their scions, but no corresponding disease symptoms were observed. There were no problems with the grafts or scions that might raise doubts about this response. Tolerance to GFV may be entirely separate from resistance, since GFV was readily detectable in the scions, but there were no corresponding phenotypic reactions. Such tolerance may also be due to environmental interaction, and may not be reproducible in whole plant studies. Tolerance might be expected to be a relatively common occurrence in a coevolving plant/pathogen complex, since tolerance would have a reduced selective impact on the pathogen compared to the selective pressure of resistance. Middle Eastern cultivars and *V. vinifera* populations should be reexamined with GFV tolerance in mind, particularly if *in vitro* tolerance can be correlated with whole plant studies.

In addition to tolerance, GFV resistance seems to be present in 2 seedlings: 87-6-39 in the O30-44 OP population, and 87-5-17 in the O30-51 selfed population. The graft and culture data

O30-44 OP

ELISA Value \ Morphological Rating	A	B	C	D	
A	1	0	0	0	1
B	0	1	0	0	1
C	2	4	14	2	22
D	0	3	31	7	41
	3	8	45	9	65

ALMERIA X O30-44

ELISA Value \ Morphological Rating	A	B	C	D	
A	0	3	12	0	15
B	0	4	15	0	19
C	0	4	17	0	21
D	0	0	3	1	4
	0	11	47	1	59

O30-51 SELFED

ELISA Value \ Morphological Rating	A	B	C	D	
A	0	1	3	1	5
B	0	0	6	0	6
C	0	10	17	1	28
D	0	1	9	0	10
	0	12	35	2	49

ALMERIA X O30-51

ELISA Value \ Morphological Rating	A	B	C	D	
A	0	0	0	0	0
B	0	10	3	1	14
C	1	25	6	0	32
D	0	5	9	0	14
	1	40	18	1	60

ALMERIA X O30-53

ELISA Value \ Morphological Rating	A	B	C	D	
A	0	0	0	0	0
B	0	3	4	0	7
C	0	9	15	3	27
D	0	12	6	0	18
	0	24	25	3	52

Fig. 3: Number of seedlings in each of 4 classes by both ELISA and morphological rating. Morphological ratings: A = seedlings with replicates having only class 1 ratings (1111), B = 1112 through 2222, C = 2223 through 3333, D = 3334 through 4444. ELISA values: A = readings ≤ 0.075 OD 405_{nm}, B = > 0.075-0.250, C = > 0.250-0.800, D = > 0.800.

taken for 87-6-39 did not reveal any reason to doubt its resistant status. ELISA did not detect GFV among the 4 replications of 87-5-17, and 3 of the 4 replications had normal morphology, while the 4th had a rating of 2 (reduced vigor and small leaves). This moderately affected replicate did not graft or grow as well as the other 3 and its abnormal morphology may not have been caused by GFV. These 2 seedlings seem to have a high degree of resistance to GFV. Although they have not yet been screened for resistance in other than a tissue culture environment, they did exhibit much greater resistance to GFV than either of the parents or any of the other seedlings.

GFV resistance seems to be genuine, but further crosses and tests are needed before the number of genes controlling resistance can be accurately determined. Paramount among considerations for the next generation of crosses and selfings is more accurate appraisal of the parental reactions to GFV, both resistant and susceptible, so that a quantitative trait could be assessed. The results suggest that GFV resistance is recessive and controlled by two unlinked genes with duplicate dominant epistasis. However, given the single environment in which the seedlings were evaluated and the seemingly ambiguous parental reactions, this conclusion is tentative at best.

This work has produced seedlings with a wide range of GFV reactions (both resistant and highly susceptible) that can be used to produce a second generation of crosses and selfings. The results from a second generation will better characterize GFV resistance, and should elucidate the heritability of GFV resistance. Tolerance seems to exist in 2 of the seedling populations, and it may or may not be associated with resistance. If *in vitro* tolerance can be verified and shown to persist in whole plant studies it may be more valuable, and in the long term more durable, than resistance. The 2 seedlings that appear to be resistant will be reexamined, by micrografting and whole plant approach grafting. Once they are better understood, they will be crossed to known sources of *X. index* feeding resistance to produce rootstocks that will resist the vector and the virus and provide long term protection against fanleaf degeneration.

References

CLARK, M. F.; ADAMS, A. N.; 1977: Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. **34**, 475-483.

HEWITT, W. B.; 1970: Viruses and virus-like diseases of the grapevine. In: FRAIZER, N. W. (Ed.): Diseases of Small Fruits and Grapevines, 195-196. University of California Division of Agricultural Science. Berkeley, California.

-- -- ; RASKI, D. J.; GOHEEN, A. C.; 1958: Nematode vector of soil-borne fanleaf virus of grapevines. Phytopathology **48**, 586-593.

KUNDE, R. M.; LIDER, L. A.; SCHMITT, R. V.; 1968: A test of *Vitis* resistance to *Xiphinema index*. Amer. J. Enol. Viticult. **19**, 30-36.

LIDER, L. A.; GOHEEN, A. C.; 1986: Field resistance to the grapevine fanleaf virus-*Xiphinema index* complex in interspecific hybrids of *Vitis*. In: 4th Intern. Symp. Grapevine Breeding, Verona, Italy. April 13-18, 1985. Vignevini 13 Suppl. al (12), 166-169.

MURASHIGE, T.; SKOOG, F.; 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 473-497.

NEGI, S. S.; OLMO, H. P.; 1966: Sex conversion in a male *Vitis vinifera* L. by a kinin. Science **152**, 1624-1625.

OLMO, H. P.; 1943: The pollination of the Almeria grape. Proc. Amer. Soc. Hort. Sci. **42**, 401-406.

RASKI, D. J.; GOHEEN, A. C.; LIDER, L. A.; MEREDITH, C. P.; 1983: Strategies against grapevine fanleaf virus and its nematode vector. Plant Dis. 7, 335-339.

SRINIVASAN, C.; MULLINS, M. G.; 1979: Flowering in *Vitis*: Conversion of tendrils into inflorescences and bunches of grapes. Planta **145**, 187-192.

VUITTENEZ, A.; 1970: Fanleaf of grapevine. In. FRAIZER, N. W. (Ed.): Diseases of Small Fruits and Grapevines, 217-228. University of California Division of Agricultural Science. Berkeley, California.

WALKER, M. A.; MEREDITH, C. P.; GOHEEN, A. C.; 1985: Sources of resistance to grapevine fanleaf virus (GFV) in *Vitis* species. Vitis **24**, 218-228.

---; WOLPERT, J. A.; VILAS, E. P.; GOHEEN, A. C.; LIDER, L. A.; 1989: Resistant rootstocks may control fanleaf degeneration of grapevine. Calif. Agricult. **42** (2), 13-14.

Grapevine breeding for resistance to powdery mildew: Bioassay system for evaluation of plant resistance and for characterization of different *Uncinula necator* strains

Y. KITAO [1]) and J.-P. DOAZAN [2])

[1]) Suntory Ltd., Osaka, Japan

[2]) Station de Recherches de Viticulture, I.N.R.A., B.P. 131, F-33140 Pont de la Maye, France

Summary: Several isolates of *Uncinula necator* were separated and kept *in vitro*. The pathogenicity of these isolates was compared by a bioassay system using small leaves issued from *in vitro* plants; 2 μl of spore suspension was inoculated on these leaves.

Significative differences in sporulation time, aggressiveness, sporulation rate and resistance to fungicide triadimenol were observed between these isolates. Host plant variety also affects some of these characters of pathogenicity.

The isolates were classified into 2 mating types concerning the aspect of perithecia formation by paired combination between 2 isolates. Productivity of perithecia varied in response to the combination of isolates and to host plant variety.

Key words: oidium, variety of vine, resistance, biotype, fungicide resistance, perithecium, bioassay, breeding.

Introduction

Powdery mildew, caused by *Uncinula necator*, is one of the most important diseases of grapevines. Susceptibility of cultivars to powdery mildew has been studied by various authors (BOUBALS 1961; POSPISILOVA 1978; DOSTER and SCHNATHORST 1985), however the levels of susceptibility reported for a given variety are not equivalent. These discrepancies could be ascribed to different causes namely the climatic conditions, the physiology of host plants and pathogens. DELP (1954), DOSTER and SCHNATHORST (1985) studied the effects of leaf maturity on development of powdery mildew and suggested that cultivar susceptibility should be compared on young leaves. BAVARESCO and EIBACH (1987) observed the influence of nitrogen fertilization on resistance to powdery mildew. Therefore studies on susceptibility should be done with stable physiological conditions of host plant under the controlled environments.

U. necator is an obligate parasite which requires a living host plant to be maintained, so that the studies on powdery mildew are difficult to be carried out in the laboratory. For this kind of obligate parasite, dual culture *in vitro* of the pathogen and its host can provide an excellent system for studying pathogen races and host-pathogen interaction. MOREL (1948) realized dual culture of downy mildew and callus tissue of grapevines and also tried for powdery mildew. Owing to successful procedures for propagation techniques of grapevines *in vitro* (GALZY 1969; BARLASS and SKENE 1978; HARRIS and STEVENSON 1982), sterile shoot culture provides a desirable system for inoculation.

Recently, LEE and WICKS (1982) applied dual culture for evaluation of systemic fungicide treatment against downy mildew and BARLASS *et al.* (1986) developed it for screening grapevines for resistance to downy mildew. ALDWINCKLE and BUTURAC (1980), KLEMPKA *et al.* (1984) established dual cultures of powdery mildew and several cultivars of grapevines. Thus dual culture would be useful for various investigations on obligate parasites under laboratory conditions.

Perithecia are observed in many viticultural regions but their formation is very erratic under natural conditions (BERNARD and MUR 1986; MAGAREY and WICKS 1986; DIEHL and HEINTZ 1987). PEARSON and GADOURY (1987) demonstrated that perithecia are the source of primary

infection in New York. But in other regions overwintering of mycelium in the dormant bud is considered as a primary inoculum (BULIT and LAFON 1978; VAN DER SPUY and MATTHEE 1977; SALL and WRYSINSKY 1982; BERNARD 1985) and till now the sexual reproduction cycle of powdery mildew is not well known.

This paper describes an *in vitro* inoculation procedure and assay system for *U. necator*, and a characterization of several isolates by their aggressiveness, their resistance to systemic fungicides and their heterothallic properties.

Materials and methods

1. Dual culture of grapevine and powdery mildew *in vitro*

One-node cuttings of grapevine cultivars Cinsaut and Muscadelle were propagated by tissue culture method (GALZY 1969) and maintained by subculturing them every 3-4 months. Isolates of various geographical origin were obtained by collecting conidia from young colonies and inoculating them onto sterile Cinsaut plants *in vitro*. Conidia of each isolate were transferred onto sterile plants at 3-4 weeks intervals.

2. Bioassay system

Host plants: Sterile shoots of Cinsaut and Muscadelle were divided into one-node cuttings including the leaf. These systems of leaf-petiole-stem were planted in Petri dishes containing agar medium (1.5 % agar in distilled water).

Preparation of inoculum as spore suspension: Some colonies (3 weeks old) were washed in sterile distilled water containing Tween 20 in order to remove conidia from conidiophore into washing liquid which was centrifuged twice. The concentration of spores was adjusted to a level of 3×10^4 conidia/ml.

Inoculation: One drop (2 μl) of the spore suspension was inoculated onto each detached leaf with a micro-pipette. Sterile filter paper (5 mm x 5 mm) was put on the drop to absorb water, and was removed 4 d later. Petri dishes were sealed and incubated in a chamber (26-27 °C during 16 h illumination, 20-22 °C during 8 h dark).

3. Characterization of isolates of powdery mildew

a) Development of sporulation

Time from inoculation to sporulation and growth of colony diameter were used as criteria for the comparison between several isolates on Cinsaut and Muscadelle leaves *in vitro*. Observations were made at 24 h intervals. Number of conidia produced were estimated 3 weeks after inoculation for average size of colony. Conidia were collected in accordance with the method of preparation of inoculum described above. Estimation of conidia number was based on the numerical values after calculation with bacterial counter.

b) Resistance to triadimenol fungicide

A study was performed to determine resistance of isolates to the systemic fungicide triadimenol which was added to the agar medium at concentrations of 5 mg/l, 0.5 mg/l and 0.05 mg/l. Several isolates (Bordeaux, Greece and 2 from Portugal, the latter supplied by Plant Pathology Research Station of INRA-Bordeaux) were inoculated onto Cinsaut leaves. Observations were made on colony growth according to the method of DESAYMARD (1968).

Table 1: The number of days from inoculation to sporulation for 11 isolates of powdery mildew on two host cultivars Cinsaut and Muscadelle

ISOLATE	NUMBER OF DAYS	
	CINSAUT	MUSCADELLE
GREECE M-1	5.33 ± 0.50(1)	6.47 ± 0.41
GREECE M-2	5.22 ± 0.32	8.20 ± 1.57
AZAMBUJA	5.47 ± 0.35	7.38 ± 0.89
MONTEMOR	5.17 ± 0.19	6.81 ± 0.49
DIJON	6.61 ± 0.52	8.06 ± 0.31
MONTPELLIER	5.88 ± 0.54	6.56 ± 0.58
ITALY	5.22 ± 0.32	6.50 ± 0.74
SWITZERLAND	4.71 ± 0.40	5.78 ± 0.51
BORDEAUX 86 M-2	5.95 ± 0.42	7.64 ± 1.13
BORDEAUX 86 M-3	6.15 ± 0.48	7.44 ± 0.80
BORDEAUX 87	7.29 ± 1.03	10.22 ± 1.95
TOTAL	5.64 ± 0.15	7.28 ± 0.28

(1) STANDARD ERROR OF THE MEAN

c) Aspects of perithecia formation

Determination of mating types: 5 clonal isolates were obtained by separation of single spores with a small fragment of sterile razor. These single spore clones were multiplied and maintained on Cinsaut leaves. Paired combinations were made by inoculating 2 of them on the same leaf of Cinsaut. Inoculation was carried out by dusting conidia onto the system of detached leaves described before. Inoculated leaves were incubated in a chamber for 4 weeks and the presence of perithecia was observed.

Effect of cultivars on perithecia productivity: 2 isolates of opposite mating types (from Greece and Bordeaux) were inoculated on Cinsaut and Muscadelle leaves with a mixed spore suspension (2 μl). Detached leaves were planted in Petri dishes and were incubated in chamber for 4 weeks. Percentage of colonies which induced perithecia formation and number of perithecia with yellow to brown color were observed periodically.

Results and discussion

1. Development of sporulation

Times from inoculation to sporulation varied according to isolates and to host plant cultivars. Times on Cinsaut leaves tended to be shorter than that on Muscadelle for all isolates. Swiss isolate sporulated in shorter time on both cultivars, isolate of Bordeaux 87 took the longest time on these cultivars. Other isolates sporulated in 5.2-6.6 d on Cinsaut leaves, whereas in 6.5-8.2 d on Muscadelle leaves (Table 1).

Evolutions of colony diameter were shown in Fig. 1. Except the isolate of Bordeaux 87 all of them increased in colony diameter more rapidly on Cinsaut leaves than on Muscadelle leaves. Generally the differences in diameter between two host plants were more evident on the 7th d than on the 11th or 14th d after inoculation. The Montemor isolate grew most rapidly among the tested

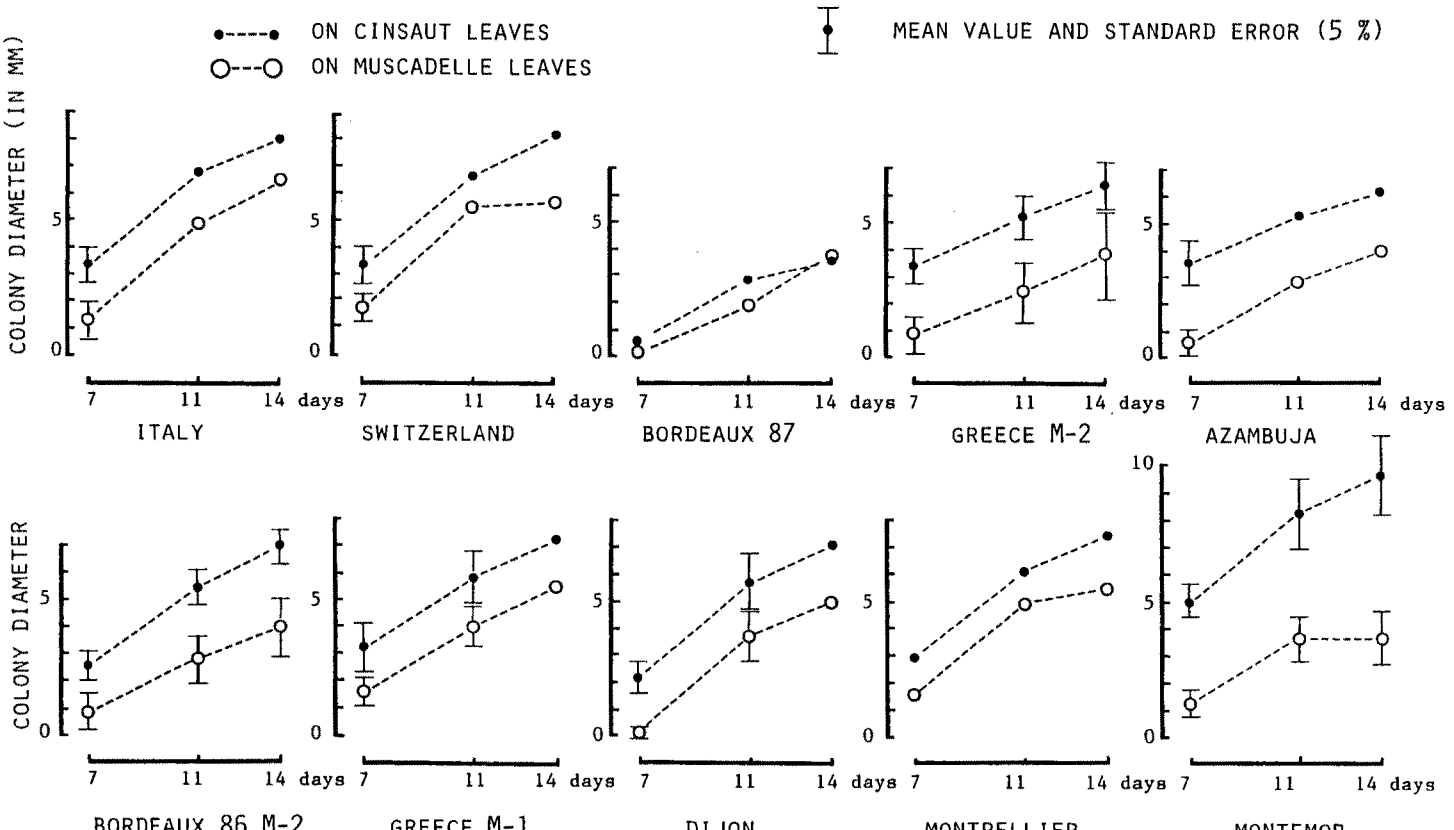


Fig. 1: Evolution of colony diameter for 10 isolates on two host cultivars.

Table 2: Estimation of conidia formation on the 21th d after inoculation on Cinsaut and Muscadelle leaves

Isolate	Number of conidia (x10^4/leaf)	
	Cinsaut	Muscadelle
Greece M-1	13.19 ± 5.30(1)	2.02 ± 1.63
Montemor	23.28 ± 5.58	1.95 ± 1.88
Dijon	12.87 ± 7.78	2.55 ± 2.29
Montpellier	11.72 ± 3.17	2.35 ± 1.50
Italy	16.24 ± 4.20	4.69 ± 4.19
Switzerland	19.17 ± 4.58	2.12 ± 2.27
Bordeaux 86 M-2	13.14 ± 3.75	2.84 ± 3.11
Bordeaux 87	1.60 ± 1.55	1.49 ± 2.01

(1) Standard error of the mean

isolates on Cinsaut leaves, whereas on Muscadelle leaves it was the Italian one. The Bordeaux 87 isolate grew very slowly on both cultivars.

The number of conidia produced for each colony was estimated on the 21st d after inoculation (Table 2). A great variability was observed according to isolates and cultivars. All isolates except Bordeaux 87 produced more conidia on Cinsaut than on Muscadelle. Some of them such as Montemor, Switzerland and Italy were very productive on Cinsaut leaves but the Bordeaux 87 isolate was less productive.

Boubals (1961) rated Cinsaut and Muscadelle as very susceptible, whereas in this study it seems that the degree of susceptibility could be affected by isolates. With Bordeaux 87 for example, these two cultivars showed the same rapidity of colony growth but the Montemor isolate grew more rapidly on Cinsaut leaves than on Muscadelle. These results show great variability in sporulation time and in colony growth in response to isolate. Doster and Schnathorst (1985) suggested that the time needed for sporulation allows susceptibility discrimination between host plants. Therefore, it could be an expression of the aggressiveness of an isolate. In our test, negative correlations ($r = -0.87^{**}$ for Cinsaut, $r = -0.86^{***}$ for Cinsaut and Muscadelle combined) were observed between time and colony diameter on the 7th d. The coefficient of correlation between time and the number of conidia estimated on the 21st d was not very clear for Muscadelle, but significant for Cinsaut ($r = -0.86^{***}$) and for Cinsaut and Muscadelle combined ($r = -0.69^{**}$).

Aggressiveness of isolates could be considered as a degree of rapidity of colony growth and at the same time as abundance of conidia production which would be an inoculum for the secondary infection. These results might suggest that the sporulation time can express the aggressiveness of isolates.

At any rate, great variability of aggressiveness was found in response to isolates on two very susceptible cultivars. This variability in aggressiveness should be taken into consideration in further studies.

2. Resistance to the triadimenol fungicide

2 isolates (Azambuja, Montemor) from Portugal could grow in the presence of higher concentration of triadimenol. Azambuja isolate sporulated at 0.5 mg/l of triadimenol as well as control. Montemor isolate also sporulated at this concentration, but colony growths were significantly lower than that of the control. Bordeaux and Greece isolates developed mycelium but did not sporulate. Sporulation of these isolates at 0.05 mg/l was less active than the control with

the Greece isolate being very sensitive to triadimenol. At 5 mg/l of triadimenol, no sporulation occurred in any isolate (Fig. 2).

STEVA *et al.* (1988) reported that some Portuguese isolates seemed to be resistant to triadimenol. Our results confirm the tendency of these isolates to be resistant to this inhibitor of the sterol biosynthesis. Azambuja isolate is the most resistant, while isolates of Bordeaux and Greece were very sensitive to triadimenol. These properties concerning resistance to a fungicide could be introduced by selection pressure of fungicide treatment in the vineyard. LEE and WICKS (1982)

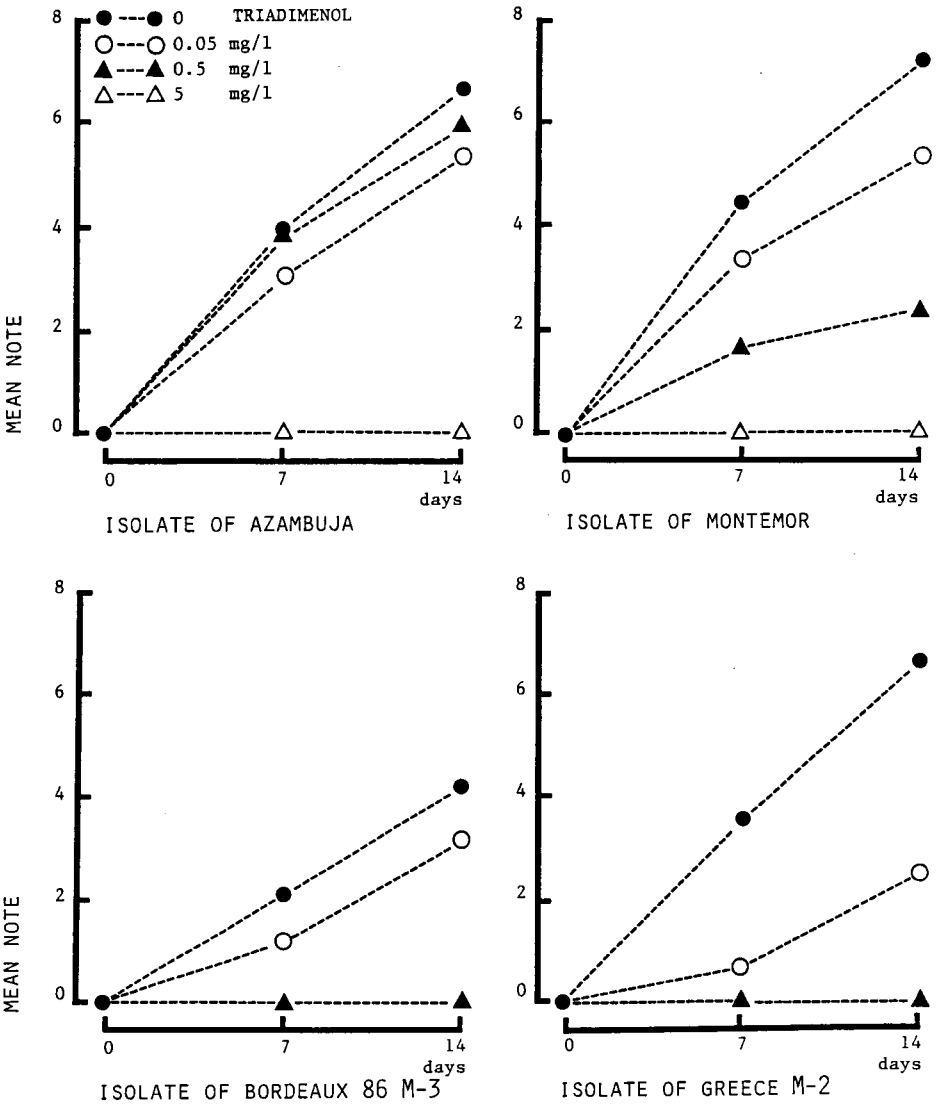


Fig. 2: Evolution of mean note of colony development for 4 isolates at several concentrations of triadimenol: Each note (note 0 to 10) represents the percentage of leaf surface infected by sporulation. Note 0: 0 %, 1: 0-2.5 %, 2: 2.5-5 %, 3: 5-15 %, 4: 15-30 %, 5: 30-50 %, 6: 50-70 %, 7: 70-85 %, 8: 85-95 %, 9: 95-97.5 %, 10: 97.5-100 %.

Table 3: Perithecia formation by paired combination of isolates

ISOLATE	BORDEAUX M-2	SWITZERLAND	GREECE M-1	GREECE M-2
BORDEAUX 86 M-1	---	---	+	+
BORDEAUX 86 M-2		---	+	+
SWITZERLAND			+	+
GREECE M-1				---

+ : PERITHECIA FORMED

--- : NON-PERITHECIA FORMATION

developed an experimental system with dual culture that can be used for fungicide studies on grapevine downy mildew. Our assay system is very convenient for the evaluation of systemic fungicides against grapevine powdery mildew.

3. Aspects of perithecia formation

a) Mating types

None of the single-spore isolates ever formed perithecia by themselves. But paired combinations of some of them induced perithecia formation (Table 3). Initiation was observed on the 14th d after inoculation. The isolates Bordeaux M-1, Bordeaux M-2 and Swiss did not produce perithecia when combined with each other. Likewise, the combination between 2 Greece isolates did not induce perithecia. Meanwhile, combinations between these two groups induced perithecia formation.

Perithecia appear late in the season in the vineyard, but their frequency varies in response to year and region (BERNARD and MUR 1986; MAGAREY and WICKS 1986; DIEHL and HEINTZ 1987). HIURA (1962) reported heterothallism for *Erysiphe graminis* and SMITH (1970) first observed this character on *U. necator*. Our results confirm this phenomenon, and 5 isolates could be divided into 2 groups. The first one includes 2 Bordeaux isolates and the Swiss isolate, the second group includes 2 Greece isolates. The combination of isolates belonging to opposite groups induced perithecia formation. HIURA (1962) reported that this phenomenon is controlled by one pair of genes in *Erysiphe*, and it is also likely to be true in *U. necator*.

The combination of two opposite mating types is a necessary condition to induce perithecia formation. But this does not account for the great variations in perithecia number. We suggest that several factors such as weather which affect the metabolism of the host and the fungus may play an important role in the frequency of occurrence of appropriate mating types on a given host, thus resulting in abundance of perithecia.

b) Effect of cultivars on perithecia

First perithecia formation was observed on the 11th d after inoculation on Cinsaut leaves and on the 14th d on Muscadelle leaves. The percentage of colonies which induced perithecia formation increased to 100% for Cinsaut leaves and 67% for Muscadelle leaves on the 21st d (Fig. 3). Perithecia productivity (Fig. 4) was significantly different between Cinsaut and Muscadelle after the 14th d of inoculation.

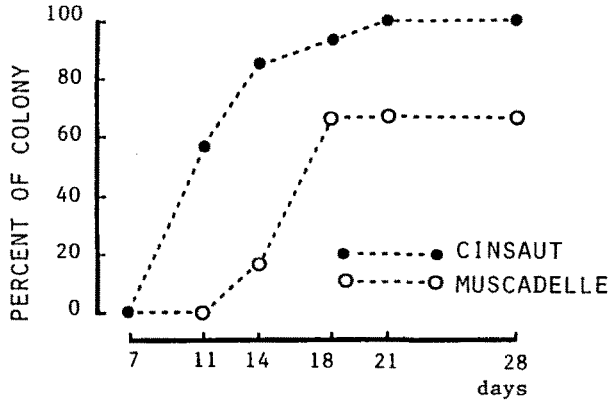


Fig. 3: Evolution of percentage of colony which induced perithecia formation on two host cultivars.

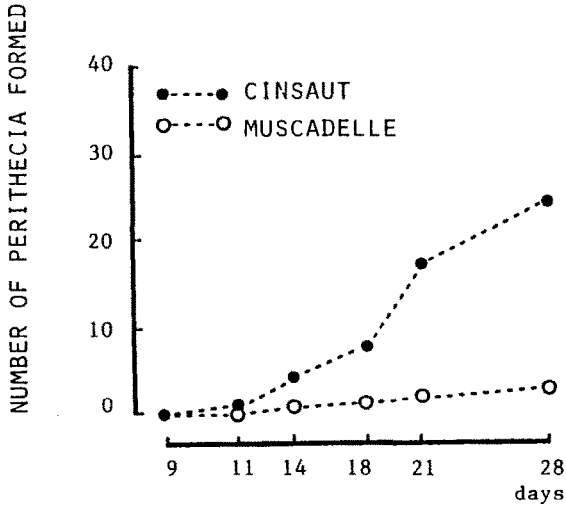


Fig. 4: Effect of host cultivars on the productivity of perithecia: number of perithecia initiated on Cinsaut and Muscadelle leaves by a paired combination of isolates (Greece and Bordeaux isolate).

Cinsaut leaves facilitated more perithecia formation than Muscadelle leaves. Diehl and Heintz (1987) reported a significant correlation between powdery mildew infections and number of perithecia under field conditions. Cinsaut and Muscadelle are rated as very susceptible (Boubals 1961), in this study, however, it seemss that the degree of susceptibility of these two cultivars is variable according to isolate: with isolates from Greece and Bordeaux, Cinsaut is significantly more susceptible than Muscadelle. It is too early for conclusions as to the relationship between susceptibility and production of perithecia. but it seems reasonable that productivity correlates with susceptibility.

Conclusion

With the *in vitro* method using detached leaves with petiole and stem, we investigated the pathogenicity of *U. necator*. This system was applied to characterization of isolates.

Aggressiveness of several isolates was compared on Cinsaut and Muscadelle leaves, and great variability on time from inoculation to sporulation, colony development and conidia production was found in response to isolate. Sporulation time correlates fairly well with colony development and conidia production. These parameters correlating with each other, sporulation time could indicate the aggressiveness of isolate. The resistance of isolates to systemic fungicide was also studied: those originating from Portugal were found to be more resistant to triadimenol than other ones. It seems that such resistance was introduced by natural selection in the vineyard under the pressure of fungicide treatment.

Perithecia formation was demonstrated in our assay system. Single spore isolates were heterothallic and they were divided into two mating type groups. Perithecia production was more abundant on Cinsaut leaves than on Muscadelle leaves, and initiation of perithecia formation was earlier on Cinsaut leaves than on Muscadelle leaves. The productivity of perithecia seems to correlate with the susceptibility of host.

The variable reactions of the host to its parasite as well as the genetic variability of *U. necator* should be carefully considered in every breeding programme for resistance.

References

ALDWINCKLE, H. S.; BUTURAC, I.; 1980: *In vitro* techniques for studying obligate pathogens of *Vitis*. Proc. 3rd Intern. Symp. Grape Breeding, Davis, 15-18 June, 1980; 87-91.

BARLASS, M.; MILLER, R. M.; ANTCLIFF, A. J.; 1986: Development of methods for screening grapevines for resistance to infection by powdery mildew. Amer. J. Enol. Viticult. **37**, 61-66.

-- -- ; SKEENE K. G. M.; 1978: *In vitro* propagation of grapevine (*Vitis vinifera* L.) from fragmented shoot apices. Vitis **17**, 335-340.

BAVARESCO, L.; EIBACH, R.; 1987: Investigations on the influence of N fertilizer on resistance to powdery mildew (*Oidium tuckeri*), downy mildew (*Plasmopara viticola*) and on phytoalexin synthesis in different grapevine varieties. Vitis **20**, 192-200.

BERNARD, A. C.; 1985: Présence du mycelium de l'oïdium (*Uncinula necator* (SCHW.) BURRIL) dans les bourgeons latents de *Vitis vinifera* cv. Carignan, pendant l'hiver. Progr. Agric. Vitic. **102**, 360-361.

-- -- ; MUR, G.; 1986: Présence des périthèces de l'oïdium, en 1985, dans le midi. Progr. Agric. Vitic. **103**, 258-261.

BOUBALS, D.; 1961: Etudes des causes de la résistance des Vitacées à l'oïdium de la vigne - *Uncinula necator* (SCHW.) BURR. - et de leur mode de transmission héréditaire. Ann. Amélior. Plantes **11**, 401-500.

BULIT, J.; LAFON, R.; 1978: Powdery mildew of the vine. In: SPENCER, D. M. (Ed.): The Powdery Mildews, 525-548. Academic Press, London, New York, San Francisco.

DELP, C. J.; 1954: Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology **44**, 615-626.

DESAYMARD, P.; 1968: Notation et méthodes de notations en phytopharmacie. Phytiatrie-Phytopharmacie **2**, 163-173.

DIEHL, H. J.; HEINTZ, C.; 1987: Studies on the generative reproduction of grapevine powdery mildew (*Uncinula necator* BERK.). Vitis **26**, 114-122.

DOSTER, M. A.; SCHNATHORST, W. C.; 1985 a: Comparative susceptibility of various grapevine cultivars to the powdery mildew fungus *Uncinula necator*. Amer. J. Enol. Viticult. **36**, 101-104.

-- --; -- --; 1985 b: Effects of leaf maturity and cultivar resistance on development of powdery mildew fungus on grapevines. Phytopathology **75**, 318-321.

GALZY, R.; 1969: Recherches sur la croissance de *Vitis rupestris* sain et court-noué cultivé *in vitro* à différentes températures. Ann. Phytopathol. **1**, 149-166.

HARRIS, R. E.; STEVENSON, J. H.; 1982: *In vitro* propagation of *Vitis*. Vitis **21**, 22-32.

HIURA, U.; 1962: Hybridization between varieties of *Erysiphe graminis*. Phytopathology **52**, 664-666.

KLEMPKA, K. C.; MEREDITH, C. P.; SALL, M. S.; 1984: Dual culture of grape powdery mildew (*Uncinula necator* BURR.) on its host (*Vitis vinifera* L.). Amer. J. Enol. Viticult. **35**, 170-174.

LEE, T. C.; WICKS, T.; 1982: Dual culture of *Plasmopara viticola* and grapevine and its application to systemic fungicide evaluation. Plant Dis. **66**, 308-310.

MAGAREY, P. A.; WICKS, T. J.; 1986: A new spore type for grapevine powdery mildew. Austral. Grapegrower Winemaker (No. 268), 92-94.

MOREL, G.; 1948: Recherches sur la culture associée de parasites obligatoires et de tissus végétaux. Ann. Epiphyt. **14**, 85-87.

PEARSON, R. C.; GADOURY, D. M.; 1987: Cleistothecia, the source of primary inoculum for grape powdery mildew in New York. Phytopathology **77**, 1509-1514.

POSPISILOVA, D.; 1978: Sensibilité des cépages de *Vitis vinifera* à l'oïdium de la vigne (*Uncinula necator* SCHW. BURR.). Ile Symp. Intern. Amélior. Vigne, Bordeaux, 14-18 juin 1977. Génétique et Amélioration de la Vigne, 251-257, INRA, Paris.

SALL, M. A.; WRYSINSKI, J.; 1982: Perennation of powdery mildew in buds of grapevines. Plant Dis. **66**, 678-679.

SMITH, G. G.; 1970: Production of powdery mildew cleistocarps in a controlled environment. Trans. Brit. Mycol. Soc. **55**, 355-365.

STEVA, H.; CARTOLARO, P.; CLERJEAU, M.; LAFON, R.; GOMES DA SILVA, M. T.; 1988: Une résistance de l'oïdium au Portugal. Phytoma (No. 402), 49-50.

VAN DER SPUY, J. E.; MATTHEE, F. N.; 1977: Overwintering of the oidium stage of *Uncinula necator* in the buds of the grapevine. Plant Dis. Rep. **61**, 612-615.

Phenol and silica incrusts in epidermal cells of *Vitis* spp. as a general defence mechanism

R. BLAICH, C. HEINTZ, G. HOOS and R. WIND

Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, F. R. Germany

A b s t r a c t : The host-parasite interactions between the grapevine *Vitis vinifera*, powdery mildew *Uncinula necator (Oidium tuckeri)* and grey mold *Botrytis cinerea* were studied by light microscopy-histochemistry and electron microscopy.

Chemical defence mechanism involves incrusting of the walls of the infected cell and of neighbouring cells with phenolic substances associated with a cell wall bound peroxidase activity. This indicates the formation of lignin-like components. In addition, silica deposits were observed in whole cell walls or parts of them. Pure, mechanically resistant silica skeletons remained after a treatment with conc. $H_2SO_4 + H_2O_2$ at 400 °C and washing with conc. HCl. They consisted of groups of 1-20 cells of the upper epidermis with adhering parts of the corresponding palisade cells or of the lower epidermis (including stomatal cells) with adhering spongy parenchyma. Not only cell walls but also wrinkles of the upper epidermis, defence papillae and fungal haustoria were silicified. Silica accumulations were greater in resistant than susceptible cultivars.

These reactions are induced not only by parasitic fungi but also by mechanical damage of the leaf. Our studies corroborate observations in other host-parasite systems and indicate the existence of an unspecific, fast-reacting mechanism serving as an early defence line which allows the activation of slower, more specific defence reactions.

Recent results in vine improvement regarding its resistance to downy and powdery mildews

M. P. COUTINHO and A. MARTINS

Instituto Superior de Agronomia, Departamento de Botanica, Tapada da Ajuda, P-1399 Lisboa, Portugal

Summary: Resistance to mildew remains very important under Portuguese conditions and induced mutations present a great interest to obtain it.

γ-rays irradiation was applied to woody material and X-ray was used on *in vitro* cultures of stem apexes and leaf explants. After doses up to 2000 rad, two resistant mutants were selected: with γ-rays, the resistance being expressed as surrounding necrotic patches; with X-rays, the plant coming out from *in vitro* culture presented small necrotic patches.

Concerning powdery mildew, we are developing new techniques for laboratorial inoculation, including a technique for sowing isolated spores. By using these, we can conduct studies on morphology and biology of the isolated colony, variability of the resistance within the *Vitis* varieties and genetic variability of the parasite. Some results concerning these areas are presented.

Key words: Plasmopara, oidium, biotype, variety of vine, clone, resistance, mutagenesis, irradiation, bioassay, geography, Portugal.

Introduction

Amongst the phytosanitary problems of the Portuguese vineyards, downy and powdery mildews still have great importance.

Last year, special weather conditions were favourable to the development of these diseases, particularly for *Plasmopara*, the damages caused being estimated at more than 30 % of the normal yield.

In spite of the higher efficacy of some new fungicides, it is still important to obtain cultivars showing resistance or semiresistance, due to the high cost of chemical treatments and the necessity of reducing to a minimum the pollution effects of pesticides.

So, we have been working on interspecific hybridization and, more recently, on irradiation mutagenesis in order to obtain semiresistant varieties (COUTINHO 1977).

Concerning powdery mildew we are trying presently to develop new techniques for inoculations and evaluation of infections under laboratory conditions. By using these techniques we intend to clarify the morphology and the kinetics of the growing process of the isolated colony coming from a single spore. In the same way we search for clonal variability of resistance within vine varieties and for genetic variability of the fungus regarding some important traits.

Material and methods

Concerning downy mildew, the experimental material chosen was cv. Touriga, largely grown both in Douro (Port wine region) and in Dao. At present this variety is being submitted to clonal selection through a national project.

Woody material, cuttings with 2 or 3 buds, and plants resulting from *in vitro* culture of stem apexes and leaf explants were utilized.

In vitro culture is, for several reasons, well suited for irradiation treatment, incrasing the mutagenic rate (COUTINHO 1987).

For γ-ray irradiation we employed the ^{60}Co equipment of the Physical and Nuclear Engineering Laboratory at Lisbon. The X-ray treatments were performed in our laboratory by means of a Baltograph apparatus.

Selection criterion was based on leaf tissue reaction to the fungus after natural infections and artificial inoculations.

For the powdery mildew studies we perform artificial inoculations on detached leaf discs by two alternate methods: detachment and deposition of the spores by air flow (COUTINHO and MARTINS 1985) and by sowing isolated conidia. The second technique consists of obtaining single spores adherent to a very thin glass stylet (≈ 30 μm diameter) and placing it by micromanipulation on the inoculation surface. This technique allows us to cultivate genetically homogeneous lines (clones) of powdery mildew, a very good way to study the biology of the isolated colony, the genetic variability of the fungus, etc. After inoculation, the spores are incubated in Petri dishes with humidified paper filter at 26 °C for periods from 5 to 10 d.

For the evaluations of artificial infections we estimate the density of the mycelium by counting the number of hyphae which cross a line segment (0.52 mm long) focused on the disc in a randomized way (COUTINHO and MARTINS 1985).

All the microscopic observations are made in a non-destructive way, allowing replicated measurements during the growth of fungus.

Results and discussion

Concerning downy mildew program, we are presently studying plants originated from woody material that has been X-ray irradiated and planted in vegetation boxes under conditions suitable for the selection tests. No significant phenological differences have been found in the plants resulting from cuttings treated with 500 and 1000 rad (Fig. 1).

In material treated with γ-rays, one mutant was detected at 1000 rad. This mutant's resistance character shows leaf infection patches of 'ringspot' type, with an infection zone rapidly surrounded by necrotic tissue avoiding the spread of the parasite. These symptoms point out to a phytoalexins reaction, as described by POOL *et al.* (1980) around the leaf parenchyma at the site of the mycelium penetration.

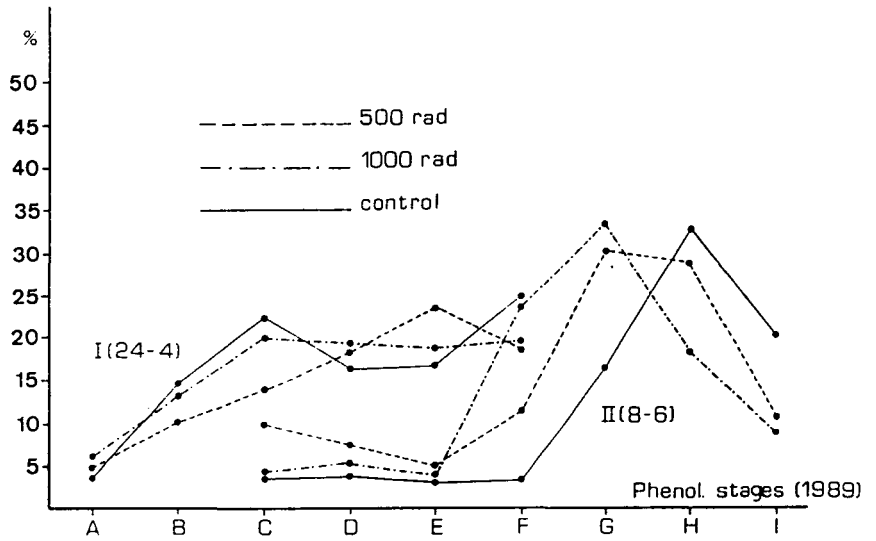


Fig. 1: Phenological stages of plants coming from cuttings treated with X-rays.

The speed of infection blocking by surrounding necrosis is clearly conditioned by weather conditions.

Plant regenerates from *in vitro* culture submitted to X-ray irradiation were transplanted to jiffypots. After inoculation, one plant of this modality showed resistance symptoms: limited and scattered small necrotic patches.

For the discussion regarding powdery mildew we shall follow the order of the objectives referred in the introduction.

Biology of the isolated colony

After deposition of the spore on the disc surface, germination begins within 6 h. The first hypha appears at one end of the spore and when its length reaches around 20 μm it inflates and originates the primary appressorium. The appressorium penetrates the epidermal cell and produces a haustorium. After this, an elongating secondary hypha grows from the appressorium and one or two more from the conidia. The hyphae grow, ramificate and produce new appressoria and haustoria (Fig. 2).

The growing rate of the hyphae depends upon the host susceptibility and environmental factors. Studies conducted on an intermediate susceptible variety, Mourisco do Douro, gave the results shown in Table 1.

Results of other evaluated morphological and biological traits of the isolated colony are presented in Table 2.

Further studies on this subject will allow a better understanding of the biological behaviour of powdery mildew and host-parasite interactions. As an example, during another study we verified that sporulation occurs when mycelium density reaches a constant value around 10 hyphae/0.52 mm, independent of the incubation period (MARTINS 1984). Therefore, if we can identify races that differ in colony compactness, this may correspond to sporulation period differences, that is differences in pathogenicity.

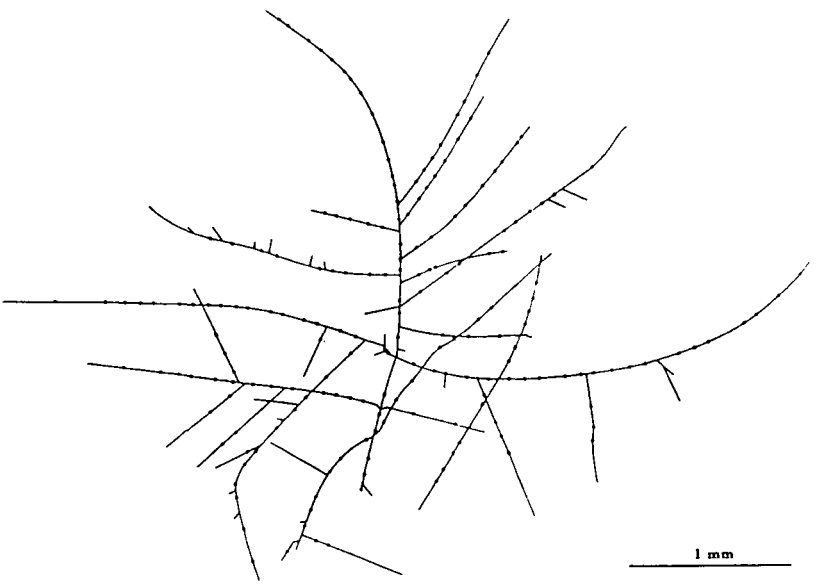


Fig. 2: Structure of the isolated colony of powdery mildew growing on detached leaf discs of cv. Mourisco do Douro during 11 d at 26 °C. • = appressoria.

Table 1: Growth rate of powdery mildew individual hyphae of isolated colonies on discs of cv. Mourisco at 26 °C

classes of daily growth (mm)	0-.09	.1-.19	.2-.29	.3-.39	.4-.49	.5-.59	.6-.69
number of cases observed	6	8	28	24	30	2	4

Table 2: Some biological and morphological traits of the isolated colony of powdery mildew growing on leaf discs of cv. Mourisco at 25 °C

GROWTH OF THE INDIVIDUAL HYPHAE	.34mm/day
MEAN LENGHT BETWEN HAUSTORIA	.1 mm
DIAMETER OF THE COLONY	5.0 mm
TOTAL AREA OF THE COLONY	25 mm2
NUMBER OF HYPHAE IN THE COLONY	42
TOTAL LENGHT OF THE HYPHAE	38 mm
MEAN ANGLE OF THE RAMIFICATIONS	66°
MEAN LENGHT BETWEEN RAMIFICATIONS	.47 mm
BEGINNING OF THE SPORULATION	14 day

Table 3: Mycelium growth rate of powdery mildew on discs of different clones of cvs Jaen and Trincadeira (inoculum density: 3.2 conidia / mm^2; incubation: 6 d at 26 °C)

JAEN		TRINCADEIRA	
clones	hyphae/.52mm	clones	hyphae/.52mm
J0566	9.4	TR1	2.2
J0609	4.1	TR2	1.7
J1612	15.3	TR3	12.4
J0656	2.4	TR5	5.4
J0771	6.6	TR12	1.7
J1402	11.1	TR15	4.2
J1417	6.7	TA1	7.1
J1602	11.4	TA3	3.9
J1626	10.3	TA4	6.6
J1636	5.6	TA5	3.0

Genetic variability of powdery mildew

Sowing isolated spores under the technique we described above enables us to cultivate genetically homogeneous lines of powdery mildew (clones) along several generations. These lines are observed in order to detect genetic differences regarding several traits: number of hyphae growing from the initial conidia of the colony; ramification angle between two hyphae; mean length between appressoria; mean length between contiguous ramifications; growing rate of the individual hyphae.

Our results point out differences in the angle of ramification and the morphology of the ramification point. These studies will be continued investigating powdery mildew strains of different geographic origin.

Variability of resistance within varieties

By artificial inoculations performed over several years with conidia carried by air flow, we verified that differences of resistance between clones exist. The results of two comparative assays (amongst much others) regarding two ancient Portuguese varieties with good morphologic homogeneity, Jaen and Trincadeira, are presented in Table 3. By comparing these results with those of many other assays we verify that clones J0656 and TR2 show highest resistance, whereas clones J1612 and TR3 are more sensible.

At present we are applying the same tests to other varieties submitted to clonal selection in order to include the response to powdery mildew in selection criterium.

References

COUTINHO, M. P.; 1977: Utilisation des rayonnements pour l'amélioration de la vigne au point de vue de la résistance au mildiou. Induced Mutations Against Plant Diseases, IAEA, Wien, SM 214/41, 233-240.

-- --; 1987: The utilisation of *in vitro* culture in a particular case of vine breeding. Experimental Mutagenesis in Plants, Plovdiv, 230-233.

-- -- ; MARTINS, A.; 1985: Quelques résultats sur la résistance de la vigne au mildiou et à l'oïdium. C. R. IVe Symp. Intern. Génétique de la Vigne, Verona, 148-152.

MARTINS, A.; 1984: Contribution to vine breeding regarding its resistance to powdery mildew [Port.]. Thesis, Techn. Univ., Lisbon.

POOL, R. M.; CREASY, L. L.; FRACKELTON, A. S.; 1980: Resveratrol and viniferins, their application to screening for disease resistance in grape breeding programs. Proc. 3rd Intern. Symp. Grape Breeding, Davis, 251-262.

Search for genotypes resistant to *Plasmopara viticola* by crossbreeding

M. Borgo, S. Cancellier and A. Costacurta

Istituto Sperimentale per la Viticoltura, Viale XXVIII Aprile 26, I-31015 Conegliano TV, Italy

Summary: Crossbreeding between *Vitis vinifera* and hybrids resistant to some grapevine diseases began in 1985 in order to create biotypes which are tolerant or resistant to *Plasmopara viticola*, *Oidium tuckeri* and *Botrytis cinerea* and show quality features similar as the European parent varieties (Chardonnay, Sauvignon blanc, Prosecco, Moscato bianco and Cabernet Sauvignon) in order to reduce chemical plant protection practices.

Analysis of the F_1 population emphasized that 20 % of the plants have a favourable tolerance to downy mildew and can be employed in back-crossbreeding with European varieties. This proves that there is a different intensity of leaf reaction to the pathogenic agent.

The above-mentioned tolerance appeared to be steady throughout the years.

An interesting tolerance to oidium was also recorded in open field and greenhouse cultivations.

Key words: Plasmopara, oidium, variety of vine, genotype, resistance, tolerance, quality, crossing, analysis, Italy.

Introduction

Vine downy mildew, caused by the pathogenic agent *Plasmopara viticola* (B. et C.) Berl. et de Toni, is the main parasitic adversity that causes serious damages to production in many Italian vineyards.

The viticultural areas of Northern Italy are considered those most subject to such risks because of summer climatic conditions which are typically rainy and humid. The frequent infections of downy mildew force viticulturists to perform a great number of treatments to prevent and restrict damages.

The growing attention towards environment problems and the consequent awareness that it is necessary to reduce chemical treatments used to control the various parasites that attack cultivations, induce research on new systems of plant protection and pest control.

For this purpose, new plant protection systems for the major cryptogamic diseases of vines are being perfected according to expected models of infection and utilizing new chemical products (Tran Manh Sung 1988).

A further contribution to the reduced use of plant protection products is provided by genetic improvement with studies aimed at creating genotypes capable of resisting or tolerating *P. viticola* and preserving organoleptic features that are equivalent and sometimes better than the best cultivated varieties.

Many researchers, in fact, emphasized the possibility of exploiting the genetic resources of vines to produce new varieties particularly resistant to downy mildew (Millardet 1891, 1894; Boubals 1956; Doazan and Kim 1978; Kim 1978; Alleweldt 1979; Becker and Zimmermann 1980; Li 1985).

The Istituto Sperimentale per la Viticoltura has also been promoting, for some years now, programmes of genetic improvement to obtain new genotypes with a certain degree of tolerance to the main vine fungus diseases (Costacurta *et al.* 1986; Borgo *et al.* 1987). The purpose is to select new individuals that require a reduced amount of chemical treatments, without, however, facing the serious risk of stimulating the diffusion of other pathogenic agents, such as *Phomopsis viticola* and *Guignardia bidwellii*, that cause excoriose and black rot.

Materials and methods

The activity of genetic improvement by crossbreeding for the above-mentioned purposes began in 1985 according to the illustrations in Fig. 1. The latter shows the European cultivars and the hybrids employed for the introduction of the character of 'resistance'.

The method of selection adopted for the F_1 generation mainly involved the degree of resistance to *P. viticola*. The plants selected for their resistance will be subject to production quality tests.

After a short period of greenhouse cultivation, F_1 populations were planted in an open field where downy mildew infections developed naturally. With regard to the type of vine protection, we must mention that these populations were only treated with fungicides during the 1st year of

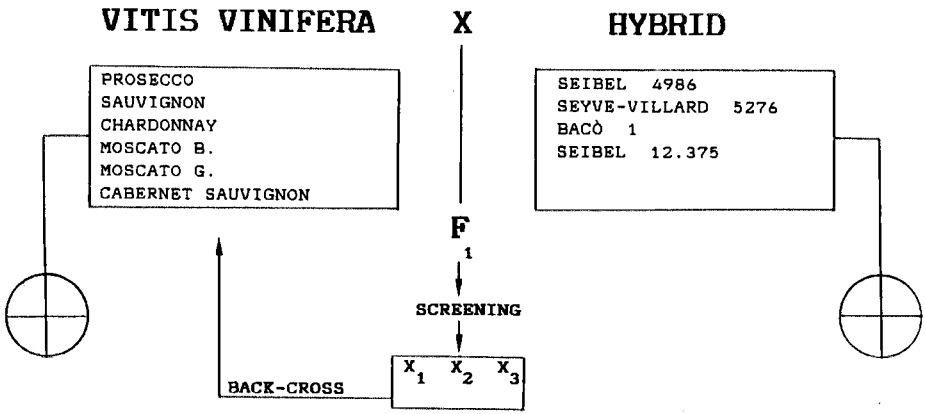


Fig. 1: Scheme of crossbreeding.

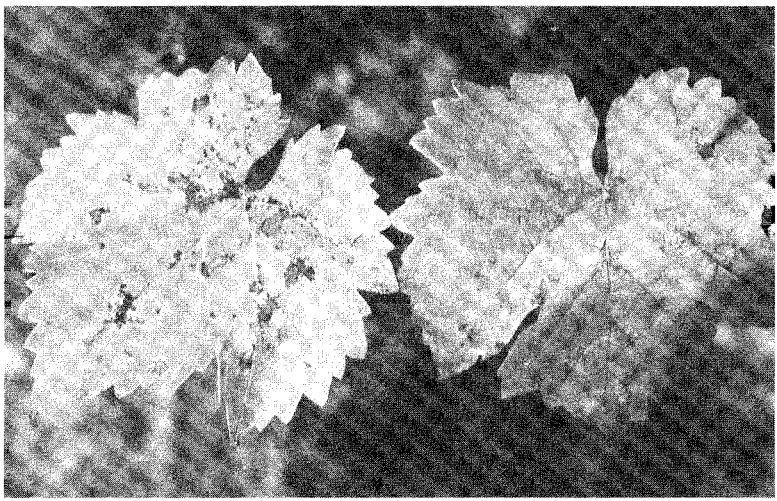


Fig. 2: Different symptomatology of downy mildew on F_1 hybrids (for further explanation see text).

Table 1: F_1 descendants of 1985 crossbreedings with different symptomatology of downy mildew recorded on leaves in the 3-year period 1987-1989

PARENTS	% DESCENDANTS	
	NECROTIZED DOWNY MILDEW	MANIFEST DOWNY MILDEW
PROSECCO X S 4986	82,6	17,4
CHARDONNAY X S 4986	78,6	21,4
SAUVIGNON X S 4986	86,0	14,0
MOSCATO B. X 4986	71,4	28,6
MOSCATO B. X SV 5276	75,8	24,2

cultivation, i. e. 1986, and that no chemical treatments were performed during the following years.

Tolerance was determined by evaluating the evidence of downy mildew on the leaves of crossbreedings, comparing them with those visible on self-fertilized control samples. Two types of symptomatologies were noticed:

- downy mildew with well defined necrotic spots of different sizes on which mosaics of sporangiophores formed only in a later stage (Type N; Fig. 2, left);
- manifest downy mildew with extensive spots and a large amount of sporangiophores (type P; Fig. 2, right).

Affected leaves and bunches were examined and classified according to a system composed of 7 classes, according to the organs involved and the extent of damage.

The results were processed according to the Townsend-Euberger formula to calculate the infection degree (I.D. %).

Results

a) Downy mildew tolerance

The F_1 descendants of crossbreedings produced in 1985 and 1986 (several hundred progenies) were repeatedly evaluated during the years of cultivation.

With regard to the results of a preliminary screening of 1985 crossbreedings, we refer to a previous report (Costacurta *et al.* 1986). In the following years, the genotypes selected for their degree of resistance were kept and observed. They proved to maintain their resistance throughout the years in which control was performed. About 80 % of the descendants constantly showed symptoms of downy mildew with necrotic type spots, whereas the residual 20 % showed mixed mildew spots, more evident under the worst climate conditions (Table 1).

The incidence of downy mildew's influence on the different groups of crossbreedings recorded in the course of the period is illustrated in graphs of Fig. 3.

The intensity of downy mildew attacks differed in the various years and was recorded in July and at the end of summer. The latter is the period that provides the most appropriate evidence of the genotypes' behaviour, especially when compared to the control testing of European varieties and their self-fertilizations grown without chemical protectant treatments, on which downy mildew affects almost 100 % of all plants with early defoliation.

The distinction between the different types of evidence of the disease's presence shows its major incidence in the group of descendants with evident signs of downy mildew on leaves in both the testing times.

Tests performed on bunches, present on 80 % of the descendants in 1989, showed that the disease occurred in 11-30 % of the individuals in all seedling populations. These amounts may be considered as acceptable according to the testing conditions; the more consistent attacks were noticed on genotypes with evident downy mildew of leaf (Fig. 4).

The results of surveys on more recent plants, planted in fields in spring 1988, are summarized in Table 2. The figures show the average number of attacks recorded at the end of 1988 and in the first 10 d of August 1989.

The genotypes with necrotic downy mildew are, on average, 28.7 % of the entire population of F_1 descendants, whereas those showing mixed necrotic and evident symptoms of the disease include about 13 % of the population.

The percentage of leaves affected is lower in the plants considered 'resistant'. Self-fertilized plants proved to be greatly affected with early defoliation.

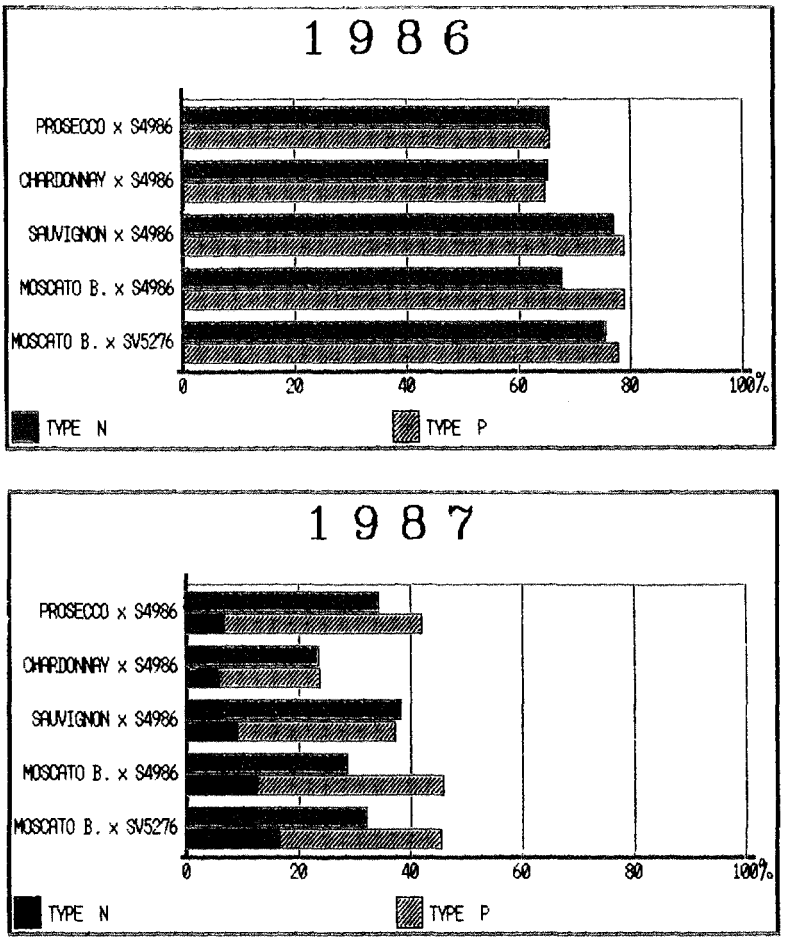


Fig. 3: Incidence of infection degrees (% I.D.) by *P. viticola* on leaves in crossbreedings F_1-1985 in several years and periods: July and August-September. (Continued overleaf.)

The figures reported show that among the European cultivars employed as parents seedlings of Sauvignon, Prosecco and Chardonnay seem to receive good resistance, in terms of frequency, to the disease, whereas amongst the donor parents the hybrid S. 4986 proves to be the best, especially when compared with S. 12.375.

b) Powdery mildew tolerance

The presence of *Oidium tuckeri* was examined on various F_1 descendants, by applying the same quantity survey systems as in downy mildew tests. The crossbreedings produced in 1985 were evaluated in field and greenhouse cultivations; those produced in 1987 were examined in the field.

Under normal cultivation conditions, powdery mildew was recorded only on a few plants (2.5 %) that seldom formed spots on some leaves and berries.

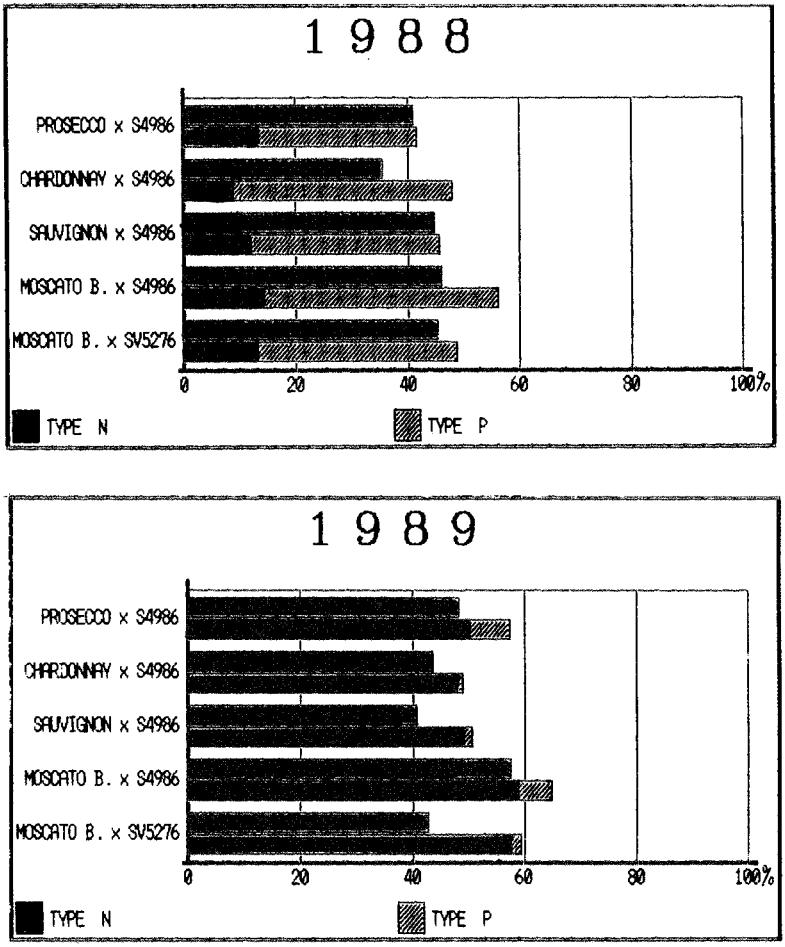


Fig. 3 (continued).

Table 2: F_1 descendants of 1987 crossbreedings divided according to different types of downy mildew on leaves

PARENTS	DOWNY MILDEW ON LEAVES					
	NECROTIZED (N)		MIXED (N-P)		MANIFEST (P)	
	GENOTYPE %	I.D. %	GENOTYPE %	I.D. %	GENOTYPE %	I.D. %
PROSECCO x S 4986	40,5	58,4	16,2	58,5	43,2	68,6
" x SV 5276	36,0	74,2	8,0	75,0	56,0	78,1
" x S 12375	18,7	45,0	18,8	47,3	62,5	63,3
SAUVIGNON x S 4986	27,6	50,3	13,8	70,0	58,6	72,9
" x SV 5276	38,9	61,9	10,7	67,3	49,7	71,7
PROSECCO x SE	35,7	55,3	21,4	59,2	42,8	86,0
CHARDONNAY x S 4986	42,8	66,7	-	-	57,2	75,0
" x SV 5276	18,2	32,0	-	-	81,8	80,0
" x S 12375	9,7	38,5	16,1	56,4	74,2	76,0
CABERNET S. x BA	18,8	48,0	31,2	36,0	50,0	77,2
AVERAGE	28,7	53,0	13,6	58,7	57,7	74,9

Table 3: Incidence of oidium attack on crossbreeding descendants of Prosecco x S. 4986 cultivated in greenhouse (Susegana, Treviso)

PARAMETERS	CROSSBREEDING DESCENDANTS		CONTROL	
	1988	1989	1988	1989
% infected plants	19,8	6,9	100	100
I.D. % on leaf	2,5	1,2	82,5	65,3
I.D. % on wood	2,2	0,8	64,7	18,7

The tests carried out on plants trained in greenhouses allowed us to better examine the behaviour of crossbreedings (Table 3). In 1988 approximately 20 % of the plants developed light infections on leaves and wood, whereas all the plants used as comparisons were strongly affected.

In 1989 there was a minor incidence of the disease, primarily due to an earlier survey.

Discussion and conclusions

The analysis of the descendants of some interspecific crossbreedings performed over several years, utilizing some of the most interesting European vine varieties cultivated in Venetia and several hybrid varieties resistant to *P. viticola*, demonstrated that this character is transmitted rather frequently to F_1 genotypes, with percentages ranging from approximately 20 to 40 %.

The results recorded so far proved that there are different behaviours for what concerns resistant parents: S. 4986 seems to be capable of transmitting resistance to *P. viticola* to a greater number of descendants, but S. 12.375 does not seem to be suitable for the same purpose. All the European varieties tested provided good results.

Over the years of observation, the selected genotypes maintained the acquired feature constantly, even when climate conditions and the epidemiology of *P. viticola* varied. The descendants affected by necrotic downy mildew recorded lower infections than those primarily bearing evident forms of the disease.

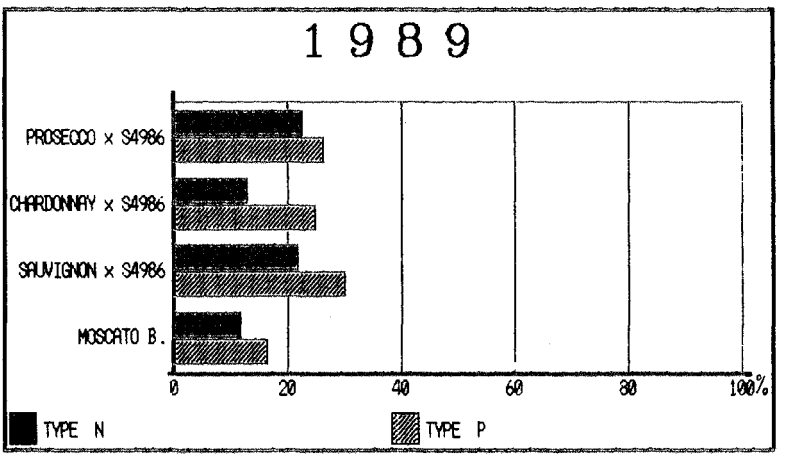


Fig. 4: Incidence of infection degrees (% I.D.) by *P. viticola* on bunches in crossbreedings F_1-1985.

Plants with intermediate features could be just as interesting considering the low incidence of downy mildew. The viticultural material selected was also of interest for what concerns its *O. tuckeri* tolerance, i. e. the number of plants affected and the extremely low incidence of disease attacks.

At present, there is no information concerning resistance to *Botrytis cinerea* PERS., as there was only a small amount of bunches on which tests could be performed in 1989.

We must emphasize that at this moment there were no other diseases due to cryptogams that might appear on unprotected plants.

Research will continue in the coming years according to the established crossbreeding programme: At the same time, new and future genotypes will be selected for resistance to grapevine diseases. The resistant genotypes will be subject to control to examine their productive and, primarily, organoleptic features.

References

ALLEWELDT, G.; 1979: Sélection de variétés résistantes en R. F. d'Allemagne. XVIe Congr. Vigne Vin, Stuttgart, 21-27 mai 1979.

AVRAMOV, L.; BABOVIC, M.; JOVANOVIC, M.; RUZEVIC, M.; 1980: Breeding plasmopara-resistant varieties in *Vitis*. Proc. 3rd Intern. Symp. Grape Breeding, Davis, 302-307.

BECKER, N.; ZIMMERMANN, H.; 1980: Wine quality of newly bred grape varieties resistant to downy mildew. Proc. 3rd Intern. Symp. Grape Breeding, Davis, 308-323.

BORGO, M.; CANCELLIER, S.; COSTACURTA, A.; 1987: Protection intégrée de la vigne avec l'amélioration génétique par croisement. Symp. Influence of Environmental Factors in the Control of Grape Pests, Diseases and Weeds. Thessaloniki, 6-8 October, 1987.

BOUBALS, D.; 1956: Amélioration de la résistance de la vigne au mildiou (*Plasmopara viticola* (B. et C.) BERL. et DE TONI). Recherche de géniteurs de résistance. Ann. Amélior. Plantes **4**, 481-525.

COSTACURTA, A.; CANCELLIER, S.; CORINO, L.; MORGO, M; 1986: Resistenza a *Plasmopara viticola* in incroci di *Vitis vinifera* con ibridi. Riv. Viticolt. Enol. **39**, 532-539.

DOAZAN, I. P.; KIM, S. K.; 1978: Recherche de génotypes résistants au mildiou dans des croisements interspécifiques. Génétique et Amélioration de la Vigne, 243-249. INRA Ed., Paris.

KIM, S. K.; 1978: Etude de croisements interspécifiques chez la vigne, transmission héréditaire de quelques caractères physiologiques et de la résistance au mildiou et au phylloxera. Thèse Doct. Ing., Univ. de Bordeaux II.

LI, H.; 1985: Etude de la relation entre le mildiou de la vigne (*Plasmopara viticola* (B. et C.) BERL. et DE TONI) et l'espèce *Vitis vinifera* L., variabilité de l'agent pathogène et de la sensibilité de l'hôte. Thèse Doct. Oen. Amp., Univ. de Bordeaux II.

MILLARDET; A.; 1891: Essai sur l'Hybridation de la Vigne. Masson Ed., Paris.

-- --; 1894: Sur les résultats généraux de l'hybridation de la vigne. Rev. Viticult. 1, 57-61, 84-88, 108.

TRAN MANH SUNG, C.; 1988: Le comportement épidémique de *Plasmopara viticola* vu au travers de modèles EPI, POM et PCOP. C. R. 2e Conf. Intern. Maladies des Plantes, Tome III. ANPP, Paris.

Vitis caribaea as a source of resistance to Pierce's disease in breeding grapes for the tropics

L. G. JIMÉNEZ A. and A. INGALLS

P. O. Box 10135, San José 1000, Costa Rica

Summary: A native Costarican vine, *Vitis caribaea*, was found growing unaffected by Pierce's disease (PD; *Xylella fastidiosa*) in the forests surrounding a dying *V. vinifera* plantation. *V. caribaea* was tested by inoculation, isolation, ELISA and DNA hybridization and in all cases no bacteria were detected. It was decided that *V. caribaea* or Agrá (its Indian name) is resistant or at least highly tolerant to PD. Crosses of *V. vinifera* and *V. caribaea* were made and no compatibility barriers were found, germination of the hybrids seeds was high and a high percentage of fertile plants were produced. Many hybrids were made and planted in the field to test them for resistance to PD.

Since some of the F_1 hybrids do transmit resistance when backcrossed to *V. vinifera*, resistance must be determined by dominant genes. Some F_1 hybrids, although apparently resistant themselves, are either not transmitting resistance or are doing so in a reduced proportion. Several hybrids developed at the University of Florida were tested, one of these, F 5-8, has led to the establishment of the first successful vineyard in Costa Rica.

Key words: Pierce's disease, bacterium, Vitis, resistance, breeding, genetics, tropics, Costa Rica, America.

Introduction

The Spanish have a very old viticultural tradition and it is remarkable that this tradition should have been today lost when they colonized tropical America. It is said that the Spanish government forbade the planting of grapes in their colonies to prevent competition with their own domestic wine production. Undoubtedly in the long history of these colonies thousands of Spanish settlers have tried to establish their own vineyards. Indeed, in our own lifetimes we have known of numerous cases of people who, having planted a vineyard, had some success at first only to be disillusioned a few years later when all the plants died. A similar experience in southern California was described by PIERCE (1892).

The symptoms of Pierce's disease (PD) are very similar to those of acute water stress: the edges of the leaves burn and later drop off, the berries shrivel up, and the plant dies. We can distinguish the symptoms of PD from simple drying if we notice that the leaves burn asymmetrically in sections, more on one side than on the other side of the leaf, and that a yellow or red band appears on the border between vital and necrotic sections. The plant seems to recover between attacks leaving a narrow dark line between each successive burn. Eventually the leaves fall from the petioles which stay connected to the stem, the wood of the vine matures unevenly leaving islands of immature wood on mature canes which are then subject to winter damage.

For one hundred years all attempts to discover the cause of this disease were unsuccessful, although much field work was done to establish its wild hosts (grasses and many weeds) (FREITAG 1951) and its vectors (leafhoppers) (HEWITT *et al.* 1942). Recently, the possibility of applying modern laboratory techniques and the discovery that tetracycline antibiotics (HOPKINS and MORTENSEN 1971) could suppress symptoms in PD diseased vines suggesting that the disease might be caused by a mycoplasma-like organism, revitalized interest in the disease. Using the electron microscope, rickettsia-like bacteria (RLB) were found to be associated with the disease (GOHEEN *et al.* 1973; HOPKINS 1973). A few years later the causal bacterium was isolated and cultivated (DAVIS *et al.* 1978). This opened up the possibility of using techniques such as inoculation, ELISA (RAJU *et al.* 1980, 1981) and DNA hybridization (JIMÉNEZ and DAVIS 1987).

In 1988 the name *Xylella fastidiosa* was proposed (WELLS *et al.* 1987) for this bacterium. Studies of host-vector-pathogen relationships have shown that PD is endemic in California, the Gulf States (HEWITT 1958), Mexico (RAJU *et al.* 1980), Central America (GOHEEN *et al.* 1979; JIMÉNEZ 1980, 1982) and Venezuela (JIMÉNEZ 1985) and it is of ancient origin.

Preliminary work

Following the discovery (GOHEEN *et al.* 1979) and confirmation (HOPKINS, unpublished; JIMÉNEZ 1980) of PD in Costa Rica, a search was made for material having resistance to this disease. Several hybrids developed by MORTENSEN in Florida were tested, one of these, F 5-8, which was never released in Florida because of its susceptibility to fungus diseases, has led to the establishment of the first successful commercial vineyard in Costa Rica. A native Costarican vine, *Vitis caribaea*, was found growing unaffected by any disease in the forests surrounding a dying *V. vinifera* plantation in Montezuma on the Pacific coast of Costa Rica. It was tested by inoculation, isolation, and ELISA and in all cases either no bacteria were found or they were so low in number that the results were untrustworthy. It was decided that *V. caribaea* or Agrá (its Indian name) is resistant or at least highly tolerant to PD and that it might be a good source of resistance if it could be crossed with *V. vinifera*. In mid forties JOSEPH FENNELL had used *V. caribaea* in some of his crosses at the Inter-American Institute of Agricultural Science in Turrialba, but when this organization was reorganized all his genetic material (FENNEL 1948) was lost to Costa Rica. Turrialba is in a wet tropical rainforest, a notoriously bad climate to grow grapes in.

Starting in 1978, new crosses were made, this time in Montezuma which is in a hot dry tropical forest just on the edge of the climatic zone of maximum dryness in which *V. caribaea* can still be found growing naturally. In some crosses female *V. caribaea* was pollinated with *V. vinifera*, in others *V. vinifera* was hand emasculated and pollinated with *V. caribaea*. No compatibility barriers were found. Germination of the hybrid seed was high and a high percentage of fertile plants were produced. Many F_1 hybrids and backcrosses to *V. vinifera* were made and planted in the field at a distance of 40 cm x 200 cm to test for resistance to PD. The field was prepared for planting by machetti in order to preserve the roots of the native hosts of PD intact. The weeds were allowed to grow freely to facilitate infection of the new plants. Weeds were chopped and insecticides were used only when absolutely necessary. We slowly began to realize that we were confronted with a happy but frustrating circumstance: Whereas in a temperate climate the spread of the disease is more or less slow, once it enters the vine, the latter succumbs in a reasonably short time (3 months to 1 year). In the tropics, however, the spread is very rapid, in 6 months over 80 % of a new planting in Montezuma was contaminated (JIMÉNEZ 1982), and yet after infection the disease develops very slowly within the plants which degenerate over a period of 2-9 years. Cardinal produced high fruit for 3 years despite the fact that typical symptoms of PD were observed throughout this time and bacteria were consistantly isolated from them. In laboratory tests, Costarican strains of PD were found to have virulence similar to those of the North American strains (GOHEEN *et al.* 1979). It is possible that a small difference in virulence not observable in the lab could correspond to a large difference in the field, but it is obviously indesirable and illegal to test this in the open. Instead, HOPKINS (private conversation) attributes this greater longevity to an increased tolerance of plants grown in the tropics: Using good cultural practices, in a tropical climate the plant can sustain constant vigorous growth under conditions of little stress, this allows it to outgrow the damage (to the xylem) caused by the bacteria almost as fast as it is produced. In fact in some microclimates (Alajuela, Costa Rica and Maracaibo, Venezuela) (JIMÉNEZ) *vinifera* grapes can be grown successfully in spite of PD. The frustrating aspect is that we can never be quite sure whether a hybrid we have made is really tolerant or whether waiting just one more year it will die. After waiting so many years to selecting a vine for resistance, it becomes rather uncertain whether it died of PD, another disease or accident.

Materials and methods

It would be very useful to know which percentage of a certain cross can be expected to survive PD in Costa Rica. In Florida, it was shown (MORTENSEN 1968) that using a model of three independent dominant genes where all three are necessary for resistance, one could predict the resistant percentage. Could we expect that Agrá has the same type of resistance mechanism as the wild grapes native to Florida that were used by MORTENSEN? Since there is no significant geographic barrier between Florida and Central America and all of this is inhabited by various species of wild grape which are probably related to each other and all are under the pressure of PD, they might share a common type of resistance. Until we had more information, we went on the hypothesis that this is so. This allowed us to use the backcross system of breeding as long as we were sure we had selected out plants that were not resistant at each generation. But this is exactly what we could not do. In our backcross population, now 8 years old, only 27 % of all seedlings have died, instead of the 88 % predicted by MORTENSEN. Many of the survivors however are loosing vigor and becoming unproductive. Fruit production is the principle stress that plants have to undergo and those that are not under stress can survive better an attack of PD. In order to select effectively, all the plants should be under the same stress. This can be achieved to some extent by considering only the plants that are capable of producing fruit:

$$\frac{\text{Plants that are still fruiting}}{\text{All the plants that would be capable of fruiting in absence of PD}}$$

Here we have entered an unmeasurable term in the denominator which is equal to:

All the plants that have produced at least once in their lifetime + the plants that could have produced but died of PD before they had time to

From previous experience with a quasi randomly selected collection of *V. vinifera* varieties donated by Dr. GOHEEN we found that less than 25 % of *vinifera* seedlings die before they can produce fruit. But about $^1/_2$ of the plants in the denominator are resistant to PD, so we have to add $^1/_2$ of $^1/_4$ or $^1/_8$ to the denominator. This correction is not large and will not be made in the following work, however at any time we can include it by multiplying the result by $^8/_9$.

Now we arrived at our measurable ratio which we will call sustainability:

$$\frac{\text{Number of plants in production}}{\text{Number of plants that have produced at some time } (x\ ^9/_8)}$$

In doing this we have largely corrected an error in the % survivors due to the fact that some plants die of other causes: extreme susceptibility to fungus, genetic weakness, and accidents. Since most of these affect the plants early in their lives they will be prevented from ever producing fruit and so these plants will not enter into the ratio. The numerator and the denominator are not fixed numbers but variables which depend upon the year of observation:

$$\frac{\text{Number in production [Year]}}{\text{Number that have produced [Based year]}} = \text{Sustainability}$$

The based year is the year we look back at our data and calculated the number of plants that have produced at some time. If we do this too early, our ratio is too high and is close to 1 because exactly the same plants appear in the numerator and denominator. If the base year is adequate, the ratio will be a function of the year observed. To illustrate this we use our oldest backcross population (8 years old) as an example. There are too few plants in each cross to get reasonable curves by cross, but by batching one row of 100 plants of mixed backcrosses we get the following data:

Year	6th	7th	8th
Dead plants	12	15	27
Degenerating	18	39	30
Producing	33	18	16
Have produced	38	38	38
ELISA*			3
% Survivors	88 %	85 %	73 %
Healthy	70 %	46 %	43 %
Sustainability	87 %	47 %	42 %

* The 3 plants showing positive readings were already degenerating.

Among other things this data serves to clear up any doubts about our use of sustainability instead of % healthy plants, here they turn out almost equal. Also it is clear that, no matter how we look at the data, we get a ratio of survivors much higher than that of MORTENSON in Florida.

Results

Fig. 1: Here we present a series of graphs showing the progression of selection for PD against time for individual backcross families.

Fig. 2: At about 3-4 years most plants have started producing fruit, but PD has not yet seriously affected the production and so the curve has its maximum at this time. After this, the curve descends as PD kills and degenerates vines, as we hoped after 6 or 7 years the curve levels off as only plants tolerant to PD will be still producing. Unfortunately this leveling off has not occurred for all crosses.

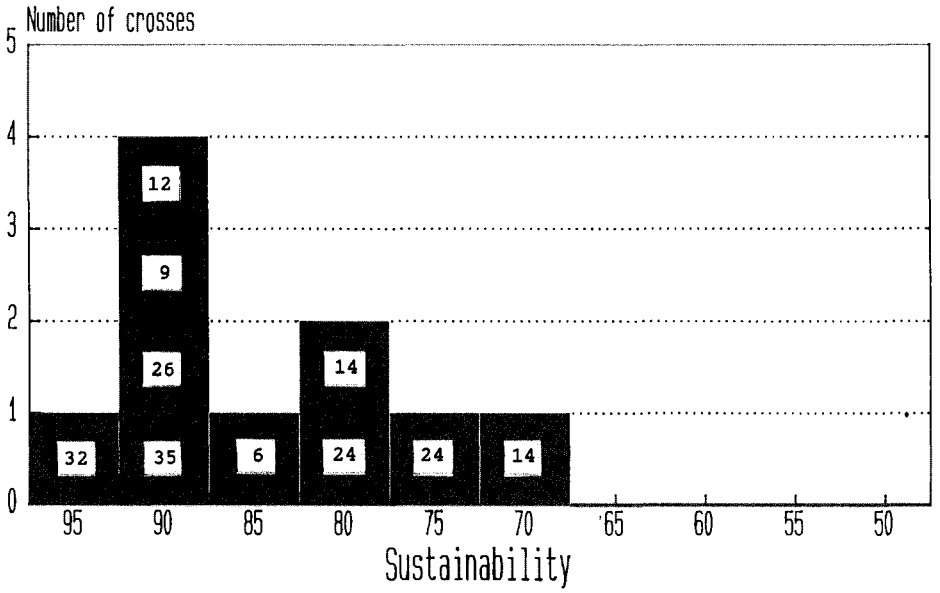


Fig. 1: Sustainability of F_1 hybrids Agrá used as resistant parent. Data from 9-year old plants used.

Fig. 3: It is too soon to tell if this curve will level off leaving a few residual plants or else drop to zero, in which case the 'resistant' parent would not have been transmitting resistance.

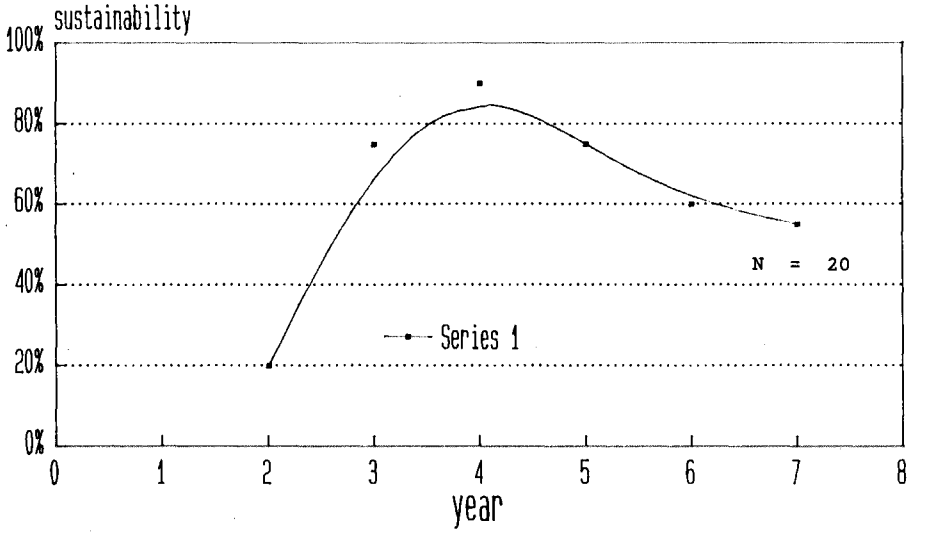


Fig. 2: Sustainability of the progeny of 75 C (32 A 10 x M. Hamburg).

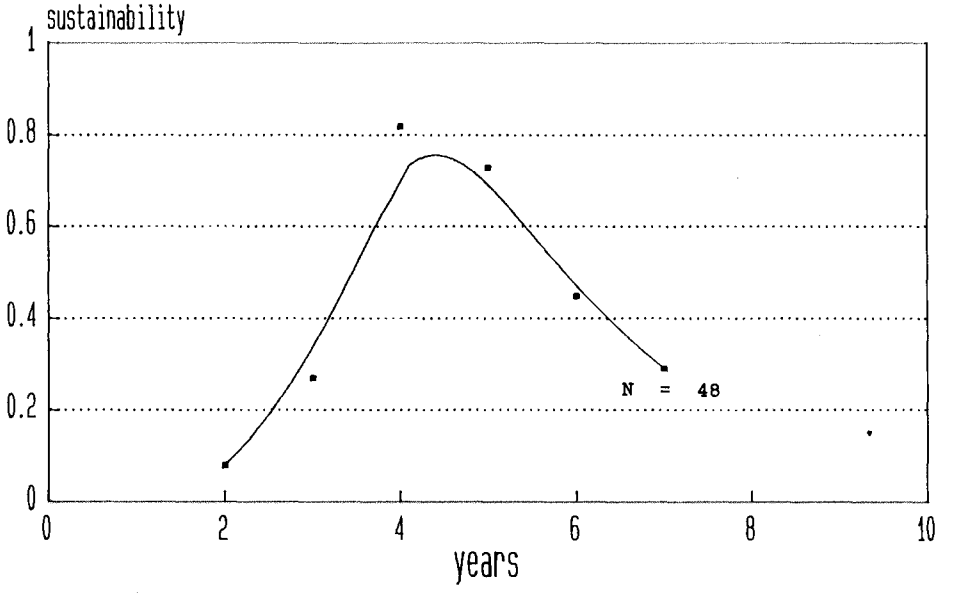


Fig. 3: Sustainability of 73 A (3 C 15 x Ruby Cabernet; 3 C 15 = Petit Sirah x Agrá).

Fig. 4: Plotting together every backcross family with over 15 plants for the denominator (to reduce sampling error) planted in 1982 we notice that the crosses can be divided into two groups, those that seem to be heading for a ratio around 50 % and those that are descending to a much lower level.

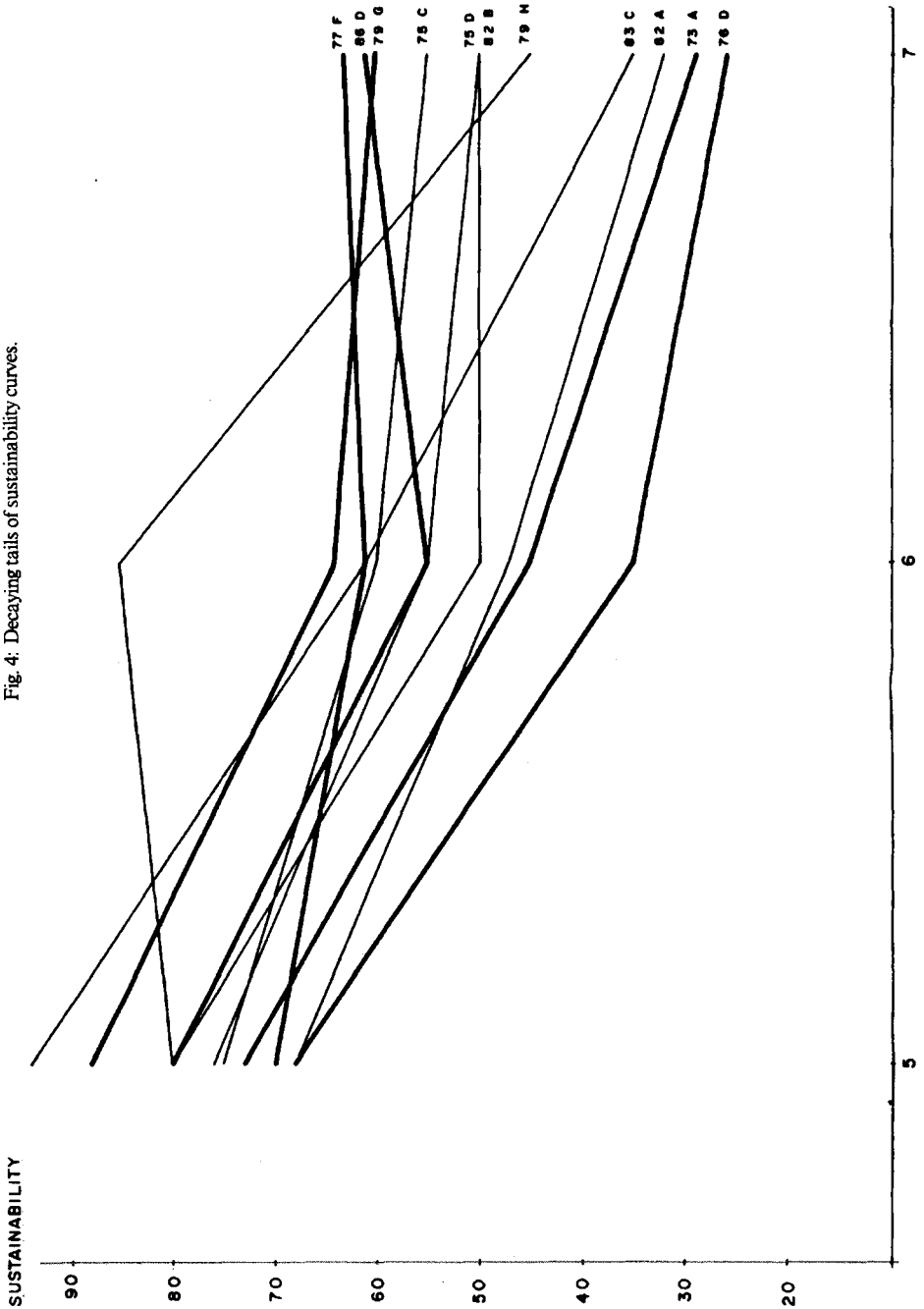


Fig. 4: Decaying tails of sustainability curves.

Fig. 5: Here is set of curves that show 3 different backcrosses all with the same 'resistant' parent 56 C 1. Although 56 C 1 (Aleatico x Agrá) is 9 years old, is still vigorous and has a negative

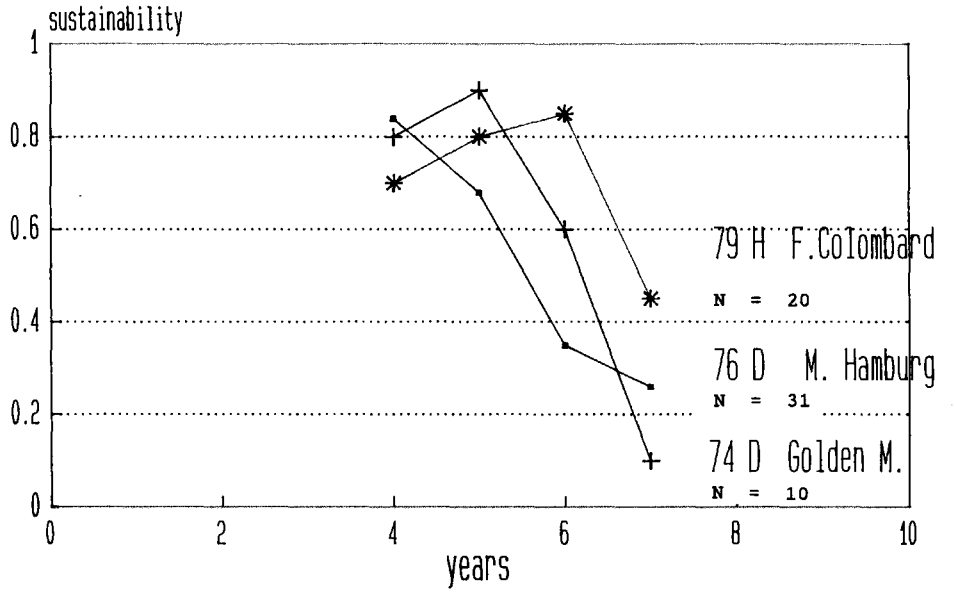


Fig. 5: Sustainability of 3 crosses using 56 C 1 (Agrá x Aleatico).

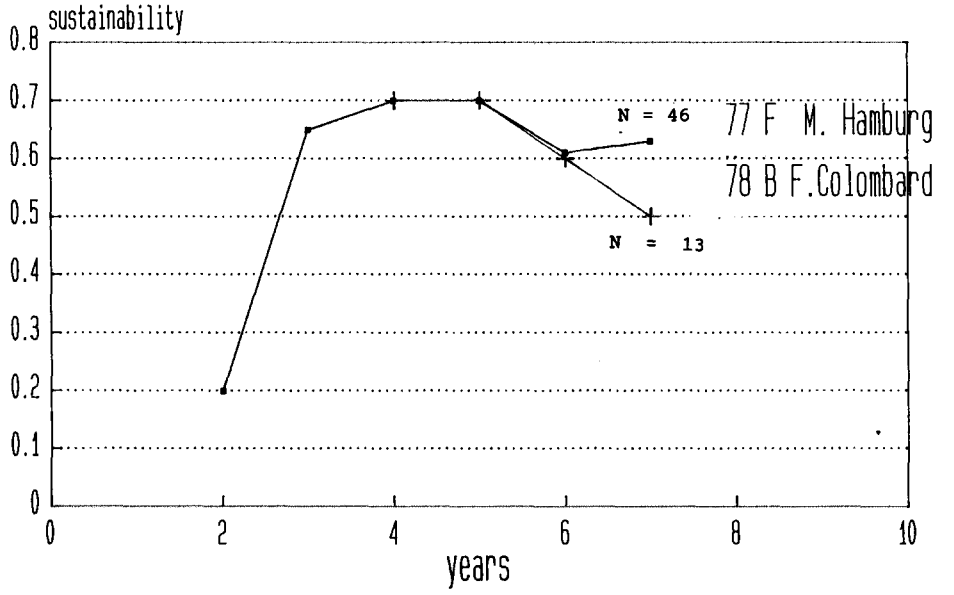


Fig. 6: Sustainability of 2 crosses using 56 C 11 (Agrá x Aleatico).

reaction with ELISA, it does not seem to be transmitting resistance to its progeny. Its productivity has been declining, however, and this seems to be the key to the early spotting of plants low in tolerance to PD. Many of the other plants that are not transmitting full resistance are, however, sustaining their productivity and there is no visible way to distinguish them.

Fig. 6: This graph shows the opposite type of behavior in the progeny of 56 C 11, a sibling to 56 C 1. 56 C 11 seems to transmit good resistance to its progeny. The ELISA test was performed on half of the cross 77 F but no new information was gained from this test: only one plant was found positive but it was already visibly degenerating. Contrasted to 56 C 1, 56 C 11 has steadily increased its production each year.

We should briefly describe the grapes produced. The F_1 hybrids produce berries that are about twice the diameter of those of Agrá and are all black although the color of those crossed with white *vinifera* is not very intense or stable in the wine. Although the fruit is high in sugar, their taste is usually acid and biting, while their aroma resembles that of *vinifera*. The backcross fruit is from 2-4 times larger than Agrá in diameter, sometimes white or red, less acid and sometimes lower in sugar than the F_1 hybrids. The average production of both the F_1 and the backcrosses is very low being less than 200 g/plant which is probably due more to the short photoperiod of the tropics than hybrid infertility. Heavier producers can be selected that give 1-2 kg/plant at the close planting distances used to test them (1 m^2/plant).

Some data showing how the extreme acidity of Agrá can be easily reduced by backcrossing to *vinifera*:

Code	Type	°Brix	T. acid*	pH	Cross
Agrá	Wild	15.8	32.5	2.79	Betty
18 B 9	F_1	20.9	22.4	3.00	Agrá x Carignane
57 E 39	F_1	22.7	17.1	2.75	Agrá x Aligoté
6 G 1	F_1	24.8	17.1	2.88	Sauvignon blanc x Agrá
22 B 8	F_1	23.9	13.4	3.18	Fernao Pires x Agrá
80 D 17	BC_1	23.4	13.3	3.00	(Sylvaner x Agrá) x F. Colombard
81 B 11	BC_1	24.0	12.7	3.20	(Chardonnay x Agrá) x M. Alex
78 G 9	BC_1	23.2	15.5	3.00	(Green Veltliner x Agrá) x Aleatico
83 C 12	BC_1	18.6	11.2	3.05	(Agrá x Carignane) x Ruby Cabernet
59 D 3	BC_1	17.6	12.2	3.22	(Agrá x Ruby Cabernet) x Carignane
77 F 48	BC_1	21.8	7.2	3.23	(Agrá x Aleatico) x M. Hamburg

* in g tartaric acid/l

Discussion and conclusions

Since some of the F_1 hybrids do transmit resistance when backcrossed to *V. vinifera*, resistance must be determined by dominant genes. Some F_1 hybrids, although apparently resistant themselves, are either not transmitting resistance or are doing so in a reduced proportion. We might call these plants partially tolerant to explain this genetically, we might hypothesize that the three genes postulated by MORTENSEN have an additive effect, which in the harsher climate of Florida was not evident. In this system, one gene will give a little longer survival time, two genes will result in a partial tolerance typified by 56 C 1, three genes will lead to full tolerance like 56 C 11; this would explain the 50 % ratio of the test crosses with 56 C 11: $^1/_8$ of their progeny would be fully tolerant and $^3/_8$ would be partially tolerant.

We will have the results of the first test of this hypothesis when we see whether the sustainability of 56 C 1 turns out to be 25 % with all the survivors partially tolerant. The existence of partial tolerance would be a burdensome impediment to the production of hybrid grape varieties for the tropics.

A much more favorable alternative would be that Agrá has two distinct resistance mechanisms, one depending on three genes that gives the low sustainability of 56 C 1 and another controlled by one dominant gene giving the 50 % ratio of 56 C 11. We will have to wait several more years to resolve this question.

It is feasible to use the resistance of V. *caribaea* to PD and many other diseases to breed resistant hybrid vines useful for the production of wine and table grapes for the tropics. Although there are many problems in selecting for resistance to such a weakly aggressive yet virulent disease in a gentle tropical climate, the lack of a viticultural tradition means that we do not have to satisfy a predetermined taste preference, we have no competition, and grape and wine prices are extremely high.

Literature cited

DAVIS, J. M.; PURCELL, A. H.; THOMSON, S. V.; 1978: Pierce's disease of grapevines: Isolation of the causal organism. Science **19**,75-77.

FENNELL, J. L.; 1948: Inheritance studies with the tropical grape. J. Hered. **39** (3), 54-62.

FREITAG, J. H.; 1951: Host range of the Pierce's disease virus of grapes as determined by insect transmission. Phytopathology **41**, 920-934.

GOHEEN, A. C.; NYLAND, G.; LOWE, G. K.; 1973: Association of a rickettsialike organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology **63**, 341-345.

– – ; RAJU, B. C.; LOWE, S. J.; NYLAND, G.; 1979: Pierce's disease of grapevines in Central America. Plant Dis. Rep. **63**, 788-792.

HEWITT, W. B.; 1958: The probable home of Pierce's disease virus. Plant Dis. Rep. **42** (2).

– – ; FRAZIER, N. W.; JACOB, H. E.; FREITAG, J. H.; 1942: Circular 353. Univ. of Cal., Coll. of Agricult., Agricult. Exp. Sta., Berkeley, Cal.

HOPKINS, D. I.; 1973: Rickettsia-like bacterium associated with Pierce's disease of grapes. Science **19**, 298-300.

– – ; MORTENSEN, J. A.; 1971: Suppression of Pierce's disease symptoms by tetracycline antibiotics. Plant Dis. Rep. **55**, 610-612.

JIMENEZ ARIAS, L. G.; 1980: El mal de Pierce de la vid, su distribucion en Costa Rica y caracteristicas fenotipicas de su agente causal. Tesis presentada para Licenciado en Fitotecnica, Univ. de Costa Rica.

– – ; 1985: Evidencia immunologica del mal de Pierce de la vid en Venezuela. Turrialba **35** (3), 243-247.

– – ; DAVIS, M. J.; 1987: DNA probe for detection of Pierce's disease bacterium and other xylem-limited bacteria. Phytopathology **77**, 1769.

JIMENEZ MORA, J. M.; 1982: Determinacion de los hospedantes alternos del mal de Pierce de la vid en Costa Rica, mediante tecnicas immunologicas de adsorcion con conjugados enzimaticos.

MORTENSEN, J. A.; 1968: The inheritance of resistance to Pierce's disease in *Vitis*. Proc. Amer. Soc. Hort. Sci. **92**, 331-337.

PIERCE, N. B.; 1892: The California vine disease. U. S. Dept. of Agricult., Div. Veg. Path. Bull. **2**.

RAJU, B. C.; AUSTIN, C.; GOHEEN, A. C.; 1981: Relative sensitivity of selected grapevine cultivars to Pierce's disease bacterial inoculations. Amer. J. Enol. Viticult. **32**, 155-158.

– – ; GOHEEN, A. C.; TELIZ, D.; NYLAND, G.; 1980: Pierce's disease of grapevines in Mexico. Plant Dis. **64**, 280-282.

– – ; NOMÉ, S. F.; DOCAMPO, D. M.; GOHEEN, A. C.; NYLAND, G.; LOWE, S. K.; 1980: Alternative hosts of Pierce's disease of grapevines that occur adjacent to grape growing areas in California. Amer. J. Enol. Viticult. **31**, 144-148.

WELLS, J. M.; BOLIGALA, C.; RAJU, B. C.; HUNG, H.-Y.; WEISBURG, W. G.; MANDELCO-PAUL, L.; BRENNER, D. J.; 1987: *Xylella fastidiosa* gen. nov., sp. nov.; Gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas* spp. Intern. J. Syst. Bact. **37** (2), 136-143.

Best combiners during 40 years of breeding *Vitis* cultivars resistant to Pierce's disease

J. A. Mortensen and L. H. Stover

University of Florida, IFAS, Central Florida Research and Education Center, Leesburg, Florida 34748, USA

Summary: By breeding for resistance to Pierce's disease in *Vitis* we have obtained useful cultivars that can be grown productively in areas formerly considered unsuitable for grape production. Reviewing the most successful recombinants from crosses made between 1945 and 1984, 6 *Vitis* clones were prominent foundation parents among those tested as primitive resistant germplasm: *V. aestivalis*, ssp. *smalliana* cvs Fla. 43-47 and Fla. 449, *V. aestivalis* ssp. *simpsoni* cvs Pixiola and Fla. 451, and *V. shuttleworthii* cvs Haines City and Kissimmee. The best combiners for productivity, fruit size, and high quality were PD susceptible cultivars Aurelia, Carolina Blackrose, Cardinal, Exotic, Golden Muscat, and Villard blanc. The best combiners for seedlessness and early ripening were susceptible cultivars Lakemont and Perlette. Selection for resistance to PD required 7 or more years each generation for exposure of seedlings to PD-carrying vectors. Inbreeding was detrimental to vine vigor but good combiners were selected among inbred progeny which were more homozygous for disease resistance. Subsequent crosses of these inbreds to large-fruited, high-quality cultivars resulted in some recombinants with restored vigor and superior traits such as Blanc Du Bois.

Key words: Pierce's disease, bacterium, Vitis, variety of vine, germplasm, resistance, breeding, inbreeding, parents, Florida, USA.

Introduction

Pierce's disease (PD) is a vascular disease of *Vitis* caused by the bacterium *Xylella fastidiosa* (Wells *et al.* 1987). PD is the major limiting factor to having long-lived and productive vineyards in Florida and the Coastal Plain areas of South Carolina, Georgia, Alabama, Mississippi, Louisiana, and Texas (Hopkins and Adlerz 1988). Resistance to PD was found in *Vitis* species native to Florida (Mortensen *et al.* 1977). Since 1945 a grape breeding program has been under way at Central Florida Research and Education Center, Leesburg, to incorporate this native PD resistance into viticulturally acceptable cultivars (Stover 1960). The purpose of this paper is to review the most successful recombinants from crosses made between 1945 and 1984 and to emphasize the best parental combinations used in the program.

Materials and methods

Sources of resistance to PD were found growing naturally in the woodlands of Florida, and were collected in 1941 and 1942 (Loucks 1942). Clones of the following Florida species were propagated from their native habitat to a vineyard located west of Leesburg where their longevity and health could be observed: *Vitis aestivalis* Michx. ssp. *simpsoni* Munson, *V. aestivalis* ssp. *smalliana* Bailey, *V. rufotomëntosa* Small, *V. shuttleworthii* House, *V. sola* Bailey, *V. vulpina* L., *V. rotundifolia* Michx. and *V. munsoniana* Simpson.

Crosses made by Loucks during the 1930s using small-berried wild species as female parents and larger berried cultivars as male parents produced all small-berried progeny of low quality (Loucks 1938). *V. shuttleworthii* had larger berries, but when crossed the F_1 hybrids had small clusters. By 1945 a cross was made by Stover between Pixiola *(V. aestivalis* ssp. *simpsoni)* and Golden Muscat which produced 20 promising seedlings. Pixiola was collected by Loucks as a non-pigmented clone with fruit that was sweet but green-colored when ripe (Stover 1951).

Crosses between 1945 and 1984 were made with the objective of combining native disease resistance derived from Florida native grapes with acceptable fruit size and quality from superior

Table 1: Clones resistant to Pierce's disease (PD) used as parents in the first generation of breeding

Native species classification	Sex	Parental clone
Vitis aestivalis Michx. ssp. simpsoni	f	Pixiola
Vitis aestivalis Michx. ssp. simpsoni	f	Fla. 451
Vitis shuttleworthii House	f	Haines City
Vitis shuttleworthii House	m	Kissimmee
Vitis aestivalis ssp. smalliana	f	Fla. 43-47
Vitis aestivalis ssp. smalliana	f	Fla. 449

Table 2: First generation of crosses with *Vitis aestivalis* MICHX. ssp. *simpsoni* MUNSON and *V. shuttleworthii* HOUSE

Year of cross	Combination	Resistant progeny selected	Main uses[z]
1945	Pixiola x Golden Muscat	W381, W382	B
		Lake Emerald	WW
1950	Fla. 451 x Golden Muscat	W1001	B
1949	V. shuttleworthii open-poll.	Mantey	B
1961	Haines City x Alden	13B-5	RS
		13C-12	RS
1973	Haines City x Ark. 1105	BD7-75	B
1979	Villard Blanc x Kissimmee	CA8-15	RW

[z]Uses: B = breeding; RS = rootstock; RW = red wines; WW = white wine.

cultivars (STOVER 1960; MORTENSEN *et al.* 1977). Seedlings were planted 2.5 ft apart in a single-wire trellis vineyard at Leesburg and were fruited to determine which were usable recombinants. Individual plant performance was recorded for sex, budbreak, vine vigor, disease resistance, longevity, fruit size and quality (including seedlessness), productivity, and earliness. Natural infection from PD vectors was usually adequate to screen for resistance to PD while seedlings grew and fruited. Selections were propagated by hardwood cuttings for a second test at wider spacing (2.3-3 m in row). Crosses made each year between 1945 and 1984 were reviewed for progeny performance to determine which combinations produced outstanding recombinants.

Results and discussion

Many selections that initially were outstanding succumbed in later years to PD or fungus diseases or else became mediocre in fruit quality, productivity, or vine vigor. Selection for resistance to PD was found to be necessary each generation through natural exposure of seedlings for 7 or more years to PD-carrying vectors. 7-10 years are thus advisable before naming and release of a selection.

Table 3: First generation of crosses with *Vitis aestivalis* MICHX. ssp. *smalliana* BAILEY

Year of cross	Combination	Resistant progeny selected	Main uses[z]
1948	Fla. 43-47 x Golden Muscat	W716	B
1948	Fla. 43-47 x Niagara	Tampa	RS
1950	Fla. 43-47 x Caco	Blue Lake	JL
1963	Fla. 43-47 x Concord	E12-59	B, JU
1977	Fla. 43-47 x Aurelia	BD5-67	B
		BD8-43	RW
1977	Fla. 43-47 x Carolina Blackrose	BD10-51	B
		CB9-23	RW
1977	Fla. 43-47 x Dunstan 236	AD1-115	RW
1950	Fla. 449 x Cardinal	W987	B
1954	Fla. 449 x Lake Emerald	W1521	B

[z]Uses: B = breeding; JL = jelly; JU = juice; RS = rootstock; RW = red wine.

Table 4: Second generation of crosses and their PD-resistant progeny

Year of cross	Combination	Resistant progeny selected	Main uses[z]
1949	W381 x Cardinal	W907	B
1961	W382 selfed	21C-31	B
1956	W987 x Lake Emerald	Norris	B
1957	W1001 x Villard Blanc	B3-83, B3-90	B
1956	Mantey x Roucaneuf	Stover	WW
1961	W716 x Buffalo	Liberty	B
1961	W716 x Sultanina	15B-23	B
1983	BD10-51 x Ruby Cabernet	AN5-75	RW
1958	W1521 x Villard Blanc	C5-50	B
1965	W1521 x Aurelia	E18-63	B
1976	W1521 x Aurelia	DC1-39, DC1-56	B

[z]Uses: B = breeding; RW = red wine; WW = white wine.

Foundation parents selected from *V. aestivalis* ssp. *smalliana*, *V. aestivalis* ssp. *simpsoni*, and *V. shuttleworthii* contributed PD resistance and tolerance to stresses such as warm night temperatures, high humidity, torrential rainfall during ripening, and low fertility soils (Table 1).

The best sources of fruit size, high quality, and productivity were Aurelia (Villard blanc x Chaouch), Caroline Blackrose (Aurelia x Blackrose), Cardinal, Exotic, Golden Muscat and Villard blanc. Lakemont and Perlette were the best parents for seedlessness and early ripening. Tables 2 through 7 present 5 generations of the best crosses and their outstanding progeny selected during the 40 years. Pedigrees may be traced by proceeding backwards through the tables from a given outstanding cultivar selected.

Inbreeding reduced vine vigor and resulted in smaller leaves and shorter internodes, but was useful in developing parents more homozygous for disease resistance. Subsequent crossing of these inbreds to large-fruited, high-quality cultivars such as Fla. F5-8 and Cardinal resulted in progeny with restored vigor and improved recombinations such as Blanc Du Bois (Mortensen 1988). New cultivars arising from the program now form the basis for commercial grape growing in Florida (Halbrooks and Mortensen 1989).

At least one parent in the combinations should be resistant to PD and grow vigorously on its own roots. Crosses where both parents require grafting for good performance usually had progeny lacking in vigor in Florida sand land. In fact, many seedlings from such parentages failed to reach the trellis wire and fruit normally. Prevalence of parasitic nematodes in non-fumigated sandy soil is

Table 5: Third generation of crosses and their PD-resistant progeny

Year of cross	Combination	Resistant progeny selected	Main uses[z]
1956	Fla. 449 x W907	A4-23	B
1961	Norris x Schuyler	D4-176	B
1963	Norris x Concord	E11-40	B
1963	Norris x Blue Lake	E9-48	B
1964	Norris x Alden	F8-35	B
1980	B3-83 x Blanc Du Bois	BD7-33	WW
1964	B3-90 x Exotic	Daytona	T
1983	Stover x NC74C039-1	RN2-21	WW
1964	C5-50 x Exotic	F5-8	T, B
1973	C5-50 x Liberty	BD6-47	B
1981	E18-63 x NY45791	CA11-17	T, B
1982	E18-63 x Lakemont	BN8-25	T
1982	DC1-39 x Himrod	BN6-85	T

[z]Uses: B = breeding; T = table; WW = white wine.

thought to be a major factor inhibiting seedling growth of the progenies. Root-knot nematodes were prevalent among roots of older seedlings when removed for discard.

One outstanding breeding clone was Fla. W1521, which contributed vigor, high budbreak percentage and longevity; resistance to PD, anthracnose (*Elsinoe ampelina* DE BARY (SHEAR)), downy mildew (*Plasmopara viticola* (B. et C.) BERL. et DE T.) and fruit rots; and adaptability to frequent summer rainfall without fruit cracking or uneven ripening. Fla. W1521 was a parent of 4 outstanding clones in Table 4, a grandparent of 6 in Tables 5 and 6, and a great-grandparent of 5 clones in Tables 6 and 7. Another outstanding breeding clone was Norris, which contributed large size of berry and cluster along with resistance to Pd and downy mildew and susceptibility to anthracnose. Norris was parent to 4 elite clones in Table 5, grandparent to 4 in Table 6, and great-grandparent to 4 in Table 7. Both Fla. W1521 and Norris are pistillate-flowered, had Lake Emerald as their pollen parents, and had Fla. 449 as their mother and grandmother, respectively (Tables 3 and 4).

Lake Emerald (a *V. aestivalis* ssp. *simpsoni* derivative) contributed PD resistance, productivity, and vigorous growth under humid, subtropical environmental stresses. Fla. 449 (a *V. aestivalis* ssp. *smalliana*) contributed resistance to PD, downy mildew, powdery mildew

Table 6: Fourth generation of crosses and their PD-resistant progeny

Year of cross	Combination	Resistant progeny selected	Main uses[z]
1961	A4-23 selfed	D6-148	B
1964	A4-23 x Perlette	F9-68	B
1973	D4-176 x F9-68	Orlando Seedless	T, B
1983	BD5-67 x F9-68	DN15-12	RW
1969	E12-59 x E11-40	Conquistador	T, JU, JL
1972	E9-48 x Ark. 1105	BD12-49	WW, T
1968	C5-50 x F8-35	Suwannee	WW, B
1980	Daytona x Stover	BD5-117	T, B
1969	21C-31 x F5-8	L9-10	WW
1982	BD6-47 x Ark. 1105	BN5-101	T, B

[z]Uses: B = breeding; JL = jelly; JU = juice; RW = red wine; T = table; WW = white wine.

Table 7: Fifth generation of crosses and their PD-resistant progeny

Year of cross	Combination	Resistant progeny selected	Main uses[z]
1968	D6-148 x Cardinal	Blanc Du Bois	WW
1983	BD7-75 x Orlando Seedless	DN21-83	B
1978	W716 x Suwannee	CA4-66, CA4-72	RW
1982	Suwannee x Verdelet	CN1-90	WW, B

[z]Uses: B = breeding; RW = red wine; WW = white wine.

(*Uncinula necator* (SCHW.) BURR.), anthracnose, black rot (*Guignardia bidwellii* (ELL.) VIALA et RAVAZ), and grape leaf folder moth (*Desmia funeralis* HÜBNER).

With careful parental selection and fruiting of progeny with population sizes of > 100 seedlings per cross it has been possible to obtain new recombinants of superior value as cultivars.

Literature cited

HALBROOKS, M. C.; MORTENSEN, J. A.; 1989: Origin and significance of Florida hybrid bunch grapes and rootstocks. HortScience **24**, 546-550.

HOPKINS; D. L.; ADLERZ, W. C.; 1988: Natural hosts of *Xylella fastidiosa* in Florida. Plant Dis. **72**, 429-431.

LOUCKS, K. W.; 1938: Investigations of fruit rots of grapes. Fla. Agricult. Exp. Sta. Ann. Rept. for **1938**, 118-119.

– –; 1942: Investigations of fruit rots of grapes. Fla. Agricult. Exp. Sta. Ann. Rept. for **1942**, 124-125.

MORTENSEN, J. A.; 1988: 'Blanc Du Bois' grape. HortScience **23**, 418-419.

– –; STOVER, L. H.; BALERDI, C. F.; 1977: Sources of resistance to Pierce's disease in *Vitis*. J. Amer. Soc. Hort. Sci. **102**, 695-697.

STOVER, L. H.; 1951: Breeding has produced better grape varieties for Florida. Proc. Fla. State Hort. Soc. **44**, 269-271.

– –; 1960: Progress in the development of grape varieties for Florida. Proc. Fla. State Hort. Soc. **73**, 320-323.

WELLS, J. M.; RAJU, B. C.; HUNG, H. Y.; WEISBURG, W. G.; MANDELCO-PAUL, L.; BRENNER, D. J.; 1987: *Xylella fastidiosa* gen. nov. sp. nov.: Gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas* spp. Intern. J. Syst. Bacteriol. **37**, 136-143.

Evaluation of interspecific populations of grapevine in breeding for complex resistance to fungal diseases and phylloxera

V. T. Usatov, L. K. Kireyeva, V. A. Volynkin, V. P. Klimenko and N. P. Oleinikov

National Institute for Grape and Products of Grape Processing 'Magarach',
31, Kirov Street, 334200 Yalta, Crimea, USSR

S u m m a r y : Roentgenoscopy was used as a method to determine the quality of hybrid seeds and to predict the development of viable plants from interspecific hybridization. The seeds were grouped into five classes of quality (embryo classes) depending on embryo size and degree of endosperm development. As the index number of a class increased, the proportion of plantlets and vigorous plants produced also increased.

In order to evaluate genotypic peculiarities of the original forms and seedlings, the seedlings were studied at the juvenile stage of ontogeny.

Analysis of development of the hybrids studied during 5-6 years under conditions of complex infection pressure at a special planting site made it possible to evaluate the degree of their resistance to phylloxera, pathogenic soil microflora and fungal diseases and to eliminate susceptible genotypes.

The heritability of resistance to fungal diseases (mildew, oidium, grey rot) and phylloxera was studied, conclusions were made concerning the combining ability of the original forms, and these forms were evaluated as donors of the desirable characters.

Using transgressive resistant hybrids as donors in backcrossing provided improved quality with a broad range of resistance variability, which made it possible to select promising genotypes.

K e y w o r d s : resistance, plasmopara, oidium, botrytis, phylloxera, hybrid, Muscadinia, seed, roentgenoscopy, screening, genetics, breeding, USSR.

Introduction

Among all worldwide cultivated fruit-bearing plants, apple trees are the most widely spread (24 mill. ha); they are followed in the decreasing order of acreages by species such as pear (7 mill. ha), peach (6 mill. ha), and plum (5 mill. ha). The spread of grapes is rather high, it amounts to about 10 mill. ha. This distribution is not occasional. Grapes have universal therapeutic properties; fresh grape as well as juice, wine, vine leaves, seeds and grape marc are of great importance (Kiskin 1984).

Generally, the demands of USSR in fresh grape are ill-satisfied. During the 10th and 11th five-year plan periods, the annual consumption of this valuable product was only 0.5 kg per capita, while according to the data of the Institute of Nutrition of the USSR Academy of Sciences the average annual physiological rate of fresh grape consumption should be 10 kg per capita. In perspective, about 13.5 kg of fresh grape per capita will be required annually to organize the rational nutrition of the country population (Railianu 1985).

As a result of the extensive selection researches, a wide range of high-quality grape varieties which satisfy consumers' demands has been obtained; but the fact that the overwhelming majority of these varieties is susceptible to frosts, phylloxera, mildew, oidium and grey rot results in significant complication of cultivation techniques due to the use of grafted cultures and numerous protective treatments with toxic chemicals and the restriction of cultivation to the southern regions of the country.

The prospects of vinegrowing should correspond to the ecological demands. This is conditioned by the present and predicted pollution of environment with chemicals used for plant protection, as well as to the increasing economic demands under conditions of increasing primary production costs and the transition to self-repayment and self-financing.

The breeding of high-quality productive grape varieties with complex resistance to pests and diseases and their wide industrial introduction will play the major role in the future intensification

of this branch of economy, and the accelerated breeding of these varieties will evidently be carried out by selecting promising combinations of crosses (specific combining ability) as well as by decreasing the duration of the selection process.

Analysis of data presented in the literature and our own experimental results show that the resistance to phylloxera, mildew and grey rot and good quality of yield are independently inherited and this fact makes it possible to choose promising genotypes using selection to resistance (GOLODRIGA *et al.* 1979).

A considerable progress in the field of breeding for resistance was due to the use of the donors of resistance in the hybridization process obtained as a result of over 100 years of breeding researches which were conducted by French originators (SEYVE VILLARD, SEIBEL, RAVAT, OBERLIN, JOANNES SEYVE etc.).

Several species of plants in Vitaceae family have been distinguished as having resistance to pests and diseases. *Vitis rotundifolia* MICHX., a resistant species, and its DRX hybrids (DUNSTAN 1962) are of particular interest in this respect. Some difficulties arising in hybridization were due to differences in the chromosome number in *V. vinifera* L. belonging to *Euvitis* with $2n = 38$ and in *V. rotundifolia* belonging to *Muscadinia* with $2n = 40$. For a long time, the breeders could not obtain fertile plants using such crosses. The first fertile hybrid between *Euvitis* and *V. rotundifolia* was obtained in the USA (OLMO 1954, 1971; PATEL and OLMO 1955). Later DRX hybrids were obtained from such crosses (DUNSTAN 1962) and introduced into France (BOUQUET 1980). In 1974, some hybrid seeds were kindly supplied to us by the French breeders.

Materials and methods

The objects under investigation in determining the quality of grape seeds, the new original material in breeding for resistance, were 18 grape seed samples obtained by interspecific hybridization of *V. vinifera* (diploids and polyploids) with pure *V. rotundifolia* and its DRX hybrids (these hybrids were encoded as 'Magarach N 100'), as well as seeds obtained by the random pollination of these hybrids. Generally, 3257 seeds were investigated with 1434 of them having full weight (those which sunk). All seeds obtained both as a result of crossings and random pollinations were included into study. At first, the seeds were dipped into water. Some of them sank. Examination of the floating seeds showed them to be hollow, i. e. without embryo and endosperm. The number of the full weight seeds was determined by counting (in % of the total number). Subsequently only sunk seeds were studied. Their quality was determined by roentgenoscopic method (NEKRASOV and SMIRNOVA 1961; SMIRNOVA 1978) using a Svetlana X-ray emitter [1]). Seeds under investigation were placed on a frame having a sticky film bottom; this frame was put under the X-ray tube of the emitter. The roentgenogram was obtained under defined conditions. The quality of seeds on roentgenograms was determined by the degree of endosperm and embryo development taking into account the intensity of their fixation on the film and classifying them into categories of seeds and classes of embryo development. The content of seeds of all classes of development (in %) was determined for each sample studied.

At the stage of plantlets, 2308 seedlings and 15 combinations with the single pollinator (cv. Podarok Magaracha) were investigated. The variability was estimated according to the following characters: germination capacity, size of cotyledons, presence of deformed cotyledons, differences in the number of cotyledons, teratologic changes, and seedling survival rate.

The juvenile gene pool screening, i. e. the mass screening of hybrids at the juvenile stages of ontogeny using simple and rapid methods for determining the morphological-phenological variability was used for the early selection of the objects studied (KLIMENKO 1986).

[1]) Investigations were carried out in cooperation with T. MUKHORTOVA and V. NOVIKOVA in Nikitsky Botanical Garden.

Resistance to phylloxera and pathogenic microflora was estimated using the method developed by P. N. NEDOV and A. P. GULER (1971); resistance to mildew was rated using the 5-point scale according to the method approved by the Section of Viticulture of the National Academy of Agricultural Sciences (Methodical Instructions Concerning the Grape Breeding, Erevan, 'Aistan', 1974).

Susceptibility to oidium was estimated using the method proposed by I. N. NAIDIONOVA (1985) at an infected planting site established in the greenhouse. Hybrid seeds and two-bud cuttings were placed in hydroponic channels. Controls were positioned along the axis of the channels. Samples were inoculated with conidia suspension (35-30 conidia/0.001 ml of suspension). Suspension rate was 25 ml/m^2 of the channel area. Inoculations were repeated every 2 weeks.

The following grape varieties investigated by a number of authors were used as controls: highly resistant varieties: SV 12-375 (BOUBALS 1961), Kishmysh Vatkana (Methodical Instructions Concerning the Grape Breeding, 1974; NAIDIONOVA 1985), Gangal kara (SOHI and SRIDHAR 1972; SHTIN *et al.* 1986); resistant varieties: Seibel 15062 (BOUBALS 1961), Kishmysh rozovy (NAIDIONOVA 1985); susceptible varieties: Ravat 6 (BOUBALS 1961), Riesling Italico (NAIDIONOVA 1985).

In order to reveal the genetically dependent grey rot resistance, a laboratory method of estimation was used (GOLODRIGA *et al.* 1979).

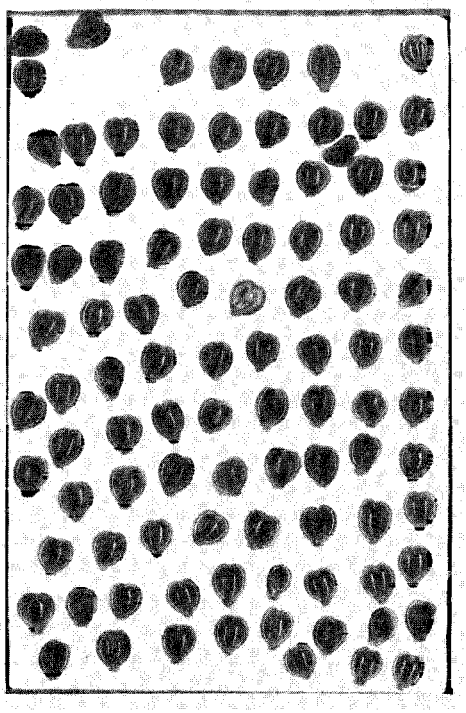


Fig. 1: Roentgenogram of the hybrid grape seeds.

Results and discussion

Potential advantages of the vast hybrid collections of the Breeding and Ampelography Department of the National Institute for Grape and Products of Grape Processing 'Magarach' in ontogeny and pathogenesis were investigated in order to develop scientific bases and concepts for complex resistance.

Promising hybrids destined for the use as original forms in the process of breeding for resistance were selected among the seedlings of heterogenous populations (GOLODRIGA and KIREYEVA 1981; GOLODRIGA *et al.* 1986). Seedlings were selected among the seed progeny of DRX x *V. vinifera* hybrids; they were characterized by high fertility and productivity and combined the great vigor and the disease resistance of muscadine varieties with berry characteristics of *V. vinifera*. So Magarach N 100-74-1-5 seedling (DRX N 60-24 x *V. vinifera*) meets the requirements of the standard European table grape varieties – it has berries and clusters of large size and gives a high yield.

The aims of the research work conducted by the breeders of the 'Magarach' Institute include the mobilization of different forms of *V. rotundifolia* and its hybrids in order to carry out hybridization with *V. vinifera* varieties and hybrids, and the investigation of the probability of introgression (when the genetic material of some species gradually penetrates into another one through incomplete interspecific isolation barrier) of *V. rotundifolia* resistance in order to form new sources of selection material. Seedlings of heterogenous populations obtained using DRX N 58-5 and DRX N 60-24 were included into the program of breeding for resistance. The degree of resistance of the most promising seedlings among those mentioned above was estimated in the complex infection planting site and these seedlings were included into the breeding program as original forms. However, as a result of genetic abnormalities in the process of interspecific hybridization non-viable seeds which absolutely do not differ in their appearance from the viable ones are frequently formed. The roentgenoscopic method allows preliminary estimation and selection of viable seeds. This method was used for the first time to determinate the quality of grape seeds. To make comparisons between the combinations, the average class of the seed development (K_{av}) was calculated by determination of the arithmetical mean of the weighted values using the expression:

$$K_{av} = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5}{100}$$

where n_1, n_2, n_3, n_4 and n_5 are the numbers of the seeds of the corresponding class as % of the total number of seeds in the sample ($n_1 + n_2 + n_3 + n_4 + n_5 = 100$).

Thereafter, seeds were germinated in the laboratory at 26-30 °C. Germination in the laboratory was determined by the number of germinated seeds (in %) compared to the total number of the seeds sown. Germinated and ungerminated seeds were sown in the hydroponic greenhouses with the aim of obtaining seedlings and investigating their biological characteristics.

The roentgenograms (Fig. 1) clearly show endosperms of different degrees of development and comparatively small embryos also of different sizes and degrees of development. Hybrid seeds obtained by crosses and the random pollination were classified into 3 categories: 1. Filled seeds which have a well developed endosperm, 2. empty seeds which have no endosperm and embryo, and 3. deficient seeds which are partly filled with an endosperm and have an embryo which is less than $^1/_8$ in volume compared to the developed endosperm of the seed; and into 5 classes of embryo development: I. Empty seeds which have no endosperm and embryo; II. seeds with embryos which are less than $^1/_8$ in length compared to the developed endosperm of the seed; III. seeds with embryos which are $^1/_8$ in length of the seed endosperm, clearly seen and not closely adjacent to the endosperm; IV. seeds with embryos which are more than $^1/_8$ in length compared to the seed

Table 1: Distribution of hybrid grape seeds from different cross combinations to embryo classes

Material studied	Total number of full weight seeds	Embryo classes, %					
		I	II	III	IV	V	K_{av}
Cross combinations							
Muscat VIRa x Mag. N 100-74-1-5	266	0	19	39	33	9	3.3
Polyvitis x Mag. N 100-79-34	32	0	36	48	8	8	3.3
Mag. N 100-74-1-5 x Podarok Magaracha	59	0	0	7	20	73	4.5
Mag. N 100-74-3-1 x Tsvetochny	28	0	14	18	18	50	4.0
Mag. N 100-74-2-1 x Tsvetochny	146	0	19	38	41	7	3.4
Mag. N 37- 77-44 x Mag. N 100-79-52	38	0	8	40	26	26	3.7
Mag. N 44-77-18 x Mag. N 100-74-1-5	109	1	8	35	41	15	3.6
Mag. N 100-74-1-5 x Tsvetochny	43	0	0	18	50	32	4.1
SV N 52-76 x Mag. N 100-79-58	23	0	0	17	35	48	4.3
Mag. N 40-69-11 x Mag. N 100-74-3-1	63	0	0	0	0	100	5.0
Mag. N 38-77-5 x Mag. N 100-79-52	101	0	11	39	27	23	3.6
Mag. N 10-69-17 x Mag. N 100-79-15	36	0	14	14	14	58	4.2
Random pollination							
Mag. N 100-74-1-5 (DRX 60-24 x Vv)	101	0	5	26	47	22	3.4
Mag. N 100-79-17 (Polyvitis x Mag. N 100-74-1-5)	81	0	4	5	54	37	4.3
Mag. N 100-74-3-3 (DRX x 58-5 x Vv)	100	0	3	12	50	35	4.2
Mag. N 100-74-3-1 (DRX 60-24 x Vv)	49	0	0	8	16	76	4.7
Mag. N 100-74-2-4 (DRX 60-24 x Vv)	37	0	3	22	35	40	4.6
Mag. N 100-79-52 (Polyvitis x Mag. N 100-74-1-5)	15	0	0	27	47	26	4.0

endosperm, they are positioned much closer to the endosperm and comparatively clearly seen; V. seeds with embryos which are 1/4 or more in length compared to the seed endosperm, white in colour, positioned close to the seed endosperm in the most cases, not clearly seen on roentgenograms and often leading to confusion when seeds are classified into classes; the section of such seeds shows a developed embryo close to the endosperm.

Among the full-weight seeds studied, the empty seeds are almost absent, generally there are developed and incompletely developed seeds. The number of developed and incompletely developed seeds varies depending on the cross combination from 35 % to 100 % and from 0 % to 65 %, respectively; in the random pollination, the number of developed and incompletely developed seeds varies from 69 % to 92 % and from 8 % to 31 %, respectively.

When hybrid grape seeds obtained from various combinations of crosses and from the random pollinations were classified according to the embryo classes, 5 embryo classes were established (Table 1). Embryo class I seeds were practically absent in all cross combinations and in the random pollination. However, the number of seeds of embryo classes II and III varies greatly depending on cross combinations in the limits of 8-36 % and 7-48 %, respectively; in the random pollination, this variation is in the limits of 3-5 % and 5-27 %, respectively. The number of seeds of embryo classes IV and V also varies: in crosses from 8 % to 50 % and from 7 % to 100 %, and in the random pollination from 16 % to 54 % and from 22 % to 76 %, respectively. All seeds belonging to embryo class V were obtained only in one combination of Magarach N 40-69-11 x Magarach N 100-74-3-1. The value of the average embryo class is also different and varies in the random pollination and in crosses from 3.4 to 4.7 and from 3.3 to 5.0, respectively.

A wide-range variation of the embryo classes indices suggests the presence of genetic abnormalities in the distant incompatible crosses. Thus, using new sources for interspecific crosses and for obtaining resistant varieties, we should carry out a careful cytogenetic investigation and determine the biological mechanism of the formation of the fully developed seeds.

Estimation of the germinated hybrid grape seeds classified according to embryo classes in the laboratory showed that the number of germinated seeds varied according to embryo classes and combinations of crosses.

In the random pollination, the number of germinated seeds of embryo classes II, III, IV and V was 0-3, 7-25, 14-33 and 44-86 %, respectively. In crosses, the number of germinated seeds of embryo classes II, III, IV and V was 6-18, 12-25, 20-34 and 32-100 %, respectively. In the random pollination and in different cross combinations, the total number of plantlets obtained was 59-89 % and 16-78 %, respectively. In the first case, the variability range was 30, and 62 in the second case. A single combination, Magarach N 40-69-11 x Magarach N 100-74-3-1, showed 100 % of germinated seeds. However, occasional germinations of embryo class I seeds were noted in Magarach N 44-77-18 x Magarach N 100-74-1-5 combination. In general, the germinated seeds belonged to embryo classes III, IV and V. The total number of germinated seeds obtained from the random pollination and from cross combinations was 20 % compared to the initial number of seeds, and 45 % compared to the number of seeds used for germination in classes I-V. The number of germinated seeds of embryo classes I-V was 0.3, 0.5, 15, 28 and 52 %, respectively.

After estimating the number of germinated seeds in the laboratory, the number of germinating seeds and their ability to produce seedlings were determined according to cross combinations and embryo classes. Embryo class I seeds failed to produce plantlets. The percentages of plantlets obtained from the seeds of the random pollination belonging to embryo classes II, III, IV and V were 0.8, 8-38, 8-66 and 0-84 %, respectively. The variability range according to embryo classes was 8, 28, 59 and 84 units, respectively. Embryo classes II-V seeds obtained from different cross combinations gave 0-36, 0-42, 0-46 and 4-100 % of plantlets, respectively, the variability range being 36, 42, 46 and 96, respectively.

Seedlings were investigated during the period from the appearance of plantlets up to the development of mature plants. The number of survived seedlings was determined according to the

embryo classes. The highest number of embryo class V viable seedlings was obtained in Magarach N 40-68-11 x Magarach N 100-74-3-1 combination. 58 viable plants were obtained from 62 plantlets. Survival rate of seedlings belonging to different embryo classes varied exceedingly.

Depending on cross combinations, the percentage of survived seedlings obtained from embryo classes III-V seeds were 0-18, 0-42, 0-48 and 4-100 %, respectively, and the variability range was 18, 42, 48, 96, respectively. The number of seedlings surviving from embryo classes II-V seeds in the random pollination was 0-8, 8-33, 15-67 and 0-77 %, the variability range was 8, 25, 52, 77, respectively. The total number of seedlings obtained was 395. The survival rate for all combinations and for the random pollination averaged 77 %.

The number of vigorous plants varies depending on cross combinations. In Magarach N 40-69-11 x Magarach N 100-74-3-1 combination, for example, 23 vigorous plants were obtained from 58 seedlings, and in Magarach N 37-77-44 x Magarach N 100-79-52 combination only 3 vigorous plants were obtained from 11 seedlings generally produced by embryo class V seeds. Most vigorous hybrid plants proved to be obtained from the seeds belonging to embryo classes III, IV and V. As a whole, 118 vigorous hybrid plants were obtained. Fig. 2 shows the hybridization data according to the classes of seed quality in breeding for resistance.

The greatest number of plantlets, viable seedlings and vigorous hybrid plants was shown to be obtained generally in all populations from classes IV and V seeds and partially from class III seeds.

Breeding for resistance in grapes as well as in other perennial plants is a labor-consuming and long-term process, therefore, rapid and simple methods of estimation of parents and their hybrid progeny must find great application in practical selection. To evaluate the grape hybridization effectiveness, it is desirable and practicable to consider the objective information as soon as possible, particularly at the stage of plantlets.

The results show that the seed germination, the number of plants with defective cotyledons, the number of plants with deviations from the normal size of cotyledons, the number of plants with teratologic changes, seedlings survival rate towards the end of the first vegetation season vary in the range of 3.8-90.9, 0-50.0, 0-9.6, 0-13.2 and 1.0-100 %, respectively (Table 2).

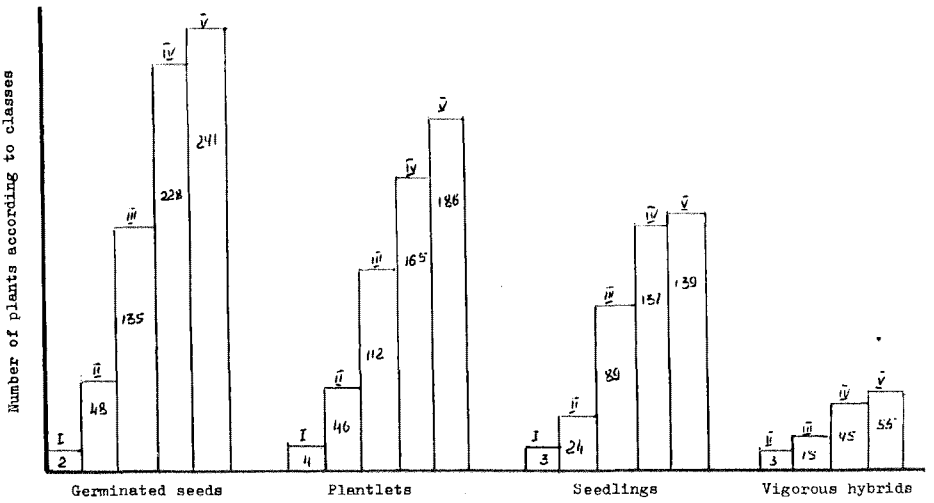


Fig. 2: Dependence of the development of viable seedlings on the class of seed quality.

Table 2: Variability of hybrid grape populations at the stage of plantlets

NN	Cross combination	Plant-lets number	Seed germination %	Cotyledon size length mm	Cotyledon size width mm	Abnormalities deformed cotyledons %	Abnormalities deviations in number of seeds %	Abnormalities teratologic changes %	Seedling surviving capacity %
1.	Tavrida x Podarok Magaracha	300	22.7	24	17	6.7	0	2.0	63.3
2.	Madeleine Angevine x - - " -	68	34.3	24	19	1.5	0	1.5	97.1
3.	Muscat blanc x - - " -	40	19.6	22	15	50.0	0	0	57.5
4.	Muscat VIRa x - - " -	32	3.8	22	15	50.0	0	3.1	84.4
5.	Sauvignon vert x - - " -	238	51.2	24	16	4.2	0	2.1	16.0
6.	M.47-68-2 x - - " -	355	45.0	25	18	11.3	0.05	2.3	46.8
7.	M. 7-69-12 x - - " -	87	73.1	24	17	0	1.2	0	100
8.	M.56-75-1 x - - " -	400	35.7	22	16	0	0	0	1.0
9.	M.37-77-41 x - - " -	69	49.3	20	10	30.4	0	0	36.2
10.	M.44-77-11 x - - " -	56	69.0	22	16	8.6	0	0	55.2
11.	M.44-77-13 x - - " -	100	50.8	24	16	15.0	0	1.0	58.0
12.	M.44-77-19 x - - " -	114	52.5	20	14	33.3	9.6	13.2	39.5
13.	M.44-77-22 x - - " -	300	45.4	14	10	26.0	1.3	1.7	61.7
14.	M.34-78-7 x - - " -	110	90.9	22	14	6.4	0	0	43.6
15.	M.34-78-4 x - - x -	37	40.2	22	14	1.1	0.5	0	73.0
Variation coefficient			47.9	12.2	16.9	107.0	272.7	185.1	56.2

While analysis at juvenile stage is based on the investigation of phenological variability, the genotypic specificity of recombinants is to a considerable degree expressed during the first year of plant development (KLIMENKO 1987). The high value of variation coefficients suggests that seed (and possibly seedling) variability is generally determined by the female genotype (OLMO 1942). The highest values of variation coefficients of the cotyledon number and teratologic changes indicate the great asymmetry in the distribution due to the genotypic determination. The highest number of abnormal plants in Magarach 44-77-19 x Podarok Magaracha hybrid population is supposed to be due to aberrations in the genome of Magarach 44-77-19 hybrid which has a mutant in its genealogy.

When planning subsequent crosses in order to get an increase in the yield of hybrid seedlings, it is necessary to exclude those parents that have low seed germination and survival rate (for example, Muscat VIRa and Magarach 56-75-1) or to use unconventional methods of obtaining seedlings from them.

Correlations between morphological characters of juvenile seedlings and economically attractive features are known to exist and to be used for the early selection of the promising hybrids (POGOSIAN and KHACHATRIAN 1983). In particular, the study of cross combinations was aimed at

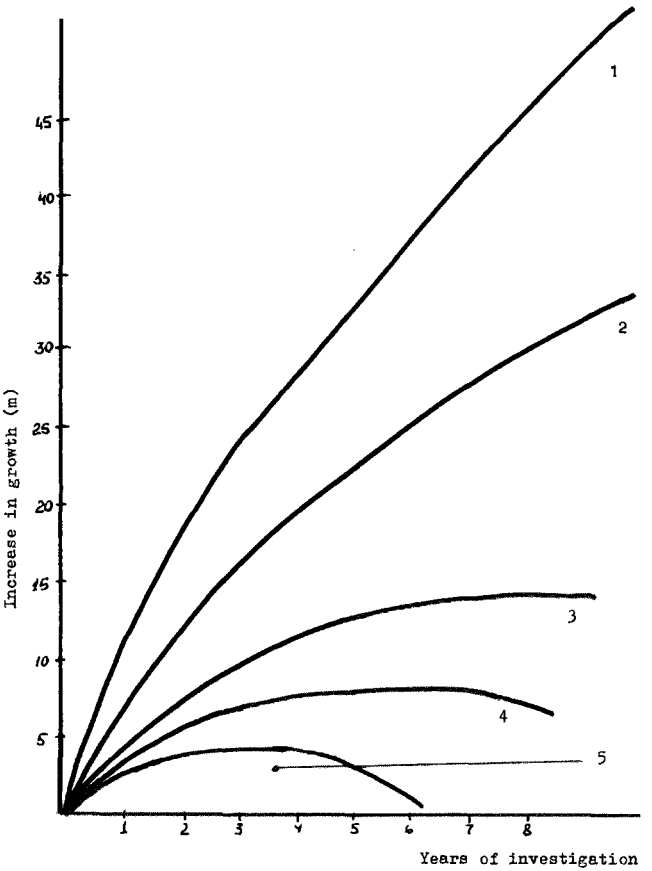


Fig. 3: Dynamics of the development of varieties and hybrids with different degrees of phylloxera resistance related to years. Resistance rating: 1-5.

Table 3: Estimation of donors of resistance as producers of the resistant progenies in breeding for mildew and phylloxera resistance

Cross combination	Hybrid number	Phylloxera resistance % according to scores						Mildew resistance % according to scores						Breeding value of the F_1 population
		population av.	1	2	3	4	5	population av.	1	2	3	4	5	
V.vinifera x S.V. 12309	58	3.2	0	25	42	22	11	2.4	12	50	29	5	4	0.16
- " - x S.V. 20366	115	3.1	0	23	48	23	6	3.1	9	32	17	26	16	0.20
- " - x Seibel 7053	51	4.0	0	11	5	58	26	3.9	4	4	26	31	25	0
- " - x S.V. 18315	109	3.7	0	7	37	32	24	3.5	0	20	34	24	22	0.03
- " - x(S.V. 18315 x V.vinifera)*	136	3.4	0	18	30	27	25	3.1	13	23	21	29	14	0.04
- " - x Seibel 13666	127	3.8	0	16	22	28	34	3.3	9	20	23	25	23	0.06
- " - x(Seib.13666 x V.vinifera)*	12	3.2	0	10	70	10	10	4.0	0	8	25	25	42	0
- " - x(Ravat 6 x V.vinifera)*	549	3.8	0	7	28	46	19	2.8	22	29	12	17	20	0.06
- " - x(S.V. 20365 x V.vinifera)*	79	3.5	0	9	45	32	14	3.3	0	3	72	16	9	0
- " - x(S.V. 20347 x V.vinifera)*	56	3.4	0	9	18	37	36	3.9	4	21	13	5	57	0.02
								Total for all populations						0.06

* Pollen mixture of the best European-Asiatic varieties

producing wine varieties but judging from the size of cotyledons, it is quite possible to obtain large-berry seedlings in Magarach 47-68-2 x Podarok Magaracha hybrid population.

The successful use of breeding for resistance is to a great extent determined by the corresponding selection of parents and by the precise evaluation of their resistance. The estimate of the resistance of parents and of a vast hybrid fund, consisting of many thousands of hybrids, to phylloxera and pathogenic microflora under conditions of the planting site complex infection during a 15-year period indicates the dependence of the dynamics of the hybrid development on the degree of resistance expressed in scores (Fig. 3). As the increase in growth is the integrated index of the phylloxera resistance which correlates well with the root system state and development, the analysis of the dynamics of the development of the hybrids studied (with the rare exceptions of genotypically weakly growing hybrids) conducted during a 5 to 6-year period provides a rather accurate estimate of their resistance degree. Hence, analysis was carried out concerning the regularities of the inheritance of the hybrid populations resistance to phylloxera and mildew (Table 3).

The main aim of this analysis was to elucidate the role played by the male parent, the donor of resistance, in transfering this character to the progeny. As the data presented concerning crosses between European-Asiatic varieties and Seyve Villard (S. V.) and Seibel hybrids show, characters of phylloxera and mildew resistance have a wide range of variability. A considerable proportion of seedlings has a high resistance to mildew (scored 1) and to phylloxera (scored 2). These seedlings are good parents for the use in selection as indicated by high indices of the value of populations (Table 3).

To improve the interspecific hybrid quality, backcrosses to European-Asiatic varieties, donors of high fruit quality, are known to be used. From this point of view, it is considerably interesting in which way the resistance will be inherited, if selected transgressive resistant hybrids obtained as a result of the first backcross of Seyve Villard and Seibel to European-Asiatic varieties are used as parents. As the data of Table 3 show, the number of resistant seedlings generally decreases in such populations; however, if the selection of parental pairs is successful, a certain proportion of hybrids will be of sufficiently high resistance.

Estimation of the varieties and the hybrid populations indicated that S. V. 13-303, S. V. 12-283 interspecific hybrids and Magarach N 100-75-1-5 hybrid showed a resistance to oidium. A highly resistant group (scored 1) consisted of Podarok Magaracha variety, a number of Seyve Villard interspecific hybrids and their hybrid progeny (Moldova, Magarach N 97-75-1, etc.) as well as Magarach N 13-88-2 and Magarach N 33-78-11 elite forms (Nimrang x Magarach N 124-66-26). Varieties such as Antei Magarachsky, Liana, Lanka, Mogis, Muscat Bessarabsky, Margaritar, Pamiaty Verderevskogo, Frumoasa Albe, Pukhliakovsky Magaracha, Nimrang Magaracha, Magarach N 17-82-1 hybrid (Magarach N 57-75-1 x Antei Magarachsky) showed an increased resistance to oidium (scored 2) (Oleinikov 1988).

Control varieties resistance was in close agreement with the literature data.

The evaluation of hybrid populations indicated that some donors of oidium resistance were of value for breeding and that Podarok Magaracha variety used as a male parent gave rise to seedlings of different degrees of resistance, but in many cross combinations plants which were scored 1 prevailed. The highest percentage of seedlings scored 1 was obtained in Madeleine Angevine x Podarok Magaracha and Magarach N 44-77-13 (Italia x Podarok Magaracha) x Podarok Magaracha combinations (12 and 11 %, respectively) (Table 4).

When crossing Magarach N 31-77-8 (Nimrang x Seibel 13666) with Ranny Magaracha variety, 16 % of seedlings were scored 1. The use of Ranny Magaracha variety as a female in crosses with Podarok Magaracha, JS 26205 and S. V. 20-366 did not result in highly resistant hybrids.

The donor of complex resistance, S. V. 12-397, in crosses with variety Madeleine Angevine allowed to obtain 4 % of hybrids scored 1. In Madeleine Angevine x Magarach N 124-66-26 and

Table 4: Heritability of oidium resistance in interspecific hybridization (seedlings from crosses made in 1986)

Cross combinations	Hybrid number	parental pairs ♀	parental pairs ♂	Oidium resistance: populations (according to scores, %) 1	2	3	4	5	av. score for a population
Madeleine Ang. x Podarok M.	41	4	1	12	32	52	4	0	2.5
N 44-77-13 x Podarok M.	53	3	1	11	15	43	25	6	3.0
N 31-77-44 x Podarok M.	13	4	1	8	38	46	8	0	2.6
N 44-72-11 x Podarok M.	15	3	1	7	27	53	13	0	2.8
N 44-77-22 x Podarok M.	144	4	1	6	29	39	21	5	2.9
N 47-68-2 x Podarok M.	156	4	1	4	10	59	18	9	3.2
Muscat VIRa x Podarok M.	22	5	1	0	9	77	14	0	3.1
Ranny M. x Podarok M.	13	2	1	0	23	64	13	0	2.9
Ranny M. x J.S. 26-205	117	2	2	0	11	63	17	9	3.2
Ranny M. x S.V.20-366	72	2	0	0	14	72	14	0	3.0
N 31-77-8 x Ranny M.	110	2	2	16	43	39	2	0	2.3
Muscat VIRa x N100-74-1-5	11	5	1	2	16	19	36	27	3.7
N 44-77-18 x N100-74-2-2	75	4	1	1	20	40	34	5	3.2
Antei Mag. x Tavria	23	2	3	0	17	44	22	17	3.4
Muscat Yant. x Antei Mag.	9	2	2	0	11	53	33	0	3.2
N 10-51-1 x Antei Mag.	28	4	2	4	11	39	32	14	3.4
N 4-57-66 x Antei Mag.	14	3	2	0	14	79	7	0	2.9
Madeleine Ang. x Antei Mag.	28	4	2	0	4	42	36	18	3.6
Madeleine Ang. x S.V.12-397	154	4	1	4	22	49	20	5	3.0
Madeleine Ang. x N124-66-26	9	4	1	0	11	78	11	0	3.0
Nimrang x N124-66-26	37	5	1	21	49	21	9	0	2.2
Bican x N124-66-26	10	4	1	0	20	60	20	0	3.0
Chaush x N 124-66-26	11	5	1	0	18	73	9	0	2.0
Tashly x S.V.20-366	55	4	2	0	20	62	16	2	3.0
N 4-68-25 x Crymskaia zhemchuzhina	22	1	4	12	23	60	5	0	2.6

Madeleine Angevine x Antei Magarachsky combinations, highly resistant plants were not produced.

Nimrang x Magarach N 124-66-26 population where the yield of resistant seedlings was 21 % proved to be the most profitable. In the progeny of crosses between Madeleine Angevine, Bican, Chaush varieties and Magarach N 124-66-26, hybrid seedlings were of low resistance.

The high yield of resistant hybrids was provided by Magarach N 4-68-25 (S. V. 20-366 x *V. vinifera*) x Crymskaia Zhemchuzhina and Magarach N 10-51-1 x Antei Magarachsky combinations. The latter was the only combination of those studied in which Antei Magarachsky variety provided the production of 4 % of transgressive hybrids concerning the resistance to oidium.

In breeding for grey rot resistance, the use of various sources of disease resistance genes may be recommended. In this respect it is possible to use species such as *V. armata* (Staudt 1980)

Table 5: Heritability of grey rot resistance in F_1 grapevines

Cross combinations		Parents resistance ♀	Parents resistance ♂	Hybrid number	In % according to scores: resistant 1	resistant 2	resistant 3	susceptible 4	susceptible 5
Moldavsky Chiorny	x Mtsvane	1	3	33	30.3	12.1	21.2	21.2	15.2
Moldavsky Chiorny	x Rkatsiteli	1	3	26	19.2	30.8	19.2	15.4	15.4
Moldavsky Chiorny	x Seyve Villard 18315	1	2	29	24.1	13.8	13.8	27.6	20.7
Moldavsky Chiorny	x Seyve Villard 20347	1	4	34	5.9	8.8	17.6	29.4	38.3
Moldavsky Chiorny	x Seibel 7053	1	5	11	0	27.2	18.2	18.2	36.4
Rkatsiteli	x Magarach 376	3	5	14	0	0	14.3	35.7	50.0
Rkatsiteli	x Magarach 2-57-72	3	2	23	0	13.0	13.0	21.8	52.2
Rubinovy Magaracha	x Magarach 6-68-27	1	1	32	28.1	28.1	21.9	12.5	9.4
Tavkveri	x Magarach 6-68-27	4	1	33	27.3	33.3	18.2	12.1	9.1
Tagobi	x Amursky Oboepoly	4	1	14	7.2	14.3	35.7	21.4	21.4
Nimrang	x Amursky Oboepoly	3	1	12	8.3	16.7	41.7	8.3	25.0
Bastardo Magarachsky	x Khindogny	4	3	14	0	7.2	42.8	35.7	14.3
Bastardo Magarachsky	x Portugieser	4	4	13	0	0	7.7	38.5	53.8

Magarach N 376 - Riesling x Muscat blanc
Magarach N 2-57-72 - Mtsvane x Sochinsky Chiorny
Magarach N 6-68-27 - Ravat 6 x pollen mixture

having practical resistance to botrytis, *V. riparia* (ALLEWELDT 1985), *V. labrusca* (VASILIEVA 1975), *V. amurensis* (TAMASHI 1964), complex interspecific hybrids of Seyve Villard, Seibel and other breeders as well as highly resistant *V. vinifera* varieties and hybrids (GOLODRIGA *et al.* 1979; SUPOSTAT 1986).

These findings are supported by the results obtained which are presented in Table 5. Crossing of variety Moldavsky Chiorny with varieties Mtsvane and Rkatsiteli is an example showing the possibility of obtaining grey rot resistant hybrids in F_1 by breeding within *V. vinifera* L. A high percentage of resistant hybrids is obtained in both cases, but in the first case the maximum number of highly resistant hybrids is observed (30.3 %), and the maximum number of resistant hybrids is detected in the second case (30.8 %). It should be noted in this context that Moldavsky Chiorny belongs to highly resistant varieties and Mtsvane and Rkatsiteli are considered to be varieties of intermediate resistance.

The use of complex hybrids (for example, Seyve Villard 18315, Seyve Villard 20347, Seibel 7053) in the hybridization with the highly resistant variety Moldavsky Chiorny resulted in F_1 hybrids which have high grey rot resistance, but the high percentage of susceptible hybrids obtained in crosses with susceptible and highly susceptible parents indicates the insufficient degree of genetic dominance of Moldavsky Chiorny in the first generation, concerning disease resistance.

Crossing between Rkatsiteli intermediately resistant females and highly susceptible Magarach 376 hybrid results in progeny where percentages of intermediately resistant and susceptible forms are 14.3 and 85.7 %, respectively; better results are obtained using Magarach 2-57-72 interspecific resistant hybrid, but in this case also practically no highly resistant forms are produced.

The use of *V. amurensis* in crosses when breeding for grey rot resistance can be considered promising, and this is supported by the example of crosses with Tagobi and Nimrang varieties.

The use of some varieties in crosses results in F_1 positive transgressions. When crossing Bastardo Magarachsky and Portugieser varieties (both are susceptible), 7.7 % of positive transgressive intermediately resistant forms were obtained in the first generation, and crossing between Bastardo Magarachsky and Hindogny (susceptible and intermediately resistant varieties) gave practically the same quantity of intermediately resistant forms (7.2 %).

So, in breeding for grey rot resistance, the resistance of parents should be taken into account and in spite of the possible production of resistant transgressive recombinants resistant forms should preferably be used.

On the basis of the data presented, analysis of the specific combining ability was possible. The highest combining ability in breeding for grey rot resistance was shown by Magarach 6-68-27 interspecific hybrid whose crosses with varieties having different resistance (Rubinovy Magaracha – scored 1 and Tavkveri – scored 4) resulted in equally large groups of resistant progenies, 78.1 and 78.8 %, respectively.

At the same time, the use of highly resistant Amursky oboepoly males results in a considerably smaller quantity of disease resistant forms compared to Magarach 6-68-27 hybrid.

Thus, a high combining ability of Magarach 6-68-27 provides a considerably higher number of resistant forms compared to Amursky oboepoly which has a lower combining ability.

Comparative analysis of Moldavsky Chiorny x S. V. 20347, Tavkveri x Magarach 6-68-27, Tagobi x Amursky oboepoly populations which can be considered as reciprocal crosses according to grey rot resistance (scored 1 x 4 x 4 x 1) suggests the lack of female effects in breeding for this character.

Conclusions

The results obtained indicate the possible use of the roentgenoscopic method in the preliminary estimation of parental forms. Using the roentgenoscopic method, data on the quality of

seeds were obtained concerning the filling with endosperm and the embryo development. Seeds were classified according to quality classes and preserved for sowing and subsequent investigation of seedlings. The development of plants obtained from different classes of seeds was monitored, the number of plantlets, developed seedlings and vigorous hybrid plants was determined.

In all cross combinations and in the random pollination, an increase in the class (I-V) resulted in an increase in the proportion of plantlets, viable and vigorous hybrid plants produced. Despite the difficulties in obtaining hybrid plants using interspecific hybridization, 3-8 % of viable plants were produced depending on cross combinations and embryo classes.

Thus, the roentgenoscopic method makes it possible to determine the seed quality and to predict the development of viable plants under conditions of conventional cultivation as well as to select seeds for the use in embryo and tissue culture.

The results of investigation of grapevine seedlings at the stage of plantlets can serve as an estimate of the parent and seedling genotypic characteristics, they should be taken into account when planning crossings and selection of hybrids at the subsequent stages in the breeding process.

Analysis of the hybrid development dynamics under conditions of the high infection pressure (phylloxera and pathogenic microflora) during a 5 to 6-year period at a special planting site provides a sufficiently accurate estimate of the degree of hybrid resistance to phylloxera and allows the rejection of susceptible genotypes. Detailed analysis of the root system should be carried out only on the isolated resistant forms.

The use of the resistant hybrids as donors of resistance in backcrosses to *V. vinifera* L. varieties allows the improvement of fruit quality while retaining a sufficiently high variability range of hybrid resistance characters, which makes it possible to select promising genotypes.

S.V. 12309, S.V. 20366 and Seibel 13666 are considered to be the most promising donors of resistance to phylloxera, pathogenic microflora and mildew.

At the oidium infected planting site established in the greenhouse, a considerable number of promising oidium resistant genotypes were for the first time isolated from the hybrid populations according to the intensity of the conidiophore development on 1-year seedling leaves in Madeleine Angevine x Podarok Magaracha, Magarach N 44-77-13 x Podarok Magaracha, Magarach N 31-77-8 x Ranny Magaracha, Nimrang x Magarach N 124-66-26, Magarach N 4-68-25 x Crymskaia Zhemchuzhina populations.

The highest yield of transgressive hybrids was provided by Nimrang x Magarach N 124-66-26 combination.

The results of our investigations made it possible to elucidate some of the problems connected with directed breeding of grey rot resistant varieties and to come to the conclusion that the increased production of the first-generation disease resistant grapevine hybrids was possible by both interspecific and intraspecific crosses and that the heritability of this character in both cases depended on the genotype structure of parental forms and on their combining ability; this provides the possibility of obtaining positive transgressions concerning the disease resistance, but the female parent effect connected with this character was not detected.

The development of the bases of the particular grapevine genetics, the evaluation of parents according to their ability to produce resistant progeny, the use of the complex infection planting site in the practical selection and the selection of promising forms at the juvenile stage will make it possible to create a wide range of complex resistant grape varieties of different uses. The wide use of resistant grape varieties in agriculture will result in a decrease in labor and energy requirements in grapevine cultivation, and a nearly complete elimination of treatments with toxic chemical substances used as means of plant protection against pests and diseases will decrease the pesticide pollution of environment and will allow the production of dietary grapes without residual quantities of toxic substances.

References

ALLEWELDT, G.; 1985: Die Resistenzzüchtung von Reben. Rebe Wein **38**, 75-77.

BECKER, H.; 1979: Results of interspecific hybridization in Geisenheim (table-wine varieties). 2e Symp. Intern. Amélioration de la Vigne, 1978, Bordeaux, 165-171. INRA, Paris.

BOUBALS, D.; 1961: Etude des causes de la résistance des Vitacées à l'oidium de la vigne, *Uncinula necator* (SCHW.) BURR. et de leur mode de transmission héréditaire. Ann. Amélior. Plantes **11**, 401-500.

BOUQUET, A.; 1980: *V. vinifera x Muscadinia* hybridization: A new way in grape breeding for disease resistance in France. Proc. 3rd Intern. Symp. Grape Breeding, Davis, 42-51.

DUNSTAN, R. T.; 1962: Some fertile hybrids of bunch and Muscadine grapes. J. Heredity **53**, 299-303.

GOLODRIGA, P. Ya.; KIREYEVA, L. K.; 1981: The use of *V. rotundifolia* hybrids in breeding for immunity [Russ.]. In: Col.: Problems and Ways of Increasing Plant Resistance to Diseases and to Extreme Environment Conditions in the Context of Breeding Aims [Russ.] **4**, 184-188.

-- --; -- --; AKISHEVA, I. V.; 1986: The use of the intraspecific and introgressive hybridization in grapevine selection at the polyploidy level [Russ.]. Vinogradarstvo i Vinodelie SSSR **5**, 18-21.

-- --; USATOV, V. T.; MALCHIKOV, Yu. A.; VOLYNKIN, V. A.; 1979: Breeding immune grape varieties [Russ.]. Vestnik Selskokhoziaistvennoi Nauki **3**, 87-91.

KISKIN, P. KH.; 1984: Therapeutic properties of grapes [Russ.]. Sadovodstvo, Vinogradarstvo i Vinodelie Moldavii **10**, 63-64.

KLIMENKO, V. P.; 1984: Investigation of grape hybrids at the early stages of their development [Russ.]. In: Methodical Recommendations Concerning Highly and Persistently Bearing Vineyard Cultivation [Russ.], 11-13. Yalta.

-- --; 1986: The screening of the grape juvenile genetic pool [Russ.]. In: Prospects of Grape Genetics and Breeding for Phytoimmunity [Russ.]. Theses of the Reports at the National Scientific Conference, 38-39. Moscow.

-- --; 1987: Preliminary selection in the grapevine populations [Russ.]. Proc. 5th Congr. Nat. Soc. Geneticists and Breeders Named after Vavilov V. I. [Russ.], Vol. **IV**, Part 4, 5.

NAIDIONOVA, I. N.; 1985: Oidium [Russ.]. In: NEDOV, N. P. (Ed.): New Methods of Phytopathologic and Immunologic Investigations in the Grape Growing [Russ.], 29-45. Shtiintsa, Kishinev.

NEDOV, P. N.; GULER, A. P.; 1971: Establishing special infected planting site in breeding for phylloxera resistance [Russ.]. Sadovodstvo, Vinogradarstvo i Vinodelie Moldavii **4**, 54.

NEKRASOV, V. I.; SMIRNOVA, N. G.; 1961: Concerning the use of roentgenographic method in the study of the development of introduced arboraceous plant seeds [Russ.]. Bulleten Glavnogo Botanicheskogo Sada **43**, 47-52.

OLEINIKOV, N. P.; 1988: Evaluation of the hybrid population resistance to oidium in the breeding of complex resistant grape varieties [Russ.]. In: Problems and Aspects of the Production of Grapes and the Products of Grape Processing. Theses of the Reports at the National Conference of Young Scientists and Specialists [Russ.], 4-5, Yalta.

OLMO, H. P.; 1942: Choice of parent as influencing seed germination in fruits. Proc. Amer. Soc. Hort. Sci. **41**, 171-175.

-- --; 1954: L'hybride *V. vinifera x V. rotundifolia* et sa valeur en obtention. Bull. O.I.V. (278).

-- --; 1971: *V. vinifera x V. rotundifolia* hybrids as wine grapes. Amer. J. Enol. Viticult. **22**, 87-91.

PATEL, L. J.; OLMO, H. P.; 1955: Cytogenetics of *Vitis*: The hybrid *Vitis vinifera x V. rotundifolia*. Amer. J. Bot. **42**, 141-159.

POGOSIAN, S. A. (Ed.); 1974: Methodical Instructions Concerning Grape Breeding [Russ.]. Aistan, Erevan.

-- --; KHACHATRIAN, S. S; 1983: Breeding Table and Wine Grape Varieties [Russ.]. Aistan, Erevan.

RAILIANU, G. A.; 1985: The role of the fresh grape in the human diet [Russ.]. Sadovodstvo, Vinogradarstvo i Vinodelie Moldavii **8**, 58-59.

SHTIN, L. T.; FILIPPENKO, I. M.; FILIPPENKO, L. I.; 1986: Results and prospects of grape breeding for complex resistance [Russ.]. In: Prospects of Grape Genetics and Breeding for Phytologic Immunity. Theses of the Reports of the National Scientific and Technical Conference [Russ.], 11-12. National Research Institute of Agricultural Sciences, Moscow.

SMIRNOVA, N. G.; 1978: Roentgenographic investigation of the leaf-bearing arboraceous plant seeds [Russ.]. Nauka, Moscow.

SOHI, H. S.; SRIDHAS, T. S.; 1972: Note on resistance and susceptibility of grape varieties to powdery mildew. Indian J. Agricult. Sci. **42**, 641-642.

STAUDT, G.; 1980: *Vitis armata*, a new source of germ plasm in grape breeding. Proc. 3rd Intern. Symp. Grape Breeding, Davis, 62-64.

SUPOSTAT, L. F.; 1986: Immunological differentiation of *V. vinifera* L. concerning facultative parasites [Russ.]. In: Prospects of Grape Genetics and Breeding for Phytologic Immunity. Theses of the Reports of the National Scientific and Technical Conference [Russ.], 14. Moscow.

TAMASHI, I.; 1964: Grape selection and directed breeding [Russ.]. Vestnik Selskokhoziaistvennoi Nauki **7**, 148-151.

VASILIEVA, Z. I.; 1975: Berry injury of the interspecific and intraspecific grape hybrids caused by grey rot compared to that of their parents and some factors of resistance to this disease [Russ.]. Author's essay for acquiring Sci. D. of Cand. Biol. Sci., Kishinev.

VOLCHEV, V.; 1985: Results of the grape interspecific hybridization [Bulg.]. Genetika i Selektsia **4**, 348-351.

Rootstocks with immunity to phylloxera and nematode resistance

H. BECKER and E. SOPP

Fachgebiet Rebenzüchtung und Rebenveredlung, Forschungsanstalt Geisenheim, D-6222 Geisenheim, F. R. Germany

Abstract: BÖRNER discovered the immunity of *V. cinerea* type ARNOLD against all known biotypes of phylloxera. No galls develop on leaves or roots of this particular wild species. It was possible to transmit this necrotic reaction through cross-breeding. BÖRNER and his team evaluated about 16,000 seedlings with genes of *V. cinerea* ARNOLD in combination with many rootstocks, crossings and other *Euvitis* species. At Geisenheim the new rootstock Na 5153-54 *(V. riparia* 183 Geisenheim *x V. cinerea* ARNOLD*)* was selected out of many seedlings and was named Boerner commemorating his work. It will be patented and taken to the German varietal list in April 1989. It is already in propagation for further grafting. In 1989 about 140,000 cuttings are grafted.

The Boerner rootstock is vigorous, winter hardy and tolerant to mildew. The take with benchgrafting is medium and might be improving with more experience. It grows in many soils and seems to be as tolerant to chlorosis as *V. berlandieri x V. riparia* stock. We have results of more than 15 years with Riesling clone 239 Geisenheim grafted on Boerner in different soils. We also tasted wines of Riesling clone 239 Geisenheim grafted on Boerner in comparison with wines from other rootstocks for many years and found no negative effects. The yields of the grafted vines on Boerner are about the same as SO4 and other rootstock clones. Dagger nematodes *(Xiphinema)* do not induce galls or swellings on roots of Boerner. The reaction of the roots of Boerner is comparable to that caused by phylloxera on roots of this vine. In contrast to Boerner, roots of *V. berlandieri x V. riparia* produce galls and bigger cells due to phylloxera feeding, but not due to dagger nematode. In our research no fanleaf virus could be transmitted to Boerner. Necrotic reactions were studied in histologic investigations of Boerner roots infested with *Xiphinema*. The results have to be confirmed by further investigations, experiments and field tests.

We expect a breakthrough in the control of nepoviruses in the next years. There are more rootstocks with *V. cinerea* genes to be studied in the future.

Influence of the rootstock and potassium fertilizer on phytoalexin synthesis in Pinot blanc grown in a calcareous soil

L. Bavaresco and M. Zamboni

Cattedra di Viticoltura, Università Cattolica S. C., Via Emilia Parmense, 84, I-29100 Piacenza, Italy

S u m m a r y : Plants of cv. Pinot blanc, grafted on *Vitis berlandieri x V. riparia* Kober 5 BB, *V. berlandieri x V. riparia* SO 4, *V. berlandieri x V. rupestris* 1103 P, were grown in pots at two levels of potassium supply (0 g K_2O/pot and 2 g K_2O/pot) in order to test the phytoalexin synthesis in the leaves.

The experimental plan included also the macronutrient, trace element and chlorophyll contents of the leaves.

The most important findings are:

a) Resveratrol synthesis decreases from Kober 5 BB to SO 4 and 1103 P, while leaf chlorophyll content increases.

b) Resveratrol synthesis is higher in the plants without potassium supply.

K e y w o r d s : rootstock, potassium, nutrition, lime, soil, phytoalexin, resveratrol, chlorophyll, mineral, Botrytis, resistance.

Introduction

Phytoalexins are low-molecular, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms (Paxton 1981). Vitaceae, including *Vitis vinifera*, synthesize stilbene phytoalexins (resveratrol and the viniferins), which on the one hand are able to inhibit *Botrytis cinerea* growth (Langcake and McCarthy 1979; Blaich *et al.* 1982; Stein and Blaich 1985), on the other hand they are used as precursors for structural defence mechanisms (Hoos and Blaich 1988).

Environmental variables, including fertilizer supply, often have a predictable effect on phytoalexin synthesis, altering plant resistance. Mineral elements are, in fact, directly involved in all mechanisms of defence as integral components of cells, substrates, enzymes and electron carriers, or as activators, inhibitors and regulators of metabolism (Mengel and Kirkby 1978).

High levels of nitrogen compared to low levels reduce the quantities of phytoalexin accumulated in grapevine hybrids and *V. vinifera* varieties (Bavaresco and Eibach 1987).

In viticulture, the mineral nutrition of the plant is greatly affected by the rootstock which has selective uptake for mineral elements, as K, Ca, Mg, Fe.

The aim of the work is to clarify the effect of three rootstocks with different K uptake and of potassium supply on the resveratrol synthesis in Pinot blanc grown on calcareous soil, which is most favourable for the production of sparkling wines.

Materials and methods

Plants of Pinot blanc grafted on three different rootstocks (*V. berlandieri x V. riparia* Kober 5 BB, *V. berlandieri x V. riparia* SO 4, *V. berlandieri x V. rupestris* 1103 P) were grown in pots (9 l volume) containing a calcareous soil. Before adding basic nutrients, the soil main characteristics were: pH = 8.4, active lime = 17 %, total N = 0.5 %, P_2O_5 = 24 ppm, exch. K_2O = 67 ppm, Fe = 96 ppm, CEC = mEq/100 g.

Potassium treatments were 0 g K_2O/pot and 2 g K_2O/pot, added as K_2SO_4 in water solution to the soil surface (1 g K_2O at the stage of 5 expanded leaves and 1 g K_2O at flowering).

At the beginning of veraison, the 5th leaf (beginning from the shoot tip) was sampled and, after washing with 1 % NaOCl solution, the leaves were investigated as follows:

Table 1: Effects of rootstock and potassium supply on resveratrol synthesis and on chlorophylls and potassium contents of the leaves

	ROOTSTOCK				SUPPLY		
	Kober 5BB	SO4	1103 P	LSD 0.05	$+K_2O$	$-K_2O$	LSD 0.05
Resveratrol (s.u.)	3390	2020	1840	1050	1980	2850	860
Tot. Chl (mg/g fresh wt)	0.82	0.85	0.96	0.06	0.83	0.93	0.05
Tot. Chl (mg/100 g dry wt)	241	265	313	19	259	287	15
Chl a/Chl b	1.96	2.03	1.92	0.08	2.02	1.93	0.06
K %	0.53	0.98	0.96	--	1.05	0.60	--

Resveratrol synthesis was obtained by exploiting the elicitor activity of mucic acid (method of STEIN and HOOS 1984). Leaf discs (17 mm Ø) were placed on filter cardboard imbued by mucic acid solution (0.01 %). After phytoalexin extraction and distillation, resveratrol was identified by using thin-layer chromatography; the amount of each sample was 2 µl. The resveratrol values were expressed as scan units (s. u.) using a Shimadzu-Chromato-Scanner CS 920, under 325 nm UV radiation.

Chlorophylls (a, b, total) were expressed as mg/g fresh wt and mg/100 g dry wt. They were extracted from leaf discs with 80 % acetone for 72 h in darkness, at +4 °C (TORRECILLAS *et al.* 1984). Corresponding equations have been used for determination of the chlorophylls (STRAIN and SVEC 1966).

Mineral elements content, macronutrients (N, P, K, Ca, Mg) and some trace elements (Fe, Mn, B) were analyzed after wet destruction of the dry matter by using the methods of COTTENIE (1980).

The statistical plan included two-way-ANOVA with interactions (the means were compared by using the LSD test at the level of 5 %) and linear regressions.

Results

Resveratrol synthesis of Pinot blanc leaves is affected by both rootstock and potassium supply (Table 1). The highest value is found in Kober 5 BB (3390 s. u.) and the lowest one in 1103 P (1840 s. u.). The plants without potassium fertilization show higher resveratrol content (2850 s. u.) than those supplied with potassium (1980 s. u.). Kober 5 BB and SO 4 show similar responses to potassium supply (resveratrol reduction), whereas 1103 P does not change stilbene synthesis in a significant way (Table 2).

Rootstock and potassium supply influence the leaf chlorophylls content significantly (Table 1). Total chlorophyll increases, changing between Kober 5 BB (0.82 mg/g fresh wt and

Table 2: Resveratrol synthesis and contents of chlorophylls and leaf mineral elements depending on rootstock and potassium supply

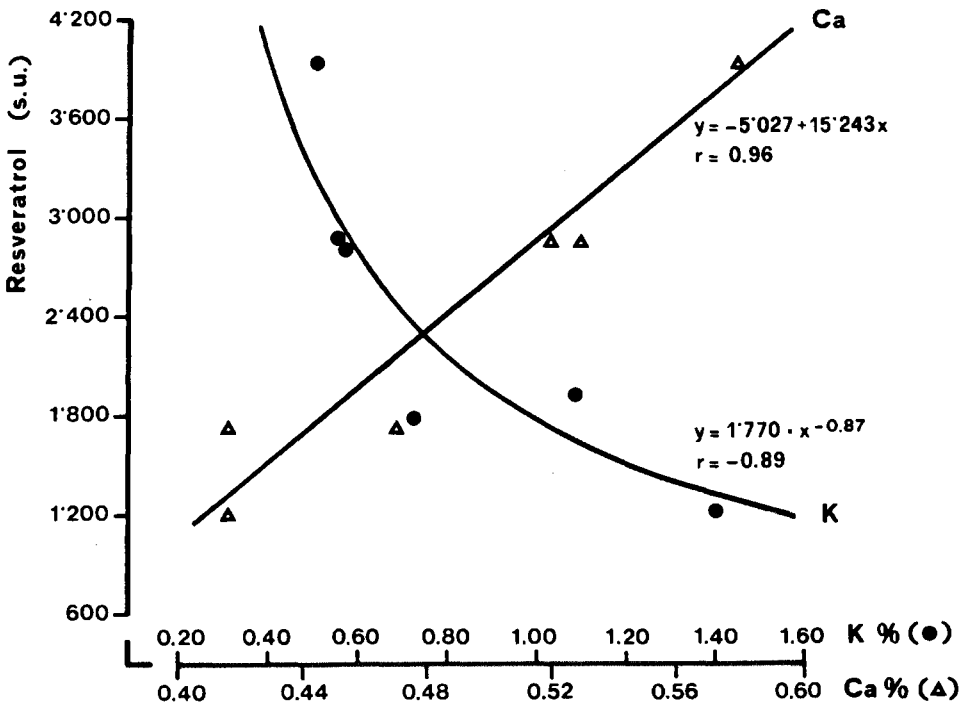
		Resvera-trol s.u.	Tot. Chl mg/g f.wt	Tot. Chl mg/100g dry wt	Chl a / Chl b	N%	P%	K%	Ca%	Mg%	Fe ppm	Mn ppm	B ppm
Pinot b./K 5BB	+K	2 850	0.72	215	1.99	2.09	0.27	0.56	0.52	0.36	75	55	7.3
	-K	3 930	0.92	266	1.93	2.60	0.28	0.50	0.57	0.64	58	47	6.4
Pinot b./SO4	+K	1 200	0.72	233	2.03	2.32	0.26	1.40	0.42	0.37	64	43	10.3
	-K	2 850	0.98	298	2.03	2.76	0.26	0.57	0.53	0.62	77	53	8.1
Pinot b./1103 P	+K	1 910	1.04	328	2.04	2.40	0.20	1.20	0.47	0.42	61	41	11.0
	-K	1 770	0.89	299	1.81	2.87	0.28	0.72	0.42	0.52	136	34	8.2
LSD 0.05		1 490	0.09	26	0.10	--	--	--	--	--	--	--	--

241 mg/100 g dry wt), SO 4 (0.85 mg/g fresh wt and 265 mg/100 g dry wt) and 1103 P (0.96 mg/g fresh wt and 313 mg/100 g dry wt).

By potassium supply the chlorophyll content decreases, changing from 0.93 mg/g fresh wt and 287 mg/100 g dry wt to 0.83 mg/g fresh wt and 259 mg/100 g dry wt, respectively.

The negative effect of potassium supply on the chlorophyll content is observed with each rootstock, except for 1103 P which has a positive influence (Table 2).

The leaf potassium content is strongly related to the rootstock genotype; the lowest value occurs with Kober 5 BB (0.53 %), the highest one with SO 4 (0.98 %) (Table 1). The plants supplied



Correlations between leaf potassium and calcium content, respectively, and resveratrol.

show a higher content (1.05 %) than those without potassium fertilizer (0.60 %): this difference is very plain in SO 4, whereas it is very weak in Kober 5 BB (Table 2).

Resveratrol synthesis is negatively related to leaf potassium content; on the other hand, the phytoalexin synthesis increases by increasing the leaf calcium content (Fig.).

Discussion

The results obtained emphasize the role of the rootstocks on mineral element uptake which modifies some physiological parameters involved in disease resistance (resveratrol) and chlorosis occurrence (chlorophylls). Kober 5 BB does not seem suitable for calcareous soils because of its susceptibility to chlorosis; besides this, the leaf potassium content is lowest, as well as nitrogen and iron with Kober 5 BB.

The high resveratrol content in case of Kober 5 BB is probably related to the leaf nitrogen content which is lowest among the genotypes. This situation can have given rise to a shift of the balance between the primary and secondary metabolic pathways onto the shikimate pathway, providing for a large pool of phenolics (GRAHAM 1983).

The behaviour of 1103 P is opposite: it seems suitable for calcareous soils because of its chlorosis tolerance and for its high nutritional level.

With 1103 P, the resveratrol level is lowest, probably due to the high nitrogen leaf content.

As a rule, resistance increases in response to potassium supply, up to an optimal level; in deficient plants, the synthesis of high-molecular compounds (proteins, starch and cellulose) is impaired, and low-molecular organic compounds (sugars, amino acids, amides) favourable to parasitic attack, accumulate (MARSCHNER 1986).

According to this assertion, some authors observed in grapevine a positive effect of potassium supply in decreasing development of or damage by downy mildew, powdery mildew and grey mould (SCHAFFNIT *et al.* 1930, GÄRTEL 1959, KIRALY 1976; quoted by PERRENOUD 1977).

Nevertheless, evidence has not yet been obtained that potassium supply affects phytoalexin synthesis in grapes.

The negative effect of potassium supply, observed in the present work, could be due to two reasons:

1. The potassium leaf content is in a deficiency range (CHAMPAGNOL 1984): potassium supply was not enough to allow an optimal element level in the leaves.
2. Potassium is an antagonist to calcium which is positively related to resveratrol synthesis. Calcium seems more effective than potassium in promoting phytoalexin synthesis.

Conclusions

The most significant findings are that:

a) resveratrol synthesis decreases from Kober 5 BB to SO 4 and 1103 P, while chlorophyll leaf content increases;

b) potassium supply has a negative effect on resveratrol synthesis.

Acknowledgements

The authors want to thank GIUSEPPE BRUZZI (laboratory staff) for his contribution to this project; Prof. G. ALLEWELDT for having allowed the use of the scanner; Prof. R. BLAICH for his suggestions on results discussion.

References

BAVARESCO, L.; EIBACH, R.; 1987: Investigations on the influence of N fertilizer on resistance to powdery mildew *(Oidium tuckeri)*, downy mildew *(Plasmopara viticola)* and on phytoalexin synthesis in different grapevine varieties. Vitis **26**, 192-200.

BLAICH, R.; BACHMANN, O.; STEIN, U. (1982): Causes biochimiques de la résistance de la vigne à *Botrytis cinerea*. Bull. OEPP **12**, 167-170.

CHAMPAGNOL, F.; 1984: Eléments de Physiologie de la Vigne et de Viticulture Générale. Dehan, Montpellier.

COTTENIE, A.; 1980: Soil and plant testing as a base of fertilizer recommendation. FAO Soils Bull. **38** (2).

GRAHAM, R. D.; 1983: Effects of nutrients stress on susceptibility of plants to disease with particular reference to the trace elements. Adv. Bot. Res. **10**, 221-276.

HOOS, G.; BLAICH, R.; 1988: Metabolism of stilbene phytoalexins in grapevines: Oxidation of resveratrol in single-cell cultures. Vitis **27**, 1-12.

LANGCAKE, P.; MCCARTHY, W. V.; 1979: The relationship of resveratrol production to infection of grapevine leaves by *Botrytis cinerea*. Vitis **18**, 244-253.

MARSCHNER, H.; 1986: Mineral Nutrition in Higher Plants. Academic Press, London.

MENGEL, K.; KIRKBY, E. A.; 1978: Principles of Plant Nutrition. International Potash Institute, Bern, Switzerland.

PAXTON, J. D.; 1981: Phytoalexins – A working redefinition. Phytopathol. Z. **101**, 106-109.

PERRENOUD, S.; 1977: Potassium and Plant Health. IPI Research Topics N° 3. International Potash Institute, Bern, Switzerland.

STEIN, U.; BLAICH, R.; 1985: Untersuchungen über Stilbenproduktion und Botrytisanfälligkeit bei *Vitis*-Arten. Vitis **24**, 75-87.

– – ; HOOS, G.; 1984: Induktions- und Nachweismethoden für Stilbene bei Vitaceen. Vitis **23**, 179-194.

STRAIN, H. H.; SVEC, W. A.; 1966: Extraction, separation, estimation, and isolation of the chlorophylls. In: VERNON, L. P.; SEELY, G. R. (Eds.): The Chlorophylls, 21-66. Academic Press, London.

TORRECILLAS, A.; LEON, A.; DEL AMOR, F.; MARTINEZ-MOMPEAN, M. C.; 1984: Determinaciòn rapida de clorofila en discos foliares de limonero. Fruits **39**, 617-622.

Selected hybrids of the wine grape variety Seibel 5279

J. Füri † and E. Visontai

Research Institute for Viticulture and Oenology, H-6001 Kecskemét, Hungary

Summary: In the resistance breeding programme of our Institute, which was begun after World War II, the French resistant hybrids Seyve Villard and Seibel, both of which resulted from crosses involving American species, were used for resistance sources.

In the resistance breeding programme at Kecskemét, the variety Seibel 5279 was used first as a male parent to transfer resistance.

From the resulting hybrid families the variety candidates RF 5 (Reflex), RF 16 (Refrén) and RF 48 (Reform) were selected because of their valuable characters. Data collected over several years demonstrate the value of these selections from both a breeding and production point of view.

This generation represents the first step in the breeding programme and the results encourage us to continue our work.

Key words: hybrid, breeding, Hungary, resistance, cold, plasmopara, oidium, botrytis.

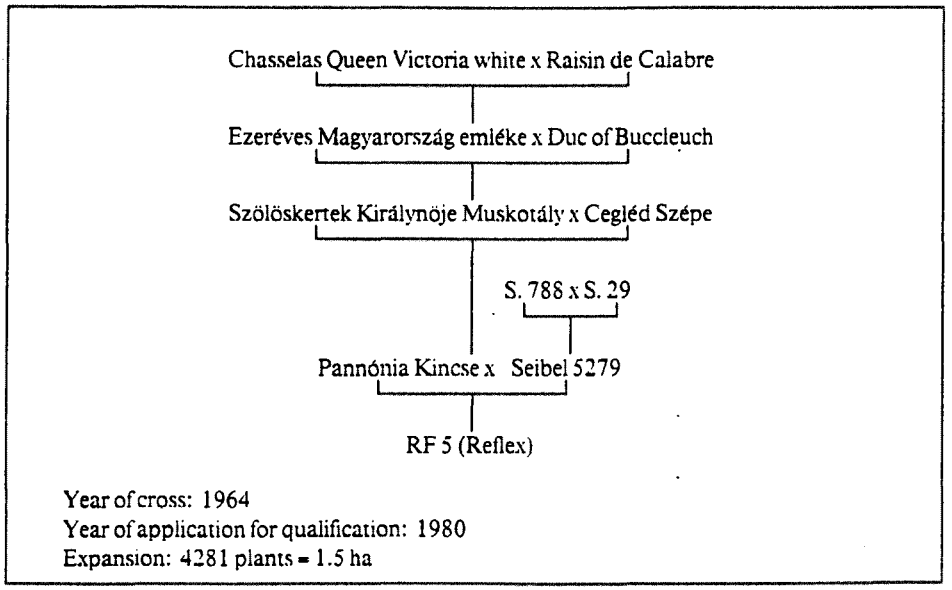


Fig. 1: RF 5 (Reflex) - production value means from 16 years (1970-1985), Kecskemét-Katonatelep.

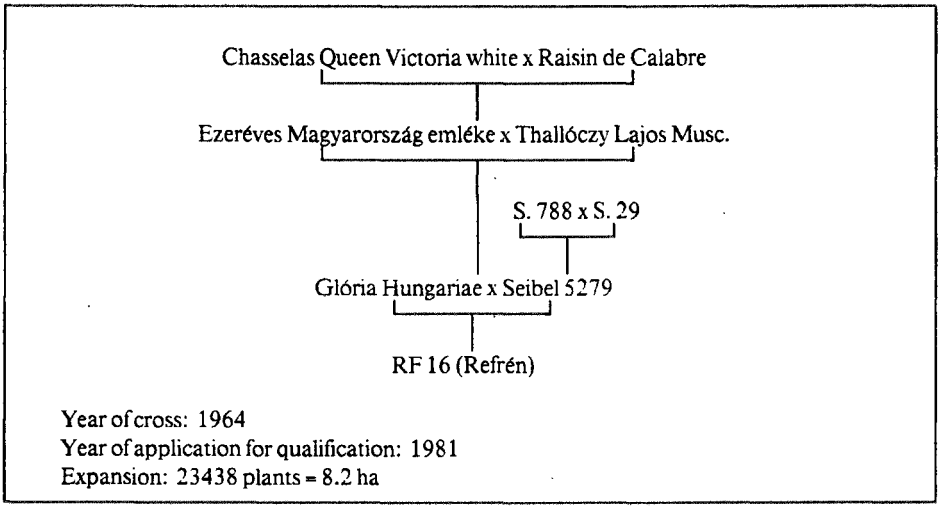


Fig. 2: RF 16 (Refrén) - production value means from 18 years (1968-1985), Kecskemét-Katonatelep.

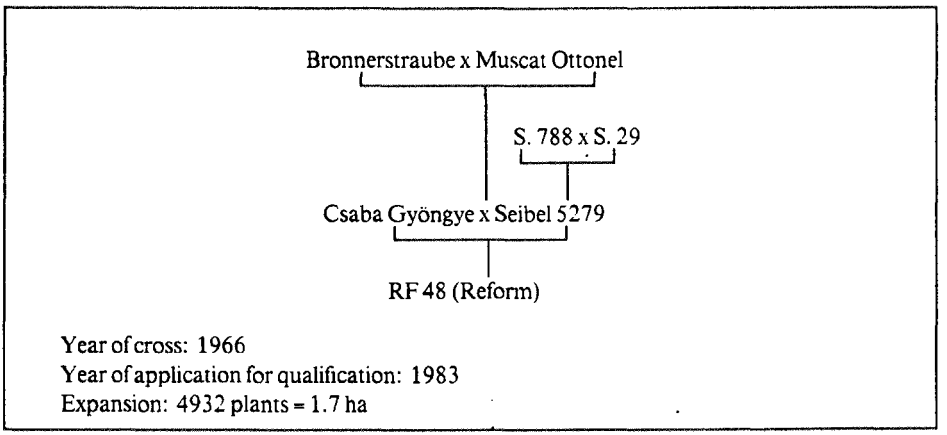


Fig. 3: RF 48 (Reform) - production value means from 9 years (1977-1985), Kecskemét-Katonatelep.

Breeding of botrytis tolerant *V. vinifera* and interspecific wine varieties

H. Becker and H. Konrad

Fachgebiet Rebenzüchtung und Rebenveredlung, Forschungsanstalt Geisenheim, D-6222 Geisenheim, F. R. Germany

Abstract: Arnsburger (Gm 22-74), a patented, intravarietal white wine variety developed at Geisenheim (Gm), is a cross of White Riesling clone 88 Gm x White Riesling clone 64 Gm and proved to be very tolerant to botrytis. The skin of the berries is tough and the stems of the clusters exhibit no stem rot, even when no botryticides were applicated. The clusters of Arnsburger are very loose and not compact. This also helps to prevent the infections of botrytis.

The variety Arnsburger was used in Geisenheim as a genetic source for tolerance against botrytis. The characteristics of this tolerance are transmitted to seedlings with *V. vinifera* and interspecific genome as well. Arnsburger was also used as mother plant because of the Riesling character of the grapes and wines. We were able to select many new types of white and even red wine varieties with high tolerance to botrytis. The new botrytis tolerant *V. vinifera* varieties were not sprayed with botryticides but with fungicides against both of the mildews. The new interspecific varieties need no spray at all and are resistant or tolerant to fungus diseases.

The best varieties are as follows:

V. vinifera varieties:

Gm No.	Crossing	Picking time	% Botrytis
Gm 711-4	Arnsburger x Reichensteiner	14.10.89	1
Gm 7110-3	Arnsburger x Faberrebe	3.10.89	1
Gm 712-7	Arnsburger x Deckrot	14.10.89	5
Arnsburger	Riesling cl. 88 Gm x Riesling cl. 64 Gm	26.10.89	15

Interspecific varieties:

Gm No.	Crossing	Picking time	% Botrytis
Gm 788-4	Arnsburger x Gf. B-6-18 (Pollux)	27.10.89	5
Gm 789-12	Arnsburger x Fr. 993-60	27.10.89	5
Gm 7813-2	Arnsburger x Landot 2282 (red wine variety)	10.10.89	0

They have been propagated for further field tests.

Section 4: Resistance/tolerance to abiotic stress factors

Investigations on some physiological parameters involved in chlorosis occurrence in different grapevine rootstocks and a *Vitis vinifera* cultivar

L. Bavaresco

Cattedra di Viticoltura, Università Cattolica S. C., Via Emilia Parmense, 84, I-29100 Piacenza, Italy

Summary: 1-year-old grapevine cuttings were grown in pots in order to test, during the growing period, the changes of some leaf compounds related to chlorosis occurrence (chlorophylls a, b and total chlorophyll, Fe^{++}, macronutrients and trace elements).

The genotypes tested were three rootstocks showing an increasing degree of chlorosis resistance (*Vitis riparia* x *V. rupestris* 101-14, *V. berlandieri* x *V. riparia* SO 4, *V. berlandieri* x *V. rupestris* 140 Ru) and a *V. vinifera* variety (Chardonnay), each of them grown in both a calcareous and a non-calcareous soil.

At the end of the growing period, the whole cuttings were analysed to test the macronutrients and trace elements content of the dry matter.

The most important findings are:

(1) During the growing period, the chlorophyll and leaf Fe^{++} content first increases and then decreases.

(2) The rootstock most susceptible to chlorosis (101-14) shows in the calcareous soil the highest Fe^{++} and total leaf chlorophyll content, while the most resistant one (140 Ru) has the lowest values. Therefore, the analysis of such parameters is not a suitable tool to screen rootstocks for chlorosis resistance.

(3) Suitable tools to judge the resistance/susceptibility to lime-induced chlorosis in ungrafted rootstocks grown on calcareous soils are: a) the dry matter production at the end of the annual growing cycle; b) the 'iron efficiency ratio' (g dry matter/mg iron) in the shoot at the end of the annual growing period.

Key words: chlorosis, resistance, rootstock, variety of vine, soil, lime, chlorophyll, iron, mineral, growth.

Introduction

Many world-wide agricultural crops, grown in calcareous soils, suffer from lime-induced chlorosis, usually recognized by yellowed intervein areas in new leaves. Plant species mainly affected include apples, avocado, bananas, barley, beans, citrus, cotton, grapes, oats, peanuts, pecans, potatoes, sorghum, soybeans and numerous greenhouse flowers (Chen and Barak 1982). The most important factor responsible for lime-induced chlorosis is the high bicarbonate (HCO_3^-) concentration in the soil solution (Boxma 1972; Mengel and Malissiovas 1981; Mengel and Bübl 1983; Mengel *et al.* 1984; Coulombe *et al.* 1984; Mengel and Geurtzen 1986; Kolesh *et al.* 1987) related to high pH (Juste *et al.* 1967). Use of soil Fe by plants is genetically controlled; a variety that can use Fe in an alkaline soil is called Fe-efficient, while a variety that develops iron chlorosis is called Fe-inefficient (Brown and Jones 1976). Mobilization of iron in the rhizosphere is due to both basic or non-specific mechanisms (independent from the iron nutritional status of the plant) and adaptive mechanisms (Marschner 1986), which are activated in Fe-efficient plants in response to iron-stress. The adaptive mechanisms differ among genotypes and they can be classified according to two strategies (Marschner *et al.* 1986).

Strategy I (exhibited by most higher plants, dicotyledons and monocotyledons except for grasses) consists of four types of response in the roots, as follows: a) enhancement of H-ions release (Marschner 1978; Landsberg 1981); b) formation of rhizodermal or hypodermal transfer cells (Kramer *et al.* 1980; Landsberg 1989); c) enhancement of ferric iron reduction to ferrous iron (Bienfait *et al.* 1982); d) enhancement of release of reducing/chelating compounds such as phenolics (Römheld and Marschner 1981; Hether *et al.* 1984).

Strategy II, occurring in barley, oats, rice and probably most other grasses, is characterized by an enhancement of release of non-proteinogenic amino acids (phytosiderophores) and by a high affinity uptake system (Römheld 1987).

Table 1: Physical and chemical characteristics of the soils

	Non-calcareous soil	Calcareous soil
pH in H_2O	6.9	8.3
pH in KCl	5.9	7.7
Sand	31%	29%
Silt	45%	55%
Clay	24%	16%
Carbonates	Absent	54%
Lime	Absent	19%
Organic Matter	1.6%	0.3%
CEC	12.9 mEq/100 g	10.1 mEq/100 g
Soluble Salts	210 µS/cm	225 µS/cm
C/N ratio	10.9	8.2
Total nitrogen	0.8%.	0.2%.
Phosphorus (P_2O_5) 1)	63 ppm	11 ppm
Potassium (K_2O) 2)	146 "	71 "
Magnesium 2)	179 "	27 "
Calcium 2)	1960 "	1920 "
Sodium 2)	11 "	9 "
Iron 3)	130 "	89 "
Manganese 3)	225 "	42 "
Zinc 3)	7 "	3 "
Copper 3)	10 "	3 "
Boron 4)	3.4 "	0.3 "

1) extracted by Olsen method

2) exchangeable cation extracted by NH_4OAc 1N at pH = 7

3) extracted by NH_4OAc 0.5 N + EDTA 0.02 M at pH = 4.65

4) extracted by Truog method and analysed by Azomethine H

Phytosiderophores are specific Fe chelating compounds such as mugineic and avenic acid (Takagi *et al.* 1984; Römheld and Marschner 1986).

The tolerant grapevine rootstocks probably have strategy I response mechanisms (Bavaresco *et al.* 1989), but vines are normally grafted and the behaviour of the whole plant towards lime-induced chlorosis is governed by two properties: 1. the ability of the roots to supply the iron needs of the leaves; 2. the leaves iron requirement to secure a normal iron nutrition of the plant (Pouget and Ottenwaelter 1973).

In the present work, the ranges of some physiological parameters involved in chlorosis occurrence in ungrafted rootstocks are discussed. It is of further interest to study the reactions of different genotypes, which are known from the field, to affect chlorosis symptoms of the scion with different intensities.

Table 2: Rootstock resistance to chlorosis based on soil IPC (from POUGET and JUSTE 1972; POUGET 1980)

Rootstock	Maximum threshold for IPC 1)
Vialla	2
Riparia Gloire	5
3309, 101-14	10
Rupestris du Lot	20
99 R, 110 R, SO4, 1103 P	30
Kober 5BB, 420 A	40
161-49, 41 B	60
333 EM	70
140 Ru	90
Fercal	120

1) $IPC = \frac{CaCO_3}{(Fe)^2} \cdot 10^4$

$CaCO_3$ = active lime (%)

Fe = iron (ppm) extracted by ammonium oxalate

Materials and methods

1-year-old grapevine cuttings (about 10 cm long) rooted in sand were grown in pots in both a non-calcareous and a calcareous soil (Table 1).

The genotypes tested were three rootstocks (related with a decreasing degree of chlorosis resistance in the scion) (Table 2) and a *Vitis vinifera* cultivar, as follows: *V. berlandieri* x *V. rupestris* 140 Ru, *V. berlandieri* x *V. riparia* SO 4, *V. riparia* x *V. rupestris* 101-14, Chardonnay clone R 8.

The shoot length was weekly gauged for each genotype in both soils.

15 d after beginning of the trial (1st sampling time), 80 d later (2nd sampling time) and 115 d later (3rd sampling time), the 4th and the 5th leaf (counting from the tip of the shoot) were collected. After washing of the leaves in 1 % NaOCl solution, the following constituents were determined:

Fe(II): It was expressed as μg/g dry wt (ppm) and μg/g fresh wt, using the method of KATYAL and SHARMA (1980). 2 g of fresh-chopped samples were added to 20 ml of 1.5 % 1,10-o-phenanthroline solution at pH 3 in 100 ml glass bottles. After 16 h, the contents were filtered and Fe(II) was estimated in the filtrate by measuring the absorbency of the Fe(II)-phenanthroline complex at 510 nm.

Chlorophylls: Chlorophyll a, b and total chlorophyll were expressed in mg/100 g d. wt and mg/g f. wt. They were extracted from leaf discs by using 80 % acetone for 72 h in the dark, at +4 °C (TORRECILLAS *et al.* 1984). The chlorophyll concentration was determined by reading the absorbencies at 665 nm and 649 nm and use of the equations given by STRAIN and SVEC (1966).

Mineral elements: Macronutrients (N, P, K, Ca, Mg) and some trace elements (B, Fe, Mn, Cu, Zn) were analysed after wet destruction of the dry matter using the methods of COTTENIE (1980).

At the end of the annual growing period, the plants were divided into leaves, shoot, trunk, roots and each part was analysed for dry matter and mineral elements content.

Table 3: Effect of sampling time, genotype and soil on the Fe(II) and chlorophyll contents of leaves

	SAMPLING TIME				GENOTYPE					SOIL		
	1st	2nd	3rd	LSD 0.05	140 Ru	SO4	101-14	Ch	LSD 0.05	n.c.	c.	LSD 0.05
Fe^{++} ppm	68	84	45	3.9	56	61	82	64	4.6	68	64	3.2
Fe^{++} μg/g fresh wt	17.7	22.8	17.0	1.11	17.2	17.7	21.2	20.7	1.28	19.6	18.8	0.79
Chl. a mg/100 g dry wt	333	491	338	36.3	344	370	423	411	41.9	382	393	NS
Chl. b mg/100 g dry wt	152	243	164	12.1	162	182	203	199	13.9	183	189	NS
Tot. chl. mg/100 g dry wt	499	734	502	39.2	506	551	629	626	45.3	573	583	NS
Chl. a/b dry wt	2.27	2.02	2.06	0.07	2.16	2.06	2.11	2.09	NS	2.11	2.10	NS
Chl. a mg/g fresh wt	0.96	1.43	1.03	0.08	0.93	1.09	1.16	1.37	0.1	1.14	1.14	NS
Chl. b mg/g fresh wt	0.42	0.71	0.50	0.03	0.44	0.54	0.56	0.64	0.04	0.54	0.55	NS
Tot. chl. mg/g fresh wt	1.39	2.14	1.52	0.13	1.37	1.63	1.72	2.01	0.15	1.67	1.69	NS
Chl. a/b fresh wt	2.27	2.02	2.06	0.04	2.16	2.05	2.08	2.16	0.05	2.13	2.10	NS

Ch = Chardonnay ; NS = not significant

n.c. = non calcareous ; c = calcareous

The statistical plan included three-way ANOVA and two-way ANOVA with interactions; the means were compared by LSD test at a 5 % level.

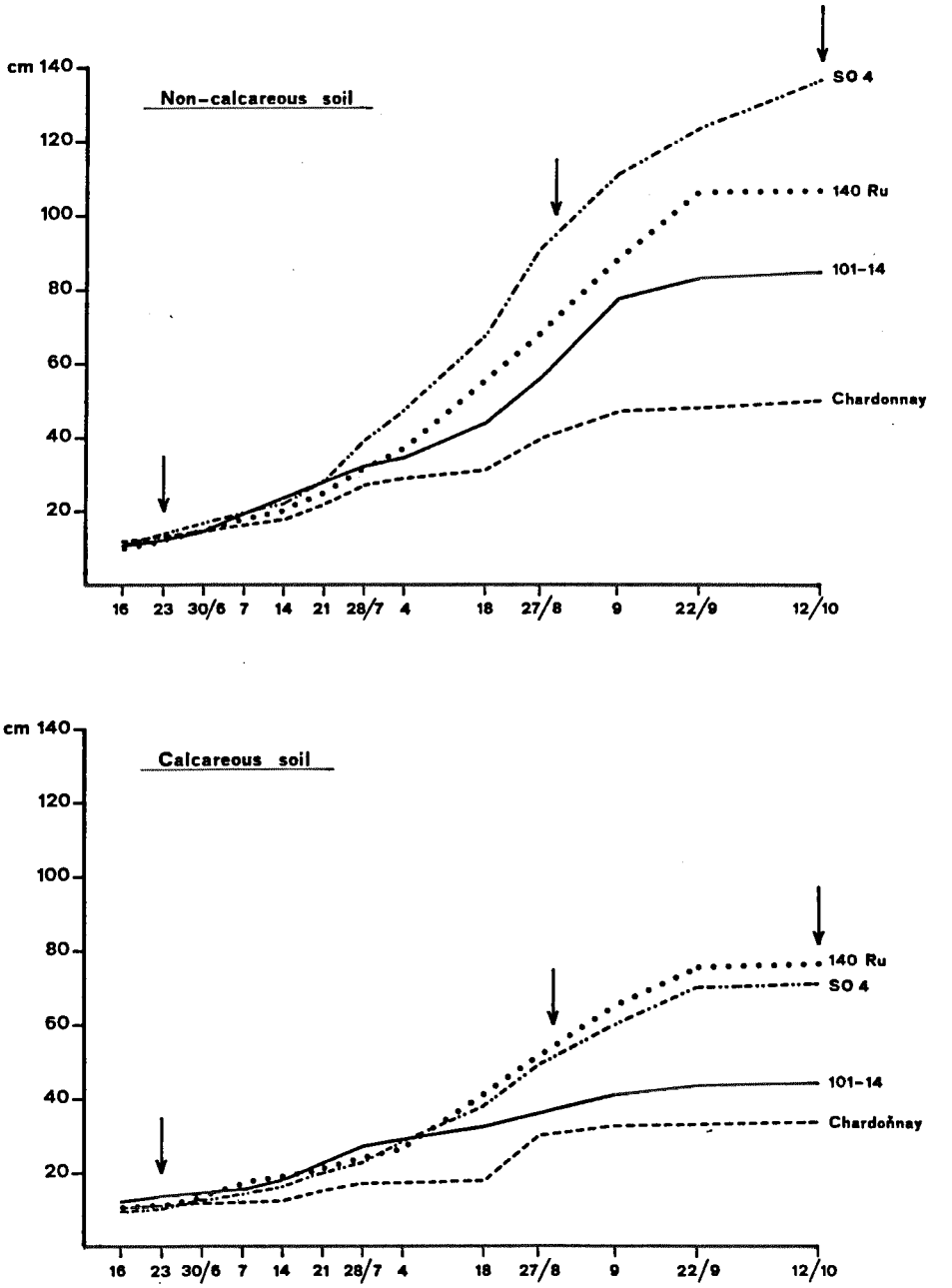


Fig. 1: Shoot growth in the two different soils depending on genotype. Arrows indicate the three sampling times.

Results

The shoot growth (Fig. 1) seems to be affected by both soil and genotype. The calcareous soil has a negative effect on the growth of each genotype.

The highest shoot length in the non-calcareous soil occurs in SO4 (136 cm), while in the calcareous one 140 Ru grows highest (76 cm). 101-14 has within the rootstocks the lowest shoot growth in both soils.

The Fe(II) content of the leaves (based on both dry and fresh weight) is affected in a significant way by the sampling time, the genotype, the soil and their interactions (Table 3). The values increase from the 1st sampling time (68 ppm and 17.7 μg/g f. wt) to the 2nd one (84 ppm and 22.8 μg/g f. wt) and then decrease at the 3rd sampling time (45 ppm and 17 μg/g f. wt).

101-14 rootstock shows the highest Fe(II) content (82 ppm and 21.2 μg/g f. wt), while 140 Ru has the lowest one (56 ppm and 17.2 μg/g f. wt).

The plants grown on the calcareous soil show a lower iron content (64 ppm and 18.8 μg/g f. wt) than those from the non-calcareous one (68 ppm and 19.6 μg/g f. wt).

The sampling time and the genotype influence the chlorophylls content in a significant way. Total chlorophyll (on the basis of both dry and fresh weight) first increases (changing from 499 mg/100 g d. wt and 1.39 mg/g f. wt at the 1st sampling time to 734 mg/100 g d. wt and 2.14 mg/g f. wt at the 2nd sampling time), then it decreases to 502 mg/100 g d. wt and 1.52 mg/g f. wt.

140 Ru rootstock shows the lowest chlorophyll content (506 mg/100 g d. wt and 1.37 mg/g f. wt); on the other hand, 101-14 shows within the rootstocks the highest value (629 mg/100 g d. wt and 1.72 mg/g f. wt).

The differences due to the two soils are not significant. The total chlorophyll and leaf Fe(II) content, on a fresh weight base, are well related when the plants are grown under stress condition (calcareous soil) (Fig. 2).

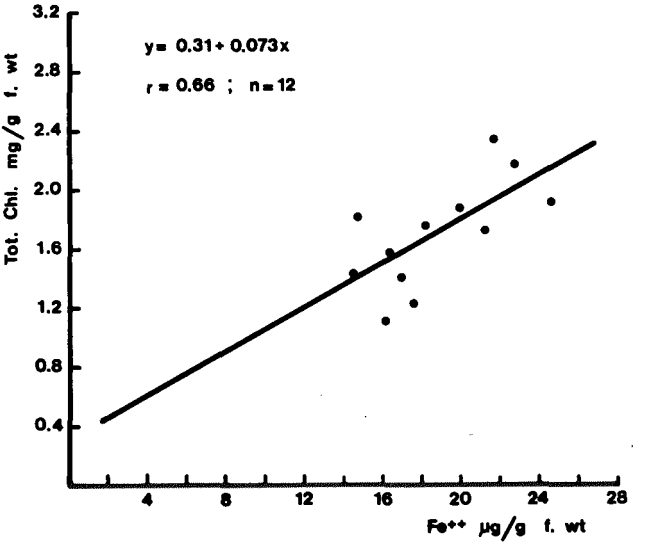


Fig. 2: Correlation between leaf Fe(II) and total chlorophyll content of the genotypes grown on calcareous soil.

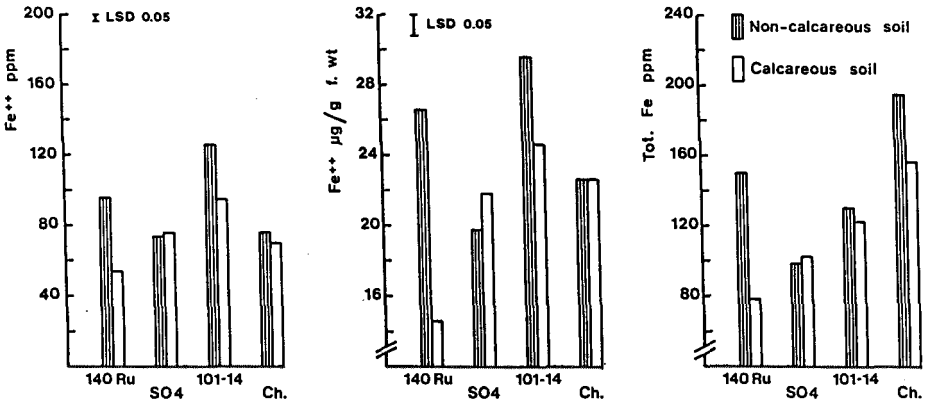


Fig. 3: Leaf ferrous iron and total iron content depending on genotype and soil at the 2nd sampling time.

When focusing the attention to the 2nd sampling time (the period of the fastest shoot growth), it is interesting to observe the behaviour of each genotype as influenced by the soil. The Fe(II) content decreases from the non-calcareous to the calcareous soil for each rootstock, save for SO 4 where it increases from 19.7 µg/g f. wt to 21.8 µg/g f. wt (Fig. 3).

In the calcareous soil, the total chlorophyll content changes within the rootstocks from 674 mg/100 g d. wt (101-14) to 742 mg/100 g d. wt (SO 4) (Fig. 4).

The effects of the sampling time, the genotype and the soil on the mineral element content of the leaves are summarized in Table 4. The behaviour of the macronutrients depending on the sampling time is different, while the trace elements have a uniform trend. Nitrogen first increases and then decreases, changing from 2.58 % to 3.20 % and 1.92 %. Leaf potassium content increases (from 0.98 % to 1.28 %), while calcium and magnesium decrease. The trace elements, except Cu, decrease with progress of the growing season.

Among the genotypes, Chardonnay variety shows the highest contents of nitrogen, calcium, manganese and zinc, while 101-14 rootstock has the highest potassium value. The leaf iron content is 155 ppm in 140 Ru, 151 ppm in Chardonnay, 133 ppm in 101-14 and 124 ppm in SO 4.

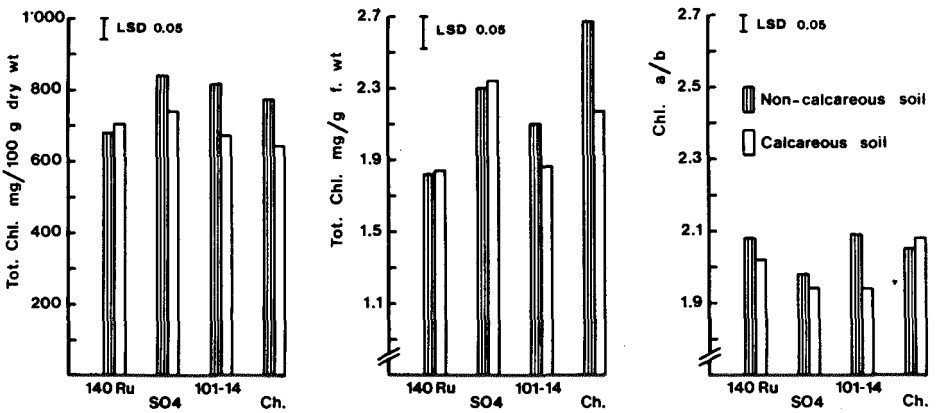


Fig. 4: Leaf total chlorophyll content and chlorophyll a/b ratio depending on genotype and soil at the 2nd sampling time.

Table 4: Effect of sampling time, genotype and soil on the mineral element content of leaves

	SAMPLING TIME			GENOTYPE				SOIL	
	1st	2nd	3rd	140 Ru	SO4	101-14	Ch	n.c.	c.
N%	2.58	3.20	1.92	2.34	2.34	2.71	2.89	2.61	2.53
P%	0.23	0.22	0.22	0.21	0.20	0.24	0.24	0.23	0.21
K%	0.98	1.14	1.28	0.96	1.08	1.31	1.20	1.18	1.09
Ca%	1.30	0.51	0.55	0.78	0.74	0.79	0.84	0.71	0.87
Mg%	0.40	0.34	0.30	0.35	0.32	0.35	0.36	0.33	0.37
Fe ppm	172	129	122	155	124	133	151	144	138
Mn ppm	106	69	53	72	71	71	89	84	67
Cu ppm	23	30	23	29	29	19	24	24	26
Zn ppm	118	39	27	58	59	58	70	55	67
B ppm	17	8	10	12	12	11	11	12	12
P/Fe	13.4	17.0	18.0	13.5	16.1	18.0	15.9	16.0	15.2
Fe/Mn	1.62	1.87	2.30	2.15	1.75	1.87	1.70	1.71	2.06

Ch = Chardonnay

n.c. = non calcareous ; c. = calcareous

The effect of the soil does not seem to be strong, save for calcium, where the plants grown on calcareous soil have a value higher than those grown on the non-calcareous one (0.87 % vs 0.71 %).

At the end of the annual growing period, the organ and the genotype affect the content of all the elements (Table 5), whereas the soil influences in an appreciable way the plant content of Ca (1.00 % and 1.51 % in the non-calcareous and calcareous soil, respectively) and Fe (377 ppm and 186 ppm, correspondingly).

Among the genotypes, the dry matter production is affected by soil above all in 101-14 (Fig. 5), which has in the calcareous soil the lowest value of the rootstocks (3.2 g). Though 101-14 in the calcareous soil has the highest leaf iron content (355 ppm) among the rootstocks (Table 6), it shows the lowest 'iron efficiency ratio' (g dry matter/mg Fe) in the shoot (Fig. 6).

Discussion

The results obtained during the growing period emphasize the role of the shoot growth stage and the genotype on some physiological parameters of the leaf involved in chlorosis occurrence.

Table 5: Percentage of dry matter, total dry matter and mineral element content of the plant depending on organ, genotype and soil at the end of the annual growing period

	ORGAN					GENOTYPE					SOIL		
	Leaves	Shoot	Trunk	Roots	LSD 0.05	140 Ru	SO4	101-14	Ch.	LSD 0.05	n.c.	c.	LSD 0.05
Dry matter (%)	27.95	37.06	50.81	35.81	2.63	37.54	36.46	37.80	39.83	2.63	38.45	37.37	NS
Dry matter (g)	4.37	3.53	6.22	4.15	0.59	5.02	5.17	4.56	3.52	0.59	5.25	3.88	0.42
N%	1.67	0.75	0.57	1.15	0.08	0.92	0.94	1.02	1.26	0.08	1.03	1.04	NS
P%	0.17	0.11	0.07	0.17	0.02	0.12	0.12	0.13	0.16	0.02	0.13	0.13	NS
K%	1.16	1.32	0.37	0.81	0.10	0.81	0.90	0.96	0.99	0.10	0.92	0.91	NS
Ca%	2.23	0.89	0.71	1.74	0.12	1.13	1.16	1.11	1.61	0.12	1.00	1.51	0.08
Mg%	0.37	0.15	0.10	0.19	0.02	0.19	0.17	0.19	0.26	0,02	0.19	0.22	0.01
S%	0.17	0.06	0.04	0.14	0.01	0.11	0.09	0.08	0.13	0.01	0.09	0.11	0.01
B ppm	22	18	13	13	1.7	16	17	16	18	1.7	17	17	NS
Fe ppm	330	61	141	594	100	256	238	233	399	100	377	186	71
Mn ppm	73	30	36	26	7	31	32	36	48	7	44	29	5
P/Fe	5.72	21.40	6.32	4.93	2.9	9.75	10.66	9.29	8.67	NS	8.62	10.57	1.94

Ch.= Chardonnay ; n.c. = non-calcareous ; c. = calcareous

Regarding the role of the genotype, 101-14 is of special interest. This rootstock, which normally induces chlorosis in the scion when growing on a calcareous soil, does not show any chlorosis symptom when it is ungrafted. Leaf Fe(II) content of 101-14 is even higher than in the other rootstocks, as well as the chlorophylls. Only at the stage of fastest shoot growth, the total chlorophyll content of 101-14, growing on the calcareous soil, is lower than in the other rootstocks, but without visual differences. This behaviour seems strange, because in trials performed on excised roots 101-14 showed a low reducing capacity and uptake rate for iron (Bavaresco *et al.* 1989). This rootstock (ungrafted) is probably able to mobilize under stress conditions from the nutrient reserves stored in the cutting a higher iron amount than the other rootstocks, thus supplying the high iron needs of the leaves. This hypothesis is supported by the data of Table 6, where a negative correlation seems to be between leaf and trunk iron of the three rootstocks growing on the calcareous soil. Besides this, not always high iron uptake capacity means high transport inside the plant to the leaves (Nerkar and Misal 1987).

Table 6: Total iron content (ppm) of the plant depending on organ, genotype and soil at the end of the annual growing period

	LEAVES			SHOOT			TRUNK			ROOTS		
	140 Ru	SO4	101-14	140 Ru	SO4	101-14	140 Ru	SO4	101-14	140 Ru	SO4	101-14
Non-calcareous soil	234	221	308	73	43	49	140	141	190	1000	833	572
Calcareous soil	240	278	355	45	52	47	137	108	85	178	230	256

Ch. = Chardonnay

The lack of yellowing in the leaves (measured in this work by the chlorophyll content) of a rootstock susceptible to chlorosis when growing in a calcareous soil was already observed by POUGET and JUSTE (1972). These authors explained this apparently paradoxical behaviour by the iron requirement of the leaves, which is different in ungrafted and grafted plants. Another explanation considers the negative effect of the grafted vine's yield on the nutrient reserves, including iron, in the woody parts of the plant (BALASUBRAHMANYAM *et al.* 1978); excessive yield induces chlorosis symptoms in the following year (MURISIER and BRIGUET 1988), depending on the reduction of the sugar reserves in the roots (POUGET 1974).

This different behaviour of a grafted and an ungrafted rootstock disappears when a seedling or a softwood cutting is tested instead of a woody cutting (BYRNE 1988; ROMERA *et al.* 1989 a, 1989 b).

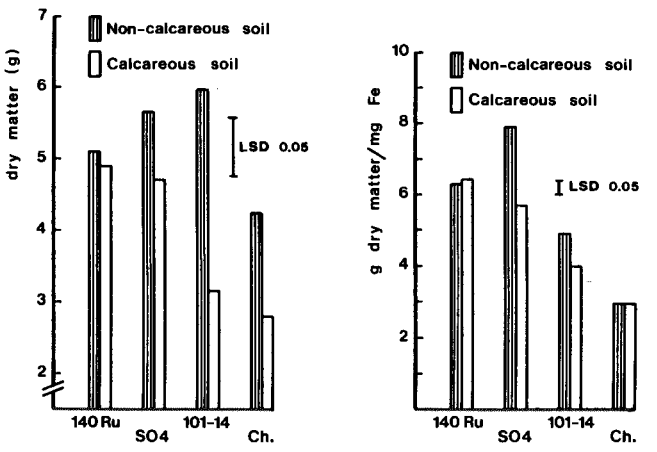


Fig. 5 (left): Dry matter production (average value of the four organs) depending on genotype and soil at the end of the annual growing period.

Fig. 6 (right): Iron efficiency ratio (g dry matter/mg Fe) in the shoot depending on genotype and soil.

Despite the lack of chlorosis symptoms, 101-14 rootstock grown on calcareous soil differs from the other two rootstocks (more resistant to lime-induced chlorosis) by having the lowest shoot growth, dry matter production (at the end of the growing period) and 'iron efficiency ratio' (g dry matter/mg Fe) of the shoot. A difference in the dry matter production between susceptible (3309) and resistant (Fercal, 140 Ru) rootstocks was also observed by CHIADMI and BRANCHARD (1987) in *in vitro* trials.

On the other hand, 140 Ru rootstock shows its characteristics of resistance by having in calcareous soil the highest shoot growth, dry matter production and 'iron efficiency ratio' of the shoot; moreover, it does not change its behaviour depending on the soil.

SO4 rootstock has characteristics intermediate between 101-14 and 140 Ru. Chardonnay (which is normally grown grafted) seems a genotype with high iron requirements, but low 'iron efficiency ratio'.

Conclusions

The most significant findings are that:

1. during the growing period, the chlorophylls and leaf Fe(II) content first increases and then decreases;
2. the rootstock most susceptible to chlorosis (101-14) shows in the calcareous soil the highest Fe(II) and total chlorophyll content, while 140 Ru rootstock (the most resistant) has the lowest values;
3. suitable tools to judge the resistance/susceptibility to lime-induced chlorosis in ungrafted rootstocks grown on calcareous soil are: a) the dry matter production at the end of the annual growing period; b) the 'iron efficiency ratio' (g dry matter/mg iron) in the shoot at the end of the annual growing period.

Acknowledgements

The author wants to thank Mr. GIUSEPPE BRUZZI (laboratory staff) for his contribution to this project.

References

BALASUBRAHMANYAM, V. R.; EIFERT, J.; DIOFASI, L.; 1978: Nutrient reserves in grapevine canes as influenced by cropping levels. Vitis **17**, 23-29.

BAVARESCO, L.; FREGONI, M.; FRASCHINI, P.; 1989: Investigations on iron uptake and reduction by excised roots of different grapevine rootstocks and a *V. vinifera* cultivar. 5th Intern. Symp. Iron Nutrition and Interaction in Plants, Kibbutz Ramat-Rachel, Israel, 11-17 June 1989.

BIENFAIT, H. F.; DUIVENVOORDEN, J.; VERKERKE, W.; 1982: Ferric reduction by roots of chlorotic bean plants: indications for an enzymatic process. J. Plant Nutr. **5**, 451-456.

BOXMA, R.; 1972: Bicarbonate as the most important soil factor in lime-induced chlorosis in the Netherlands. Plant Soil 37, 233-243.

BROWN, J. C.; JONES, W. E.; 1976: A technique to determine iron efficiency in plants. Soil Sci. Soc. Amer. J. **40**, 398-405.

BYRNE, D. H.; 1988: Comparative growth of two peach seedling rootstocks under alkaline soil conditions. J. Plant Nutr. **11**, 1663-1669.

CHEN, Y.; BARAK, P.; 1982: Iron nutrition of plants in calcareous soils. Adv. Agron. **35**, 217-240.

CHIADMI, N.; BRANCHARD, M.; 1987: Mise au point d'un test 'in vitro' permettant la détermination précoce de la sensibilité de porte-greffes de vigne à la chlorose calcaire. 3e Symp. Intern. Physiol. Vigne, Bordeaux, 42-44.

COTTENIE, A.; 1980: Soil and plant testing as a base of fertilizer recommendation. FAO Soils Bull. **38** (2).

COULOMBE, B. A.; CHANEY, R. L.; WIEBOLD, W. J.; 1984: Bicarbonate directly induces iron-chlorosis in susceptible soybean cultivars. Soil Sci. Soc. Amer. J. **48**, 1297-1301.

FREGONI, M.; SCIENZA, A.; 1975: Ricerche sull'assimilabilità del ferro in vigneti italiani. Vignevini **6**, 7-10.

HETHER, N. H.; OLSEN, R. A.; JACKSON, L. L.; 1984: Chemical identification of iron reductants exuded by plant roots. J. Plant Nutr. 7, 667-676.

JUSTE, C.; POUGET, R.; BRUZAU, F.; 1967: Influence du pH et de l'anion bicarbonique sur l'absorption du fer par les racines de vigne. C. R. Acad. Sci. **264**, 2781-2784.

KATYAL, J. C.; SHARMA, B. D.; 1980: A new technique of plant analysis to resolve iron chlorosis. Plant Soil **55**, 105-119.

KOLESCH, H.; HOFNER, W.; SCHALLER, K.; 1987: Effect of bicarbonate and phosphate on iron chlorosis of grape vines with special regard to the susceptibility of two rootstocks. Part. II: pot experiments. J. Plant Nutr. **10**, 231-249.

KRAMER, D.; RÖMHELD, V.; LANDSBERG, E.; MARSCHNER, H.; 1980: Induction of transfer-cell formation by iron deficiency in the root epidermis of *Helianthus annuus* L. Planta **147**, 335-339.

LANDSBERG, E. C.; 1981: Organic acid synthesis and release of hydrogen ions in response to Fe-deficiency stress of mono- and dicotyledonous plant species. J. Plant Nutr. **3**, 579-591.

– – ; 1989: Proton efflux and transfer cell formation as response to Fe-deficiency of soybean in nutrient solution culture. Pland Soil **114**, 53-61.

MARSCHNER, H.; 1978: Beziehung zwischen der Eisenversorgung von Weinreben und dem pH-Verlauf in der Nährlösung. Vitis **17**, 152-160.

– – ; 1986: Mineral Nutrition in Higher Plants. Academic Press, London.

– – ; RÖMHELD, V.; KISSEL, M.; 1986: Different strategies in higher plants in mobilization and uptake of iron. J. Plant Nutr. **9**, 695-713.

MENGEL, K.; BÜBL, W.; 1983: Verteilung von Eisen in Blättern von Weinreben mit HCO_3^- induzierter Fe-Chlorose. Z. Pflanzenernähr. Bodenk. **146**, 560-571.

– – ; BREININGER, M. TH.; BÜBL, W.; 1984: Bicarbonate the most important factor inducing iron chlorosis in vine grapes on calcareous soil. Plant Soil **81**, 333-344.

– – ; GEURTZEN, G.; 1986: Iron chlorosis on calcareous soils. Alkaline nutritional condition as the cause for the chlorosis. J. Plant Nutr. **9**, 161-173.

– – ; MALISSIOVAS, N.; 1981: Bicarbonat als auslösender Faktor der Eisenchlorose bei der Weinrebe *(Vitis vinifera)*. Vitis **20**, 235-243.

MURISIER, F.; BRIGUET, C.; 1988: Rendement et chlorose de la vigne. Rev. Suisse Vitic. Arboric. Hortic. **20**, 165-172.

NERKAR, Y. S.; MISAL, M. B.; 1987: Rice breeding for tolerance to iron chlorosis. J. Maharashtra Agricult. Univ. **12** (3), 385-386.

POUGET, R.; 1974: Influence des réserves glucidiques sur l'intensité de la chlorose ferrique chez la vigne. Conn. Vigne Vin **8**, 305-314.

– – ; 1980: Breeding grapevine rootstocks for resistance to iron chlorosis. Proc. 3rd Intern. Symp. Grape Breeding, Davis, California, 191-197.

– – ; JUSTE, C.; 1972: Le choix des porte-greffes de la vigne pour les sols calcaires. Conn. Vigne Vin **6**, 357-364.

– – ; OTTENWAELTER, M.; 1973: Etude méthodologique de la résistance à la chlorose calcaire chez la vigne: principe de la méthode des greffages réciproques et application à la recherche de porte-greffes résistants. Ann. Amélior. Plantes **23**, 347-356.

ROMERA, F. J.; ALCANTARA, E.; DE LA GUARDIA, M. D.; 1989 a: Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution. I. Effect of bicarbonate. 5th Intern. Symp. Iron Nutrition and Interaction in Plants, Kibbutz Ramat-Rachel, Israel, 11-17 June 1989.

– – ; – – ; – – ; 1989 b: Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution. II. Response mechanisms. 5th Intern. Symp. Iron Nutrition and Interaction in Plants, Kibbutz Ramat-Rachel, Israel, 11-17 June 1989.

RÖMHELD, V.; 1987: Different strategies for iron acquisition in higher plants. Physiol. Plant. **70**, 231-234.

– – ; MARSCHNER, H.; 1981: Iron deficiency stress induced morphological and physiological changes in root tips of sunflower. Physiol. Plant **53**, 354-360.

– – ; – – ; 1986: Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. Plant Physiol. **80**, 175-180.

STRAIN, H. H.; SVEC, W. A.; 1966: Extraction, separation, estimation, and isolation of the chlorophylls. In: VERNON, L. P.; SEELY, G. R. (Eds.): The Chlorophylls, 21-66. Academic Press, London.

TAKAGI, S.; NOMOTO, K.; TAKEMOTO, T.; 1984: Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. J. Plant Nutr. **7**, 469-477.

TORRECILLAS, A.; LEON, A.; DEL AMOR, F.; MARTINEZ-MOMPEAN, M. C.; 1984: Determinaciòn rapida de clorofila en discos foliares de limonero. Fruits **39**, 617-622.

Use of differential thermal analysis to quantify bud cold hardiness of grape selections and clones [1])

R. M. Pool, B. I. Reisch, M. J. Welser [2])

Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, New York, 14456, USA

Summary: Differential thermal analysis (DTA) was used to characterize primary bud mid-winter cold hardiness of *Vitis* spp. Bud hardiness reached a maximum and was rather stable during the months of January and February at Geneva, New York. Because cold tolerance increases during periods of prolonged cold, observed freezing temperature was adjusted on the basis of the freezing temperature of cv. Concord on the day of observation. DTA gives reproducible and meaningful estimates of bud freezing temperature. Such data account for at least 50 % of the among-cultivar variance in overall vine cold hardiness.

Key words: cold resistance, analysis, bud, variety of vine, clone, USA.

Introduction

Winter cold establishes the northern limit of wild grapes as well as that of many other genera (Burke and Stushnoff 1979). The northern limit is approximately coincident with the latitude at which the homogeneous ice nucleation temperature can be expected (George *et al.* 1974). Genera which have this northern limit frequently share a freeze avoidance mechanism to endure winter cold. In such species, when critical tissues freeze, they die. Differential thermal analysis (DTA) detects the temperature at which tissues freeze by detecting the heat of fusion which is released when liquid water becomes ice. DTA has been used to measure the killing temperature of *Vitis*. Various claims have been made regarding the relevance of DTA signals to cold damage of cane tissues (Pierquet and Stushnoff 1980; Barney 1987), but there is general agreement that DTA can accurately estimate the temperature at which dormant grape buds are killed. We have developed a micro-computer assisted DTA apparatus to estimate the freezing temperature of excised grape buds (Wolf and Pool 1986).

With grapes as with other perennial fruits, progress in breeding for polygenetically controlled traits becomes more certain when the trait in question can be accurately and objectively measured. This is especially true of winter cold hardiness. Traditionally vine hardiness is evaluated by noting the damage sustained in the field during the winter. Such estimates are difficult to interpret because field survival is subject to many influences. The 'dosage' of winter cold is not uniform from year to year nor is it uniform within a given site. Cold hardiness is influenced by both viticultural and meteorologic factors. Field hardiness may differ greatly from potential hardiness because of differences in yield or growth during the previous summer or because of the impact of mid-winter climate. Prolonged sub-freezing temperature increases cold hardiness while mid-winter thaws sometimes reduces it. DTA reduces the impact of at least some of these variables. Impact of vine-to-vine variation can be reduced by sampling mature wood within the vine (Howell and Shaulis 1980) and meteorologic influences can be accounted for because hardiness is measured on a specific day rather than observing the impact of an entire winter.

To test the usefulness of DTA for grape breeding programs, we have used the technique to study the killing temperature of a wide variety of grape germplasm.

---

1) Research supported in part by grants from the New York State Wine and Grape Foundation and the U.S. Department of Agriculture.

2) Respectively, Professor of Viticulture, Associate Professor of Grape Breeding and Staff Support Specialist, Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, New York, 14456, USA

Materials and methods

Differential thermal analysis

The basic apparatus has been described (WOLF and POOL 1986). Heat of fusion is detected by mounting excised buds containing a small amount of attached cane tissue on a thermoelectric (TE) module (Melcor™, Trenton, New Jersey). TE modules are attached to an analogue to digital acquisition board through a digital relay device which allows multiple plates to be connected. The plates are placed in a freezer and cooled at a constant rate using the micro-computer to control the temperature of a heat sink. For our standard tests, 6 replicate buds were excised from nodes 4-6 of well matured canes. They were mounted on dampened paper which in turn was placed on a TE module. After 1 h at −2 °C, the temperature was lowered at 3 °C/h. Exotherms were detected by plotting the TE signal against the heat sink temperature. The high temperature exotherm (HTE), which was caused by extracellular ice, was induced between −3 and −7 °C by seeding a moist cotton string in contact with the paper mount with ice. Low temperature exotherms (LTE's) caused by primary buds were 2-10 times larger than other LTE's. The temperature at which the median primary bud LTE per plate was recorded (if an even number of LTE's were found, the mean of the temperature of the two central LTE's was used). Most often three replicate modules were used per test, but sometimes only duplicates were run and four replicates were used to derive the seasonal curves.

Seasonal changes in cold hardiness

DTA was done at least bi-weekly during the months of September to April for 3 consecutive years (1976/78, 1977/78 and 1978/79) on three cultivars: Concord, White Riesling and Cabernet Sauvignon. Median LTE's were obtained and the data plotted. These data were used to determine the period of maximum hardiness. The data for Concord was used to standardize observed LTE temperature values in other experiments so as to account for variations cause by continuous freezing (PROEBSTING and ANDREWS 1982).

DTA of grape cultivars and species accessions

A test of American hybrid table grape cultivars has been underway at Geneva, New York, since 1978. It consists of both named cultivars of North American origin and selections from the

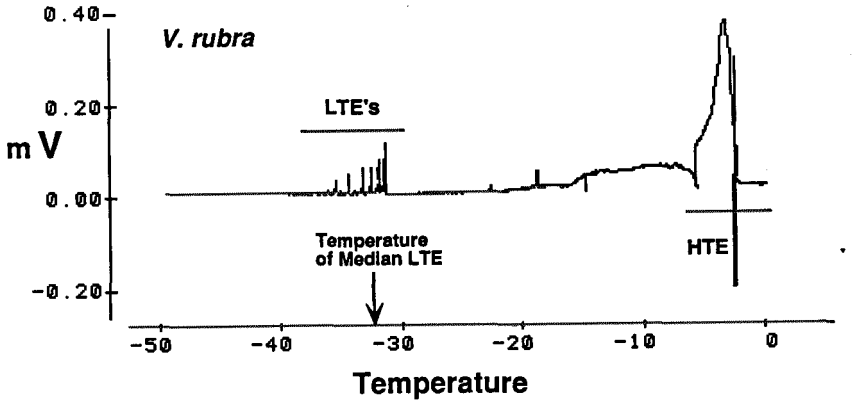


Fig. 1: DTA data for six replicate buds of *Vitis rubra* mounted on a TE module.

Geneva grape breeding program. During the months of January and February 1987 three replicate samples of 6 buds were subjected to DTA. Concord was used as a standard, winter hardy cultivar.

During January and February of 1978 median LTE temperature of cultivars and clones of cultivars of *V. vinifera* L. growing at the New York State Agricultural Experiment Station vineyards were estimated using three replicate 6 bud samples. These data were compared with an overall hardiness rating for these varieties growing at Geneva. Estimates for Pinot noir clone LTE temperatures were compared to data on field survival following the winters of 1987/88 (little cold stress) and 1988/89 (severe cold injury to Pinot noir). During January and February of 1980 duplicate samples of wine and table grape interspecific hybrid cultivars and accessions of *Vitis* species which are part of the collection of the National Apple and Grape Clonal Repository at Geneva, New York, were measured.

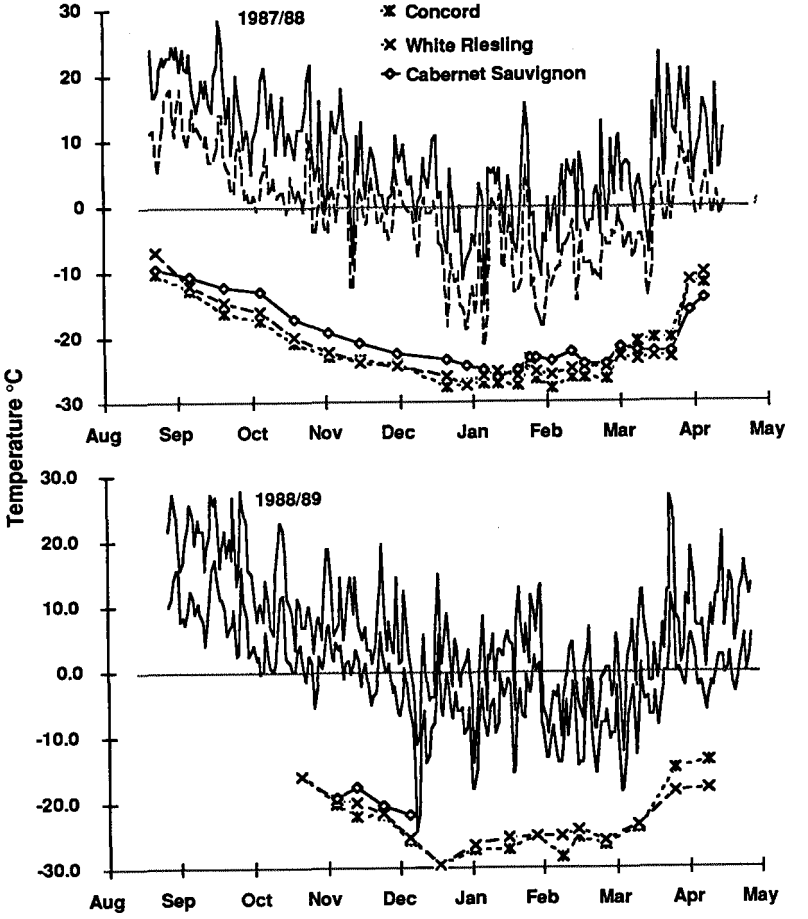


Fig. 2: Daily maximum and minimum temperatures and median LTE temperatures for three cultivars, Cabernet Sauvignon, White Riesling and Concord, growing at Geneva, New York, for the winter seasons 1987/88 and 1988/89.

Results

Seasonal changes in cold hardiness

Fig. 1 shows a DTA profile of 6 nodes from a selection of *V. rubra*. The median LTE of this sample is −32.5 °C. The occasional spikes found at higher temperatures are thought to be due to drops of water on the plate or to the freezing of previously killed buds.

The seasonal cold hardiness of the cultivars Concord, White Riesling and Cabernet Sauvignon for the years 1987/88 and 1988/89 are shown in Fig. 2. The data for Cabernet Sauvignon hardiness for 1988/89 stop after December 12, 1989. At that time the curve of minimum temperature intersected the cold hardiness curve for Cabernet Sauvignon. As a result primary bud kill exceeded 95 % and no further data were collected for that cultivar that year. The observed pattern of hardiness was not only found in 3 years of sampling at Geneva, but also with samples obtained in other states using the same cultivars and protocol. Three stages of hardiness can be seen. Acclimation begins at about the time visible periderm forms and continues until late December when maximum hardiness is reached. January and February is a period of hardiness maintenance. During this time hardiness, especially that of the cultivar Concord, is increased when the maximum daily temperatures do not exceed 0 °C for more than a few days. This has been observed before (ANDREWS *et al.* 1984), and for that reason data for other cultivars are reported in two ways, observed and adjusted. Adjustments are based on the deviation of Concord hardiness from the period mean median LTE for the year. At the end of February all cultivars begin to de-acclimate. Shortly before bud burst the buds lose their ability to form LTE's. In every year identical differences were noted among cultivars. White Riesling and Concord begin to acclimate early and reach maximum hardiness before Cabernet Sauvignon does. Concord de-acclimates earlier and more rapidly than do White Riesling and Cabernet Sauvignon.

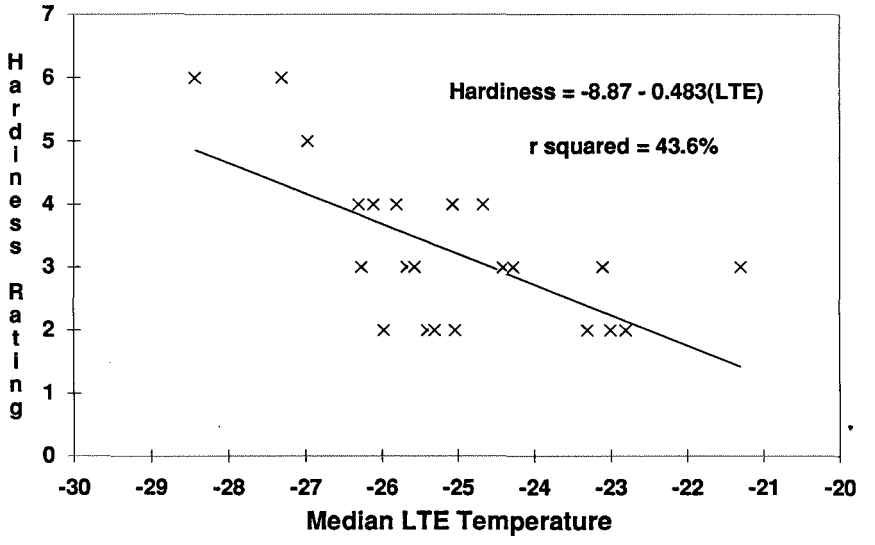


Fig. 3: Relationship between median LTE temperature and field cold hardiness ratings for *Vitis vinifera* and 2 *V. labruscana* cultivars. Each point is a different cultivar. Hardiness ratings range from 1 = very cold tender to 6 = very cold hardy at Geneva, New York.

Table 1: Hardiness of 22 *Vitis vinifera* and 2 *V. labruscana* cultivars as measured by DTA or field evaluation

Variety	Date Sampled	Mean LTE	Adjusted LTE[y]	Field Hardiness Rating[z]
Muscat Ottonel	22-Jan	-21.3	-21.9	3
Portugieser Blau	27-Jan	-22.8	-23.5	2
Reichensteiner	9-Feb	-22.8	-22.1	2
Merlot	13-Jan	-23.0	-22.9	2
Ehrenfelser	22-Jan	-23.1	-23.8	3
Sauvignon blanc	9-Feb	-23.3	-22.6	2
Pinot gris	27-Jan	-24.3	-25.0	3
Gewurztraminer	15-Jan	-24.4	-24.3	3
Nobelssa	27-Jan	-24.4	-25.1	3
Comtessa	15-Jan	-24.7	-24.6	4
Metternich	15-Jan	-25.0	-24.9	2
Limberger	22-Jan	-25.1	-25.8	3
Optima	13-Jan	-25.3	-25.2	2
Sylvaner	13-Jan	-25.4	-25.3	2
Scheurebe	9-Feb	-25.6	-24.7	3
Baccus	9-Feb	-25.7	-24.8	3
Siegerrebe	29-Jan	-25.8	-25.4	4
Perle	15-Jan	-26.0	-25.9	2
Rieslaner	29-Jan	-26.1	-25.7	4
Morio Muscat	22-Jan	-26.3	-27.1	3
Melon	13-Jan	-26.3	-26.2	4
Cabernet franc	29-Jan	-27.0	-26.6	5
Niagara	27-Jan	-27.3	-28.1	6
Delaware	13-Jan	-28.4	-28.3	6

y. Adjusted by standardizing to the LTE for Concord on the date of sampling.

z. Hardiness field ratings 1 = very tender 6 = very cold hardy

Evaluation of *Vitis vinifera* cultivars

Table 1 lists the observed and adjusted temperature of the median LTE of 22 cultivars of *V. vinifera* and 2 *V. labruscana* cultivars. An evaluation of overall field hardiness is also given. The 2 *V. labruscana* cultivars had the lowest observed and adjusted LTE temperatures and the highest hardiness rating. The LTE temperature data were regressed against the hardiness rating (Fig. 3). A highly significant relationship was obtained ($P = 0.01$) but the relationship explained only 43.6% of the variance. Field survival data and 1987/88 estimates of the LTE temperatures were obtained for 8 Pinot noir clones (Fig. 4). In 1987/88 the low temperatures did not approach that of the median LTE. There was severe primary bud injury on December 12, 1988, when temperatures ranged from −24.5 to −25.3 °C in the vineyard. At this time the vines were not fully acclimated and field injury ranged from 32 to 85 % barren nodes in 1989. Generally the observed injury related well with the LTE estimates. A major exception was the Clevner Mariafeld clone which had the lowest LTE temperature and the greatest bud injury.

Table 2: Observed and adjusted median LTE temperatures for interspecific American table grape selections and cultivars

	Observed Temperature of the LTE	Adjusted Temperature of the LTE[z]
Seedless Selections[y] and cultivars		
NY 65.479.1	-23.5	-22.7
NY 46290	-23.0	-23.1
Suffolk Red	-23.3	-23.1
NY 65.143.1	-23.2	-23.3
Lakemont	-24.1	-23.9
NY 65.483.2	-25.2	-24.3
Canadice	-24.6	-24.5
Interlaken	-24.8	-24.6
Reliance	-24.9	-24.8
Remaily Seedless	-24.6	-25.2
Einset Seedless	-25.2	-25.6
Mars	-25.7	-25.8
NY 65.077.2	-25.1	-26.0
Himrod	-25.5	-26.1
NY 63.878.6	-25.3	-26.2
Seeded Selections[y] and cultivars		
Alden	-23.6	-23.7
Yates	-25.4	-24.5
Buffalo	-25.6	-24.7
Steuben	-24.8	-24.9
NY Muscat	-25.6	-25.0
NY 65.112.1	-25.3	-25.4
Concord	-25.5	-25.5
Seneca	-25.2	-25.5
Price	-27.4	-26.5
Swenson Red	-27.7	-27.0
Alwood	-28.4	-27.4
Bath	-28.7	-27.7
Sheridan	-29.0	-28.0

[y]. Parentage

NY 46290	(Buffalo X Himrod)
NY 63.878.6	(Fredonia X Canner)
NY 65.077.2	(Vineland 52084 X Ruby Seedless)
NY 65.112.1	Vineland 52082 X Flame Tokay
NY 65.143.1	(Dunstan 210 X NY 45945 (Athens X NY 33873)
NY 65.479.1	((Muscat Hamburg X Hubbard) X (Ontario X Black Monukka))
NY 65.483.2	((NY 10782 X Muscat Hamburg) X (Suffolk Red(Ontario X Black Monukka))

[z]. LTE temperature adjusted to reflect value of Concord on day of measurement.

Evaluation of table grape cultivars and selections

Seedless cultivars and selections LTE temperatures generally were no higher than those of seeded cultivars (Table 2). 6 of the seedless cultivars (NY 65.479.1, Suffolk Red, NY 6290,

NY 65.143.1, Lakemont and NY 65.483) were more than 1 °C less hardy than the standard, Concord. The others had LTE temperatures within 1 °C of Concord. Seeded cultivars, Yates and Alden, which are rated as only moderately hardy were more than 1 °C less cold hardy than Concord. Price, Swenson Red, Alwood, Bath and Sheridan were all 1 °C or more hardy than Concord. Sheridan and Bath were more than 2 °C hardier than Concord.

Germplasm evaluation

Germplasm tested from the repository was divided into 6 categories (Table 3). In order of increasing mean hardiness they were: American *(V. labruscana)* seedless cultivars, American wine cultivars, American seeded table cultivars, interspecific wine cultivars, species selections and rootstock cultivars. The order of mean hardiness by category is not very important as the samples do not necessarily represent the range of germplasm in a category and because differences within category, which ranged from 1.4 to 12.9 °C, greatly exceeded the range of means among the categories which was only 1.6 °C. There were 5 American wine selections which ranged from −24.0 for cv. Wine King to −25.5 °C for cv. Concord. Most of the American seeded table cultivars had median LTE's within one degree of that of Concord. The exceptions were Yates and Alwood which were less hardy and Bath, Century I and Price which were more hardy than Concord. Century I and Price have been rated as only moderately cold hardy in field tests at Geneva. With the exception of the cultivar of southern origin, Venus, the American seedless cultivars were close to the value for Concord, more recently released cultivars were more cold tolerant than older ones. Only a few interspecific wine cultivars were tested. The most widely planted of these in New York state is Seyval (S. V. 5-276) which had the highest median LTE temperature of the group, 1.5 °C higher than that of Concord. The other cultivars, including the wine cultivar most recently released by the New York State Agricultural Experiment Station - Melody (Reisch *et al.* 1986) - were equal to or more hardy than Concord.

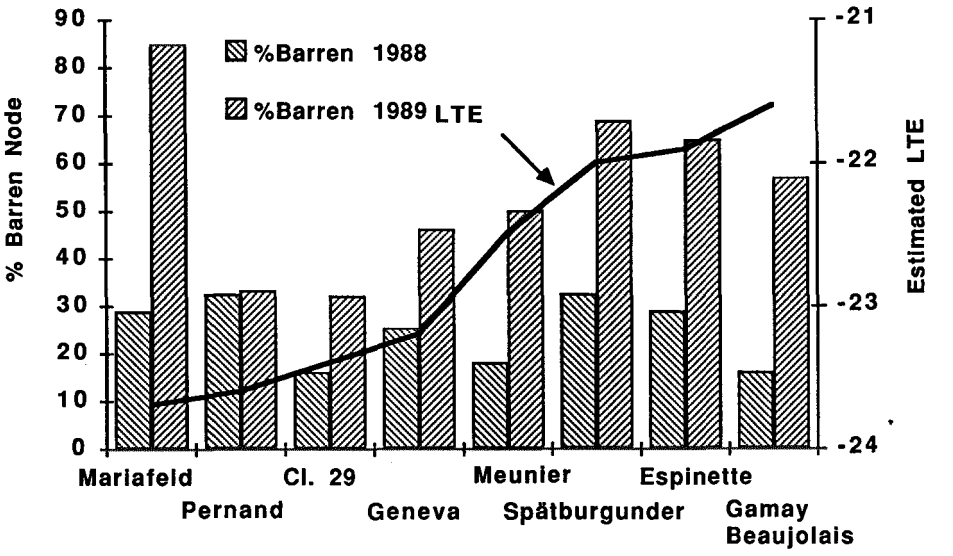


Fig. 4: Observed median LTE temperature and observed 1988 and 1989 barren node percentage for Pinot noir clones growing at Geneva, New York.

Table 3: Temperature of the median LTE of cultivars and species accessions growing in the collection of the National Apple and Grape Repository, Geneva, New York

Category	Plant Name	Repository Designation	Temperature of the Median LTE	Adjusted Temperature of the Median LTE[z]
American	Wine King	131	-23.2	-24.0
Wine or Juice	Carmen	588	-23.3	-24.1
	Diamond	124	-24.0	-24.8
	Delaware	52	-26.6	-25.0
	Concord	51	-25.6	-25.5
	Mean		-24.5	-24.6
American Table	Yates	113	-24.6	-22.9
	Alwood	*	-22.2	-24.0
	Swenson Red	439	-25.9	-24.2
	Stueben	111	-26.7	-25.1
	Ontario	45	-24.6	-25.4
	Golden Muscat	*	-24.0	-25.8
	McCampbell	44	-24.4	-26.3
	Bath	109	-24.9	-26.7
	Century 1	985	-26.3	-27.1
	Price	98	-26.7	-28.6
	Mean		-24.8	-25.6
American Seedless	Venus	573	-22.4	-20.8
	Himrod	*	-24.1	-24.0
	Glenora	*	-22.7	-24.5
	Einset Seedless	470	-24.9	-26.7
	Mean		-23.5	-24.0
Interspecific Wine	Seyval	534	-21.3	-23.0
	Seibel 2583	339	-21.3	-23.1
	Seibel 1077	535	-22.0	-23.8
	Melody	581	-27.5	-25.8
	Ravat 34	354	-24.0	-25.8
	Seibel 880	559	-24.3	-26.1
	Chancellor	43	-25.4	-27.1
	Mean		-23.7	-24.9
Rootstock	C157-11	979	-24.1	-21.8
	Mill. et Grasset 219A	598	-24.3	-22.0
	Rup. du Lot	592	-22.7	-22.5
	Mill. et Grasset 125-1	606	-23.0	-23.3
	Teleki 5C	79	-23.2	-23.7
	Richter 110	266	-23.5	-23.9
	Kober 5BB	70	-23.6	-24.1
	C 18-815	118	-23.7	-24.2

Table 3 (Cont.)	Mill. et Grasset 420	605	-26.2	-24.3
	SO4	119	-24.6	-25.1
	R. gloire	265	-24.8	-25.3
	SORI	95	-25.1	-25.6
	C 3309	87	-25.5	-25.9
	C 1616E	114	-26.2	-26.7
	C 3306	264	-27.1	-27.6
	Mill. et Grasset 101-14	63	-27.5	-28.0
	Azita	263	-25.1	-28.5
	Shakoka	73	-28.5	-29.0
	Sonona	152	-31.2	-34.7
	Mean		-24.9	-25.6
Species	V. cinerea (C66-6)	625	-22.9	-20.6
	V. berlandieri	261	-17.1	-20.6
	V. cordifolia (Rem 30-77)	1013	-24.0	-21.7
	V. rubra (Ru-66-10)	168	-24.5	-21.7
	V. argentifolia	1003	-22.3	-22.1
	V. riparia (Quebec)	612	-24.6	-22.3
	V. champini (Salt Creek)	622	-22.3	-22.8
	V. argentifolia(Rem 46-77)	970	-25.2	-22.9
	V. labrusca (Rem 26-75)	1023	-22.7	-23.0
	V. argentifolia	214	-21.4	-23.1
	V. riparia (Manitoba)	401	-25.1	-23.4
	V. labrusca (Rem 33-75)	967	-23.5	-23.8
	V. labrusca (Rem 43 -75)	1029	-23.6	-23.9
	V. cordifolia(B17)	171	-27.1	-24.3
	V. rupestris (Ganzin)	285	-26.7	-24.4
	V. riparia (Minnesota)	400	-26.2	-24.5
	V. argentifolia(Rem NE 19)	896	-26.9	-24.6
	V. cinerea (C66-14)	236	-27.0	-24.7
	V. riparia (Montreal)	193	-26.4	-24.7
	V. cordifolia(B18)	184	-27.8	-25.0
	V. labrusca (Rem 46-75)	1026	-24.8	-25.1
	V. longii	1026	-24.9	-25.2
	V. coignetiae (Pulliat)	18	-27.9	-25.6
	V. cinerea	170	-25.2	-25.7
	V. rubra	239	-25.9	-25.7
	V. argentifolia(Rem NE 4)	994	-28.1	-25.9
	V. riparia (Montana)	417	-27.8	-26.1
	V. rupestris (Pillans)	202	-24.2	-26.2
	V. champini	172	-24.6	-26.3
	V. riparia (Montana)	418	-29.2	-27.5
	V. rubra	174	-24.1	-27.5
	V. riparia (Colorado)	773	-29.2	-27.5
	V. andersonii	701	-27.8	-28.3
	V. riparia (Pulliat)	224	-31.9	-29.1

Table 3 (Cont.)

V. riparia (Montreal)	193	-25.6	-29.1
V. solonis	158	-29.1	-29.6
V. argentifolia	928	-27.2	-30.6
V. rupestris(Tiefenback)	249	-29.2	-32.7
V. longii	138	-29.3	-32.8
Mean		-25.7	-25.4

The range in rootstock hardiness was greater than observed for cultivars grown for fruit. 9 rootstock cultivars had median LTE's more than 1 °C higher than Concord. They included Rupestris du Lot, Teleki 5C, Richter 110 and Kober 5BB. SO 4, Riparia Gloire, Sori and C. 3309 had LTE values similar to that of Concord. C. 1616E, C. 3306, M. GT 101-14 were hardier than Concord and 2 rarely used rootstocks which share northern *V. riparia* parents, Shakoka and Sonona, were more than 3 °C hardier than Concord.

The range in median LTE temperature was much greater among species selections (14.5 °C) than among the cultivars. Essentially all of the fruiting cultivars were of northern origin. Among the species, with a few exceptions, those which were much less hardy than Concord shared a southern origin. A *V. riparia* selection from Quebec, Canada was more than 3 °C less hardy than Concord, but these plants have symptoms suggesting a virus infection. There were also some other northern selections such as the *V. labrusca* selection Remaily 26-75 and the *V. argentifolia* selection Remaily 46-77 which were less hardy than the standard, Concord. In terms of very hardy material *V. riparia* dominated, but some southern representatives had cold hardy buds. They included *V. solonis* and the *V. rupestris* selection Tiefenback.

Discussion

The data on seasonal fluctuations in median LTE temperature of primary buds show that the changes in hardiness are reproducible among years and cultivars. When the field temperature fell below the predicted hardiness level, Cabernet Sauvignon buds died. This confirms that the data are meaningful. The fluctuation in temperature of the median LTE observed during the period of maximum hardiness in the 3 years is clearly related to prolonged freezing of non-vital bud tissues which lowers the LTE temperature (Pool *et al.* 1985). This variability associated with mid-winter conditions is the reason that the observed LTE temperatures need to be adjusted to reflect the status of the standard cultivar, Concord.

The data with standard wine and grape cultivars demonstrates both the validity and the limit of the technique in predicting field cold hardiness. LTE temperature accounted for only 43 % of the variance associated with our field ratings. The lack of agreement can be ascribed to several factors. First our ratings tend to be more conservative for cultivars with which we have had little experience. An example is the cultivar Morio Muscat which had hardy buds, but only a moderate hardiness rating. With more years of experience, we may well revise the field rating of Morio Muscat. A second factor is that before DTA can be used, the buds must be 'mature'. The physiological factors responsible for the 'mature' node condition are poorly understood, but it is clear that the buds of many cultivars poorly adapted to New York fail to develop the ability to supercool. Such buds die at a temperature higher than −8 °C. DTA is only suitable to measure hardiness of the 'mature' bud fraction and thus DTA may overestimate hardiness for varieties which do not reliably produce buds capable of supercooling. A third complication is that we measure maximum mid-winter bud hardiness with DTA. A cultivar like Cabernet Sauvignon, in which acclimation is delayed, may sustain early season cold injury before its buds become fully hardened. The final factor that causes bud LTE temperature to sometimes differ from field hardiness is the tissue evaluated. Some

cultivars such as Perle, Century I and Price form cold hardy buds, but often fail to produce cold hardy trunks. That produces a situation in which the buds may survive, but the trunk injury is so severe that the above ground portions of the vine die.

While DTA cannot give a complete assessment of cold hardiness, it does give reproducible results concerning bud hardiness. The data produce good agreement between assessments made in different years except for three cases. Himrod, Alwood and Swenson Red produced lower median LTE temperatures in the table grape assessment than in that of the repository collection. This may have been due to the age and size of the vines in question. For the table grape assessment many mature vines were available from which to select canes. In the repository collection most of the vines were only 1 year old and only duplicate vines are planted. Thus, we may have inadvertently selected less hardy wood from the smaller population of canes.

The results of the DTA assessments of the repository collection are very interesting. Seyval is one of the most widely planted interspecific hybrid wine cultivars in New York, but its buds were not very hardy. It has been observed that primary buds of Seyval are frequently killed in the field, but that its fruitful secondary and base buds allow an adequate crop to be produced (Pool *et al.* 1978). Similarly the rootstock data related well to planting recommendations for New York (Lider and Shaulis 1974). None of the rootstock cultivars which had higher LTE temperatures than Concord are recommended for use in New York vineyards. The data for species was of course most variable. In general, southern species had higher LTE temperatures than did those from northern locations. However, several of the southern species produced very hardy buds. The 'southern' distribution of species like *V. rupestris* and *V. berlandieri* does not mean that these cultivars are not exposed to very low winter temperatures. Frequently their failure to tolerate northern winters seems to relate to their adaptation to regions with long summers rather than to regions with warm winter temperatures. Such vines will often fail to mature their wood or buds in the short growing season of northern New York state.

DTA appears to be a very useful tool for the grape breeder. While it will not measure overall vineyard hardiness, it will precisely measure the mid-winter freezing point of grape buds. Thus it produces the kind of information grape breeders require to plan their crossing strategies and to make objective assessments of their progenies. We plan to complete the evaluation of the collection of the National Apple and Grape Clonal Repository. The data will become part of the grape descriptors available on the Genetic Resources Inventory Network (GRIN), a plant information database operated by the United States Department of Agriculture.

Literature cited

Andrews, P. K.; Sandidge, C. R. III; Toyama, T. K.; 1984: Deep supercooling of dormant and deacclimating *Vitis* buds. J. Amer. Soc. Enol. Viticult. **35**, 175-177.

Barney, D. L.; 1987: An investigation of freezing injury in dormant, acclimated *Vitis* canes. PhD Thesis, Cornell Univ., Ithaca, N.Y.

Burke, M. J.; Stushnoff, C.; 1979: Frost hardiness: A discussion of the possible molecular causes of injury with particular reference to deep supercooling of water. In: Mussel, H.; Staples, R. C. (Eds.): Stress Physiology in Crop Plants, 199-226. John Wiley and Sons, New York.

George, M. F.; Burke, M. J.; Pellet, H. M.; Johnson, A. G.; 1974: Low temperature exotherms and woody plant distribution. HortScience **9**, 519-522.

Howell, G. F.; Shaulis, N. J.; 1980: Factors influencing within-vine variation in the cold resistance of cane and primary bud tissues. J. Amer. Soc. Enol. Viticult. **31**, 158-161.

Lider, L. A.; Shaulis, N. J.; 1974: Resistant rootstocks for New York vineyards. NY Food Life Sci. Bull. no. 45.

Pierquet, P.; Stushnoff, C.; 1980: Relationship of low temperature exotherms to cold injury in *Vitis riparia* Michx. J. Amer. Soc. Enol. Viticult. **31**, 1-6.

Pool, R. M.; Pratt, C.; Hubbard, H. D.; 1978: Structure of base buds in relation to yield of grapes. J. Amer. Soc. Enol. Viticult. **29**, 36-41.

– – ; WOLF, T.; WELSER, M. J.; 1985: Dormant season cold hardiness of five grape cultivars growing in New York as assessed by differential thermal analysis [Abstr.]. HortScience **20**, 531.

PROEBSTING, E. L. Jr.; ANDREWS, P. K.; 1982: Supercooling and *Prunus* flower bud hardiness. In: LI, P. H.; SAKAI, A. (Eds.): Plant Cold Hardiness and Freezing Stress, 529-539. Academic Press, New York.

REISCH, B. I.; POOL, R. M.; WATSON, J. P.; ROBINSON, W. B.; COTTRELL, T. H. E.; 1986: 'Melody' grape. HortScience **21**, 158-159.

WOLF, T. K.; POOL, R. M.; 1986: Microcomputer-based differential thermal analysis of grapevine dormant buds. HortScience **21**, 1447-1448.

Winter frost resistance of grapevine varieties belonging to different ecological and geographical groups

D. Zunic, L. Avramov and N. Todorovic

Faculty of Agriculture, Nemanjina 6, YU-11081 Zemun, SFR Yugoslavia

Summary: The influence of frost temperatures on survival of the buds was investigated *in situ* during 3 winters. The behavior of 375 grapevine varieties belonging to different ecological-geographical groups was studied at 3 locations.

The rate of buds killed by frost ranged from 5.4 to 100 %. The varieties of the group convar. *occidentalis* exhibited the greatest frost resistance of buds during 3 winters with very low temperatures. In this group the percentage of killed buds was significantly lower than in the group convar. *pontica* and much less than in the group convar. *orientalis*.

Key words: cold resistance, winter frost, bud, variety of vine, convarietas, location, year, climate, Yugoslavia.

Introduction

Many factors influence the resistance to low winter temperatures which has been shown by many authors (Lazic and Zorzic 1956; Kondo 1959; Pogosian 1967; Nagru 1971; Ratkovic 1979; Turmanidze 1981; Cindric 1984; Avramov and Zunic 1986; Avramov *et al.* 1987; Milosavljevic *et al.* 1987; Tadijanovic *et al.* 1988, and others).

Starting from the existing knowledge about the resistance of grapevine to low winter temperatures, we report the results from an *in situ* investigation on the response of different grapevine varieties to low temperatures during the period from 1984 to 1987 in the areas where ampelographic collections are located.

Materials and methods

The investigations were performed at 3 locations with existing ampelographic collections. The number of varieties was different in each collection. At 'Radmilovatz', 'Svetozarevo' and 'Bela Crkva' 286, 73 and 34 varieties, respectively, were investigated.

The age of the plants was different. The number of developing and non-developing buds was checked *in situ* after winters with extremely low temperatures.

Analysis of variance was utilized to determine significant differences between the percentages of killed buds in the same varietal group as well as between the different varietal groups taking into consideration the year and location.

Also checked was the significance of correlations between the temperature limits (0, -10, -15, -20 and -25 °C) and the degree of killed buds for each of the different groups of varieties. The dependency is presented by linear regression.

During the 3 years of our investigation, extreme air temperatures were recorded daily from October to April. We also must note that during the winter 1986/87 at the location 'Svetozarevo' an extraordinarily low temperature (-32 °C) was recorded for several hours.

Results and discussion

For clarity the results are presented graphically (Figs. 1, 2 and 3). We analyzed and presented examples only for 5 varieties which are considered models for their ecological-geographical groups (Tables 1, 2 and 3).

a) Group convarietas *orientalis*

The varieties of this group – Dattier of Beyrouth, Red Adacalca, Chirey Bayan, Hindogni and Tavriz – at all 3 locations during the 3 years of the investigation had very high average percentages of killed buds. The results of investigations are summarized in Table 1; the data show:

- During the winter 1986/1987 in all varieties of this group the highest percentages of killed buds were determined at all 3 locations.
- Statistically significant differences between the varieties of this group for the 3 years at individual locations were not determined.
- At the different locations in the same year, the same varieties exhibited significant differences in the percentages of killed buds. This can be explained by the different low temperature extremes and different times of exposure to these temperatures.

Behavior similar to that of the chosen examples was observed with the other varieties of this ecological-geographical group (Fig. 1).

b) Group convarietas *pontica*

The varieties of this group – Bagrina, Plovdina, Prokupatz, Skadarka and Vranatz – exhibited a middle-high average percentage of killed buds. The results of investigations are summarized in Table 2; they show:

- The lowest average percentages of killed buds were determined at 'Radmilovatz' in 1985/1986 and at 'Bela Crkva'in 1984/1985.
- In the varieties of this group at the same locations and years of investigations, significant differences were determined. The variety Vranatz exhibited a significantly higher percentage of killed buds than the other varieties of this group.
- Between the locations and the years of the investigations statistical differences were apparent by analysing the percentage of killed buds.
- All buds in this group of varieties were completely killed during the winter 1986/1987 at the location 'Svetozarevo'.
- At all locations the varieties exhibited the highest percentage of killed buds during the winter 1986/1987.
- Comparing the varieties of the group convar. *orientalis* with the varieties of the group convar. *pontica*, the latter were found to have few killed buds after winters with extremely low temperatures at almost every lcoation investigated. From the varieties of the group convar. *pontica*, only Vranatz is similar to the varieties of the group convar. *orientalis*.

Behavior similar to that of the chosen examples was observed with the other varieties of this ecological-geographical group (Fig. 2).

c) Group convarietas *occidentalis*

The varieties of this group – Cabernet Sauvignon, Cabernet franc, Pinot noir, White Riesling and Italian Riesling – exhibited the lowest average percentage of killed buds taking into consideration the locations and years investigated, compared with the varieties of the groups convar. *orientalis* and convar. *pontica*. The results of the investigations which are summarized in Table 3 show the following:

- In the varieties of the group convar. *occidentalis* the percentage of killed buds was from 5.4 % (Cabernet Sauvignon) to 32.1 % (Italian Riesling).

Table 1: The influence of low winter temperatures on the average percentages of killed buds in varieties of convarietas *orientalis*

Location/Year	V a r i e t i e s				
	Dattier of Beyrouth %	Red Adacalca %	Shirey Bayan %	Hindogni %	Tavriz %
1.Location:"Radmilovatz"					
1984/1985	37,94	26,74	26,22	22,65	23,78
1985/1986	62,o5	65,18	64,9o	64,o2	68,97
1986/1987	9o,38	89,83	96,o6	87,78	88,o7
2.Location:"Svetozarevo"					
1984/1985	61,66	48,32	59,7o	56,4o	52,oo
1985/1986	24,3o	25,13	48,3o	32,o8	3o,4o
1986/1987	loo,oo	loo,oo	loo,oo	loo,oo	loo,oo
3.Location:"Bela Crkva"					
1984/1985	62,36	56,o2	61,o2	6o,o8	68,9o
1985/1986	--	--	--	--	--
1986/1987	82,4o	8o,3o	76,4o	69,4o	65,4o

$LSD_{o,ol}$

$LSD_{o,o5}$

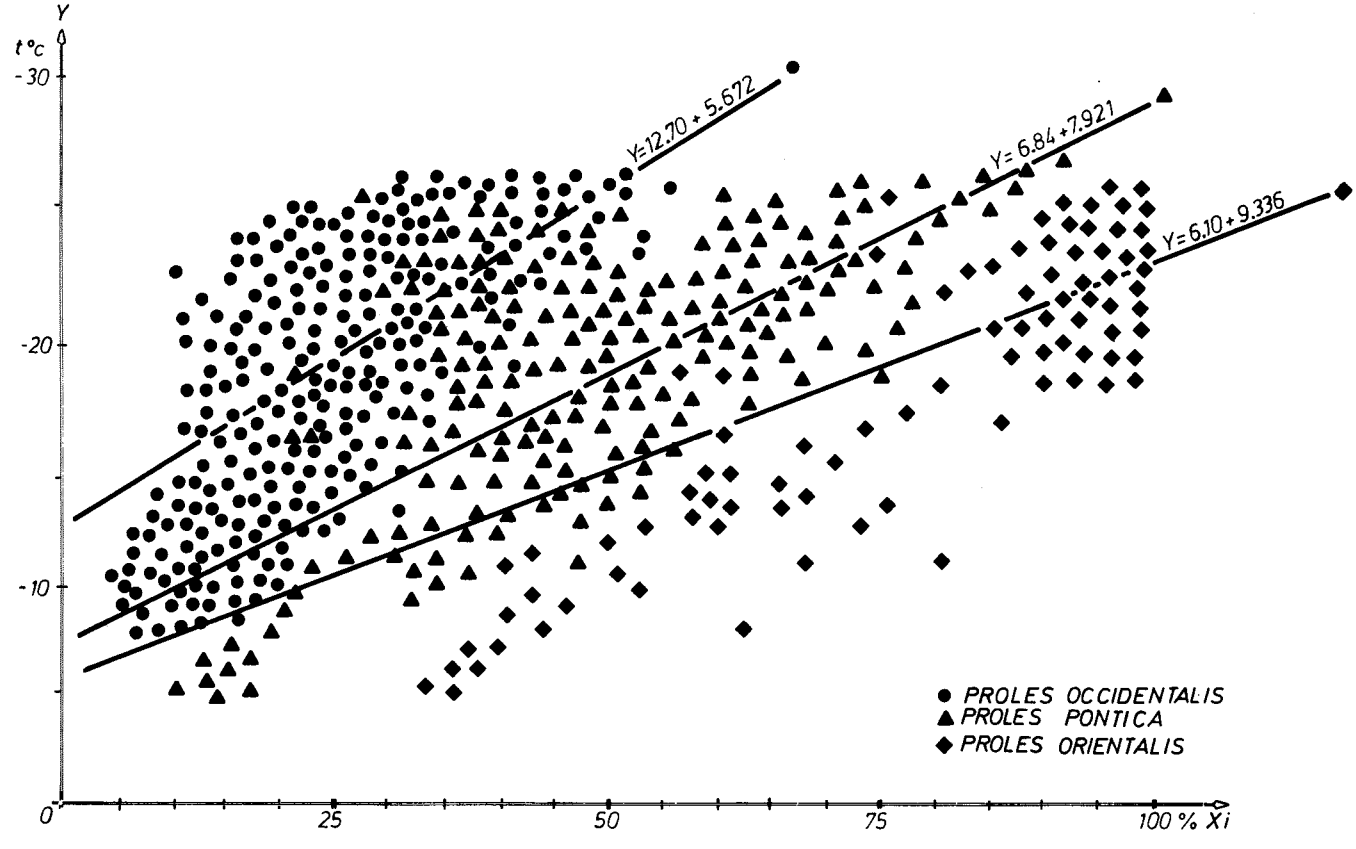


Fig. 1: Percentages of killed buds dependent on low winter temperatures in varieties belonging to different ecological-geographical groups. Location 'Radmilovatz'.

Table 2: The influence of low winter temperatures on the average percentages of killed buds in varieties of convarietas *pontica*

Location/Year	V a r i e t i e s				
	Bagrina %	Plovdina %	Prokupatz %	Skadarka %	Vranatz %
1.Location:"Radmilovatz"					
1984/1985	26,63	46,15	3o,91	31,48	58,77 ++
1985/1986	42,6o	46,12 +	38,32	31,6o	64,88 ++
1986/1987	84,44	93,51	82,14	78,55	96,41 ++
2.Location:"Svetozarevo"					
1984/1985	36,25	42,o1	38,3o	32,6o	46,42 ++
1985/1986	28,42	36,2o +	36,2o +	30,o8	49,6o ++
1986/1987	loo,oo	loo,oo	loo,oo	loo,oo	loo,oo
3.Location:"Bela Crkva"					
1984/1985	38,42	4o,2o	5o,oo	46,13	58,4o ++
1985/1986	--	--	--	--	--
1986/1987	64,o6	66,3o	61,9o	68,4o	74,o9 ++

$LSD_{o,o1}$

$LSD_{o,o5}$

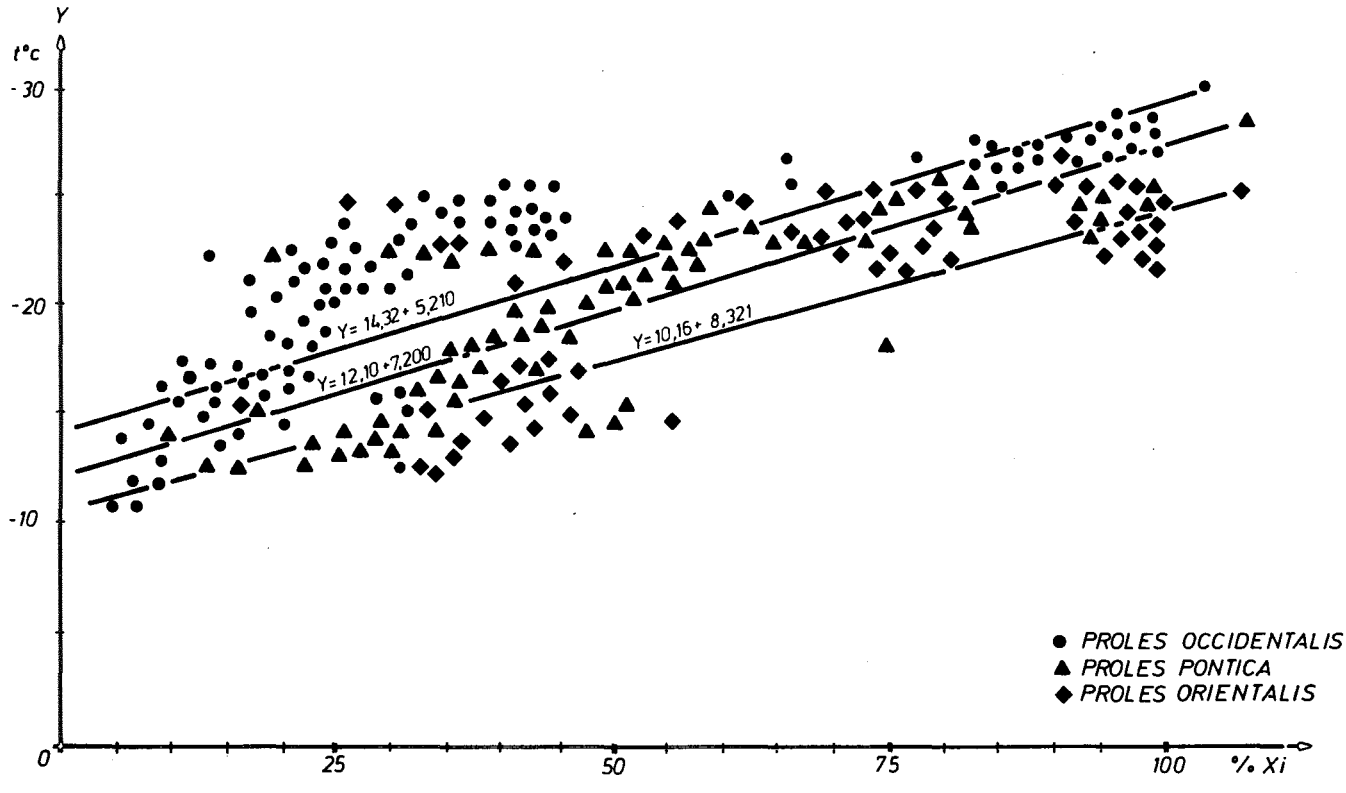


Fig. 2: Percentages of killed buds dependent on low winter temperatures in varieties belonging to different ecological-geographical groups. Location 'Svetozarevo'.

Table 3: The influence of low winter temperatures on the average percentages of killed buds in varieties of convarietas *occidentalis*

Location/Year	Varieties				
	Cabernet sauvignon %	Cabernet franc %	Pinot noir %	White Riesling %	Italien Riesling %
1.Location:"Radmilovatz"					
1984/1985	12,29	11,oo	11,26	14,59	11,o7
1985/1986	1o,o8	6,89	11,26	15,24	19,87
1986/1987	5,41	1o,6o	15,9o	15,5o	28,3o
2.Location:"Svetozarevo"					
1984/1985	14,32	16,o8	16,99	2o,oo	24,2o ++
1985/1986	6,3o	8,5o	1o,2o	11,6o	15,3o
1986/1987	1oo,oo	1oo,oo	1oo,oo	1oo,oo	1oo,oo
3.Location:"Bela Crkva"					
1984/1985	12,45	18,o2	14,82	2o,6o	3o,6o ++
1985/1986	--	--	--	--	--
1986/1987	15,66	21,65	2o,oo	2o,86	32,13 ++

$LSD_{o,ol}$

$LSD_{o,o5}$

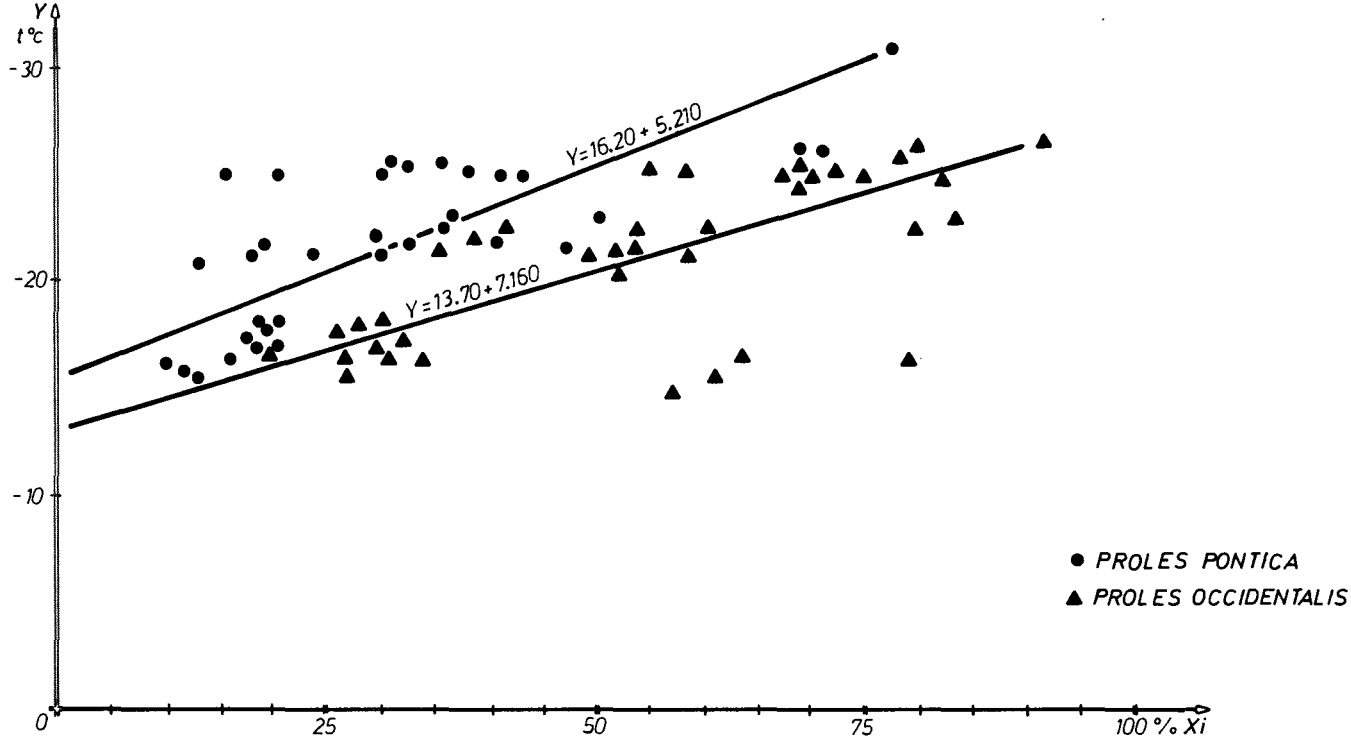


Fig. 3: Percentages of killed buds dependent on low winter temperatures in varieties belonging to different ecological-geographical groups. Location 'Bela Ckrva'.

- All buds in the varieties of this group were killed during the winter 1986/1987 at 'Svetozarevo'.

- Significant differences between the percentages of killed buds in this group were not determined comparing individual locations in the same years of investigation and comparing the years at the same locations.

- Significant differences were apparent between different varieties. The variety Italian Riesling exhibited in all investigated years at the same locations significant and much higher percentages of killed buds compared with the varieties Cabernet Sauvignon, Cabernet franc, Pinot noir and White Riesling.

Behavior similar to that of the chosen examples was observed with the other varieties of this ecological-geographical group (Fig. 3).

Otherwise, our investigations show that the *Vitis vinifera* varieties exhibit different resistances to low winter temperatures depending on their origin. The most resistant varieties belong to the group convar. *occidentalis*, and most sensitive are the varieties of the group convar. *orientalis*. The varieties which are by origin from the same ecological-geographical group also exhibit different resistance to winter temperatures.

The frost resistance of buds of grapevine varieties is also conditioned by pruning severity (Cindric and Briza 1982), trunk height (Jurcevic and Nakalamic 1969; Milosavljevic and Nakalamic 1969), position on the cane (Avramov *et al.* 1987) and the water status of the organs during winter (Cindric 1975; Tadijanovic *et al.* 1988). Other significant factors include the length of exposure of grapevine to low temperatures and the character of the low temperatures (snow, ice, wind).

The genetically conditioned resistance to low temperatures also depends on the method of fertilization (nutrition) just before winters with extremely low temperatures (Milosavljevic *et al.* 1987).

Conclusions

Taking into consideration our investigations and published data about the percentage of killed buds of the varieties belonging to different ecological-geographical groups during winters with extremely low temperatures, we can conclude:

1. The temperature conditions during the years of the investigations at all 3 locations with ampelographic collections resulted in significant percentages of killed buds. Extremely low winter temperatures were apparent during 1986/1987 when -25 °C was the usual daily low temperature and at 'Svetozarevo' -32 °C was observed during a short interval.

2. The varieties of the ecological-geographical group convar. *occidentalis* exhibited the greatest bud frost resistance during 3 winters with very low temperatures, which was significantly lower than the percentage of killed buds of the varieties of group convar. *pontica* and much less than the percentage of killed buds of the varieties of group convar. *orientalis*.

3. At location 'Svetozarevo' during the winter 1986/1987 all buds were completely killed.

4. The investigated varieties exhibited the highest percentage of killed buds during the winter 1986/1987 at all locations.

5. In conclusion we can say that the resistance of buds of a variety to low temperatures is conditioned by a complex of many factors. The ecological-geographical origin of the variety is also responsible for the resistance of the variety to low winter temperatures under our ecological conditions. This is also very important when choosing parents for the creation of new adapted varieties.

Literature

AVRAMOV, L.; TODOROVIC, N.; ZUNIC, D.; RADULOVIC, D.; 1987: Izmrzavanje okaca raznih sorti vinove loze tokom zime 1986/1987 u belocrkvanskom vinogorju. Savremena poljoprivreda **35** (11-13), 565-672.

-- -- ; ZUNIC, D.; 1986: Ocena otpornosti nekih domacih, novo stvorenih, standardnih i novo introdukovanih stonih i vinskih sorti vinove loze na niske zimske temperature tokom 1984/1985 godine u uslovima svetozarevackog vinogorja. Jugoslovensko vinogradarstvo i vinarstvo (7-8), 15-17.

-- -- ; -- -- ; 1986: Prilog oceni otpornosti sorti vinove loze na niske temperature kalemljenih i gajenih na sopstvenom korenu u belocrkvanskom vinogorju. Jugoslovensko vinogradarstvo i vinarstvo (5), 6-12.

CINDRIC, P.; 1984: Otpornost vinove loze prema niskim temperaturama. Fiziologija vinove loze. Srpska akademija nauka, Beograd, 147-174.

-- -- ; JAZIC, Lj.; RUZIC, N.; 1983: Ispitivanje vrednosti introdukovanih sorata iklonova vinove loze u fruskogorskom vinogorju. Vinogradarstvo i vinarstvo (37-38), 29-40.

KONDO, I. M.; 1959: Morozoustoicivost sortov i vozmoznost razsirenia ploscadi neukrivnoi kulturi vinograda v severnoi Azii. Trudi NIIV 'Magarac', 8.

LAZIC, S.; 1954: Zimski mrazevi u 1953/1954 godini i njihovo dejstvo na vinovu lozu u Vojvodini. Poljoprivreda Vojvodine **11**, 88-96.

-- -- ; SUPICA, M.; ZORZIC, M.; 1968: Prilog poznavanju centara porekla sorti vinove loze. Arhiv za poljoprivredne nauke, Beograd, **72**, 86-96.

-- -- ; ZORZIC, M.; 1956: Zimski mrazevi u 1955/1956 godini i njihovo dejstvo na vinovu lozu u Vojvodini. Poljoprivreda Vojvodine **12**, 70-85.

LUNTZ, O.; HAGU, G.; 1986: Ös evjarät natäsa szölötermesztesünkre a fajtak viselkendese. Szölötermesztes es Boraszat **12**, 14-18.

MILOSAVLJEVIC, M.; VUJINOVIC, D.; SOSKIC, S.; 1987: Mineralna ishrana i otpornost zimskih okaca sorte smederevke prema niskim zimskim temperaturama. Savremena poljoprivreda (9-10), 419-424.

NAGRU V.; 1971: Fiziologija zimostoikosti i zasuhoustoicivosti plodovih i vinograda. Kisinev.

NEGRUL, A. M.; 1946: Klasifikacia vinograda. Ampelografia SSSR, Tom I.

POGOSIAN, S.; 1967: Fiziologiceskie osobenosti morozoustoicivosti vinogradnogo rastenia. Erevan.

RATKOVIC, R.; 1979: Uticaj meteoroloskih faktora na prinos i kvalitet grozdja nekih sorti vinove loze. Vinogradarstvo i vinarstvo (29-32), 75-87.

TADIJANOVIC, Dj.; PULJIZ, M.; BJEKIC, S.; GASIC, N.; 1988: Iznalazenje nekih faktora otpornosti sorti vinove loze prema niskim temperaturama vazduha u fazi mirovanja. Savremena poljoprivreda (7-9), 449-460.

TURMANIDZE, I. T.; 1981: Morozoustoikost i uslovi prezimovki vinograda. Klimat i urozai vinograda, 26-33. Erevan.

ZUNIC, D.; 1988: Ekolosko geografska pripadnost vinskih sorti u SR Srbiji. (Magistarska teza).

Frost resistance of grapevine cultivars of different origin

P. CINDRIC, N. KORAC

Faculty of Agriculture, University of Novi Sad, YU-2100 Novi Sad, Yugoslavia

S u m m a r y : The tests of resistance to low temperature which included a large number of grapevine cultivars showed that the cultivars bore sign of their ecological-geographical and genetic origins with respect to the resistance to low temperature.

The tests, conducted over several years, consisted of exposing cuttings of annual shoots to low temperature in a cold chamber. The tests were repeated three times each winter, following the uniform method and time, in order to be able to distinguish relative differences in the degree of resistance between the cultivars tested.

Most cultivars from Western Europe (*occidentalis* NEGR., *gallica* NÉM.) had a high degree of resistance to low temperature. They tended to reach the peak of the resistance in mid winter.

The cultivars from the continental part of the Balkans (*pontica* NEGR., *balcanica* NEGR.) were unanimously sensitive to low temperature. The cultivars from the warm Mediterranean climate of Southern Europe (*pontica* NEGR., *balcanica* NEGR. and *occidentalis* NEGR., *iberica* NÉM.) were still more sensitive than the cultivars in the previously mentioned group.

The wine cultivars developed from interspecific crosses of European grapevines and American species exhibited a high degree of resistance in the middle and at the end of winter while the hybrids *vinifera x amurensis* were highly resistant at the beginning and in the middle of winter. Both groups can be used as donors of resistance to low temperature in programs of breeding cold hardy grapevine cultivars.

The tested table cultivars were found to be sensitive to low temperature, with the exception of the well-known cultivars Muscat Hamburg and Chasselas and the new cultivars Strugurash and Moldova.

K e y w o r d s : cold , resistance, breeding, genetics, selection, geography, ecology, wine grape, table grape, variety of vine.

Introduction

In many grape-growing regions consistent production depends largely on the occurrence of low temperatures in winter. However, grapevine cultivars differ considerably in resistance to low temperature (ZILAI 1981, 1987). These differences are primarily due to genetic factors but they may also depend on the method of growing as well as on actual climatic conditions in the period of dormancy.

If it is intended to screen for a degree of inherited frost resistance, it is necessary to standardize all other factors, i. e. the location and method of growing and the time and method of screening. Even then it is not feasible to assess accurately the absolute values of frost resistance in individual grapevine cultivars. If, however, all factors are made uniform and a uniform low temperature treatment is applied, it is possible to obtain information on the relative frost resistance of the cultivars compared. Low temperatures are a potential threat over a period of several months. Grapevine cultivars tend to vary in the degree of frost resistance during that period. Some cultivars are less resistant at the beginning, some in the middle and some at the end of the period. This circumstance imposes a need for repeated screenings for frost resistance in the course of winter (EIFERT 1975).

The direction and intensity of biochemical and physiological processes which in fact determine the degree of frost resistance in overwintering plant parts are directly affected by actual temperature conditions preceding the occurrence of critical low temperatures (KONDO 1970; POGOSJAN 1975; REUTER 1975; MARUTJAN 1978). As temperature conditions vary from one year to another, screenings should be repeated for several years in order to secure reliable results.

The objective of this investigation was to establish differences in the degree of frost tolerance among grapevine cultivars differing in geographic and genetic origins.

Investigated cultivars and number of years investigated

Group	Cultivar prime name	Synonyms (parents)	Number of years investigated
1. Cultivars iriginating from West Europe	1. Pinot gris	Ruländer	9
	2. Pinot blanc	Weissburgunder	9
	3. Pinot noir	Blauer Spätburgunder	8
	4. Chardonnay	-	9
	5. Traminer	-	9
	6. Riesling	Rhein Riesling	9
	7. Italian Rizling	Welschriesling	9
	8. Müller-Thurgau	-	9
	9. Sauvignon	-	9
	10. Muscat Ottonel	-	9
	11. Cabernet sauvignon	-	9
	12. Merlot	-	9
2. Ancient Balkan cultivars cultivated in continental parts of the Balkans	1. Medenac beli	Honigler	7
	2. Ezerjo	-	9
	3. Slankamenka crvena	Plovdina crvena	9
	4. Slankamenka bela	-	5
	5. Kreaca	Banati rizling	7
	6. Bagrina	-	6
	7. Smederevka	Dimjat	9
	8. Kevidinka	Ružica, Crvena Dinka	9
	9. Prokupac	Zarcsin	8
	10. Plovdina crna	Pamid	7
	11. Izsáki	Izsáki sárfehér	5
	12. Sremska zelenika	Szerémi zöld	7
3. Cultivars cultivated in south of Yugoslavia and south of Europe	1. Blatina	-	7
	2. Vranac	-	8
	3. Kratošija	-	5
	4. Stanušina	-	5
	5. Aramon	-	7
	6. Carignan	-	7
	7. Refošk	Terano d'Istria	5
	8. Barbera	-	5
	9. Malvasia blanc	-	5
	10. Ugni blanc	St. Emillion	5
	11. Žilavka	-	8

(Continued overleaf)

Methods

In vitro test similar to that used by MUELLNER and MAYER (1970), GUZUN *et al.* (1972), EIFERT (1975), CSERNOMOREC (1976) and SCHÖFFLING (1980) was used in this investigation.

Cuttings of annual shoots were exposed to low temperature in a cold chamber. After sampling 30 representative buds from each cultivar, the cuttings were first kept at -5 °C for 24 h and then the temperature was lowered to -21°C at the rate of 3°C/h. That temperature was maintained for 12 h and then raised gradually to room temperature. The tested materials were kept at room temperature for 1 week and then scored for the number of survived winter buds. Central buds and lateral buds were scored separately.

The test was repeated three times in the course of several winters: in late December, second half of January and second half of February. These dates corresponded to cultivars resistance to frost at the beginning, the middle and the end of winter.

Table (continued)

Group	Cultivar prime name	Synonyms (parents)	Number of of years investigated
4. Cultivars originating from interspezific hybridzation	1. SV 12-375	Villard blanc	8
	2. SV 18-315	Villard noir	7
	3. Seibel 70-53	Chancellor	7
	4. Seibel 49-86	Rayon d'or	7
	5. Bianka	(SV 12-375 x Bouvier)	7
	6. Göcsei zamatos	(SV 18-315 x Medoc noir)	6
	7. Zalagyöngye	(SV 12-375 x Csaba gyönge)	9
	8. Kunleany	(BC_1 V.vinifera x V.amurensis)	9
	9. Kunbarat	(")	9
	10. SK 77-12/6	Kunleany x Ruländer	8
	11. SK 77-5/3	Kunbarat x Pinot noir	8
	12. SK 77-14/17	Kunleany x M.Ottonel	8
5. Table grape cultivars of different origin	1. Cardinal	-	9
	2. Queen of vineyard	Szölökertekkirálynöje	9
	3. Chasselas	Gutedel; Fendant	9
	4. Italia	-	9
	5. Dattier de Beyrouth	Afuz-ali; Regina	9
	6. Ribier	Alfonse Lavallée	9
	7. Muscat Hamburg	-	9
	8. Muscat Pölöskei	(Zalagyönge x /Gloria-hungarie x Erzsébet kiralyne emléke/)	7
	9. Ljana	(Csaus x Pierelle)	9
	10. SV 20-473	Muscat de St.Vallier	9
	11. Strugurash	(Koarna njagra x Pierelle)	9
	12. Moldova	(SV 12-375 x Guzalj kara)	9

Plant samples to be tested were taken from the ampelographic collection at Sremski Karlovci in which all cultivars are grown according to the latest cultivation methods (CINDRIC *et al.* 1986). The investigation was commenced in 1980/81 when the plants started to bear fruit and it was continued for several years. Most cultivars were tested for 9 years, as presented in the table.

Let it be emphasized once again that all cultivars were tested uniformly with respect to method and time. Therefore, the results should indicate those differences among the cultivars which are genetically controlled.

The table presents some basic data for the cultivars tested. The cultivars had been grouped according to their origin, location of large-scale commercial production or their use.

Results

1. Cultivars originating from Western Europe

According to their ecological-geographical classification, the cultivars from Western Europe belong to convarietas *occidentalis* NEGR. (NEGRUL 1956), subconvarietas *gallica* NÉM. (NÉMETH 1976; CSEPREGI and ZILAI 1976). In general, these cultivars exhibited a relatively high degree of frost resistance (Fig. 1). Regarding the three dates of testing, their resistance was highest in mid winter and lowest at the beginning of winter. A high level of resistance was exhibited by the cultivars Riesling, Traminer and the cultivars from conculta Pinot (P. gris, P. blanc, P. noir and Chardonnay). A somewhat lower resistance was displayed by Sauvignon, Italian Riesling and Müller Thurgau. The cultivars Muscat Ottonel and Cabernet Sauvignon had a different pattern of resistance in the course of winter – they reached the peak of the resistance in the second half of February and not in mid January as the other cultivars in this group. CSEPREGI and ZILAI (1976) did not class Muscat Ottonel in the same group with the other cultivars but in convarietas *orientalis* NEGR., subconvarietas *caspica* NEGR., along with the cultivar Chasselas. This appears to be correct since Muscat Ottonel and Chasselas share not only a number of morphological characters but also the pattern of frost resistance which is characteristic for the cultivars of subconvarietas *caspica* NEGR. (CINDRIC *et al.* 1987). This supports the opinion of GALET (1958) that Muscat Ottonel draws origin from Chasselas.

It should be mentioned that the cultivar Merlot had a perceptibly lower resistance in the middle and at the end of winter than Cabernet Sauvignon, in spite of a number of similarities between the two cultivars.

2. Old Balkan cultivars

Fig. 2 shows the results of the cold tests for cultivars grown in the continental part of the Balkans. According to their ecological-geographical classification, NÉMETH (1976), CSEPREGI and ZILAI (1976) classed them in convarietas *pontica* NEGR., subconvarietas *balcanica* NEGR. In general, their resistance to low temperature was low. Namely, most of them exhibited an exceptionally low degree of resistance at the beginning of winter and a somewhat higher resistance in the second half of winter. In the climatic conditions of their original habitat, these cultivars could be grown successfully only when hilled before winter. After the introduction of modern cultivation methods which imply high training and the omission of hilling as a regular agrotechnical practice, the productivity of these cultivars became quite unstable. This, in addition to a somewhat lower wine quality, was an important reason why these cultivars were abandoned from commercial production in the last several decades.

3. Cultivars originating from southern parts of Yugoslavia and Southern Europe

Fig. 3 shows the tested cultivars which are grown in warm regions of Southern Europe. Those were well-known Southern French cultivars Aramon and Carignan, classed in convarietas

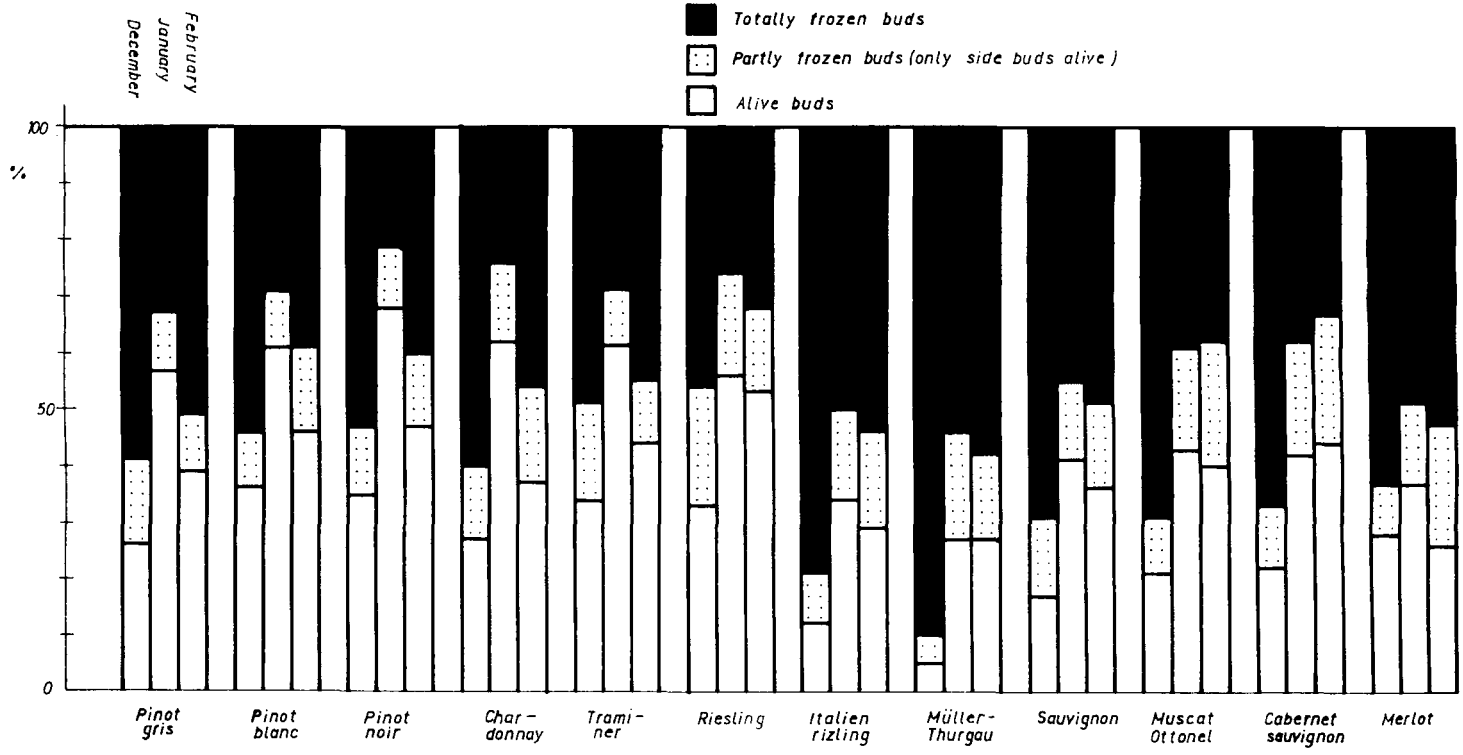


Fig.1: Results of cold tests with wine grape cultivars originating from West Europe.

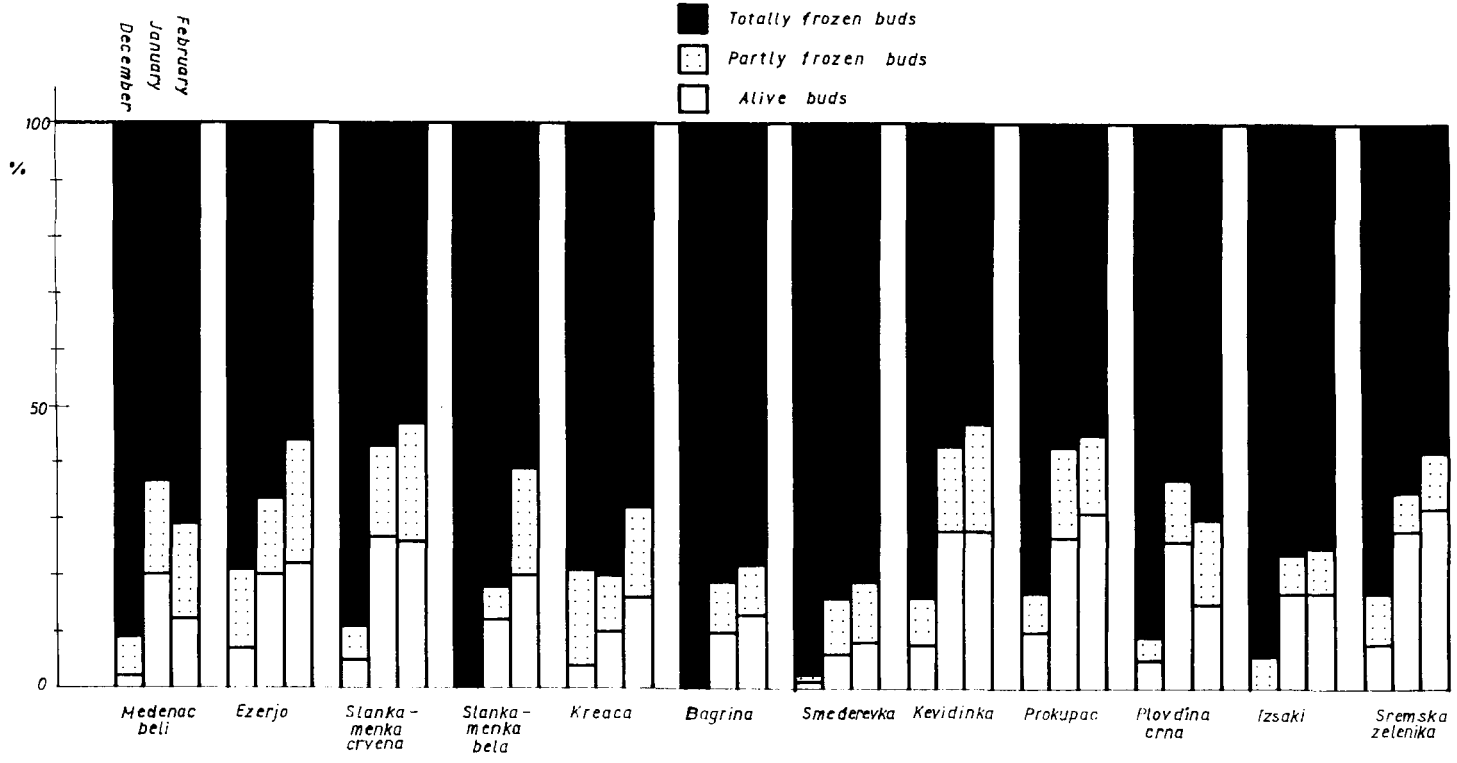


Fig. 2: Results of cold tests with ancient Balkan cultivars grown in the continental part of the Balkans.

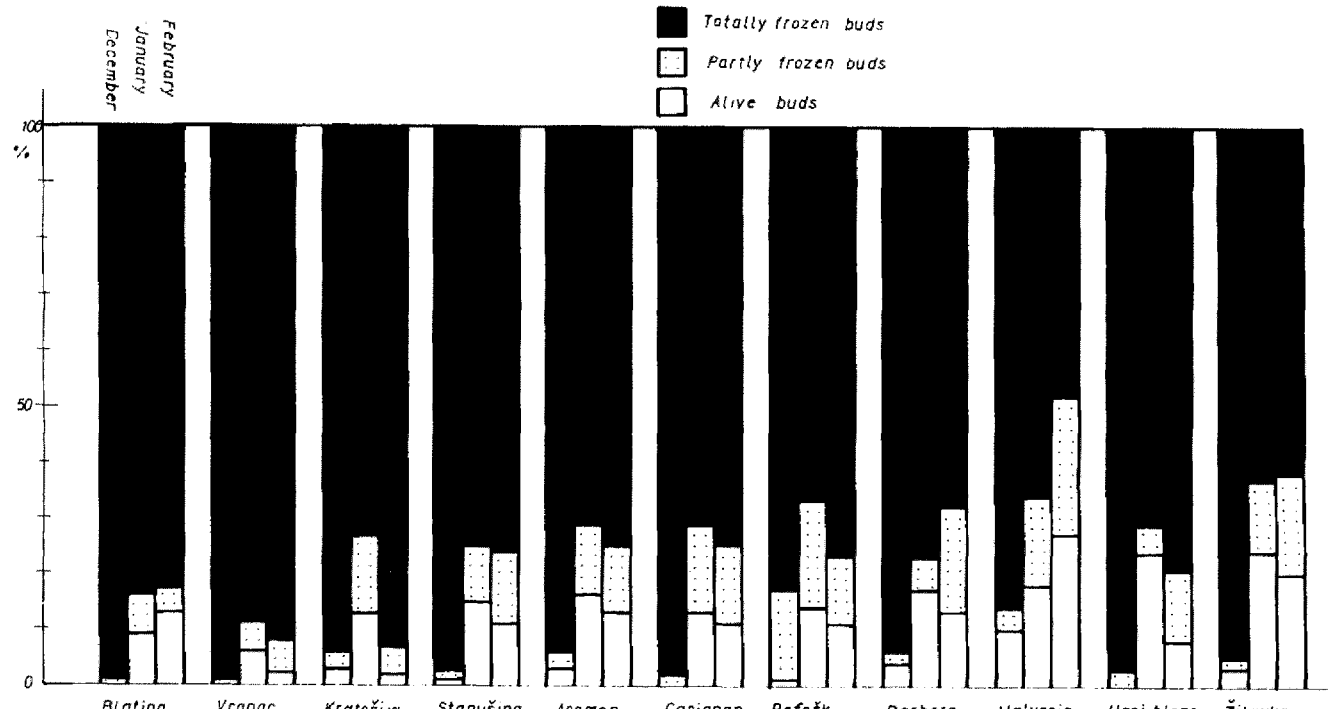


Fig. 3: Results of cold tests with wine grape cultivars originating from south of Yugoslavia and South Europe.

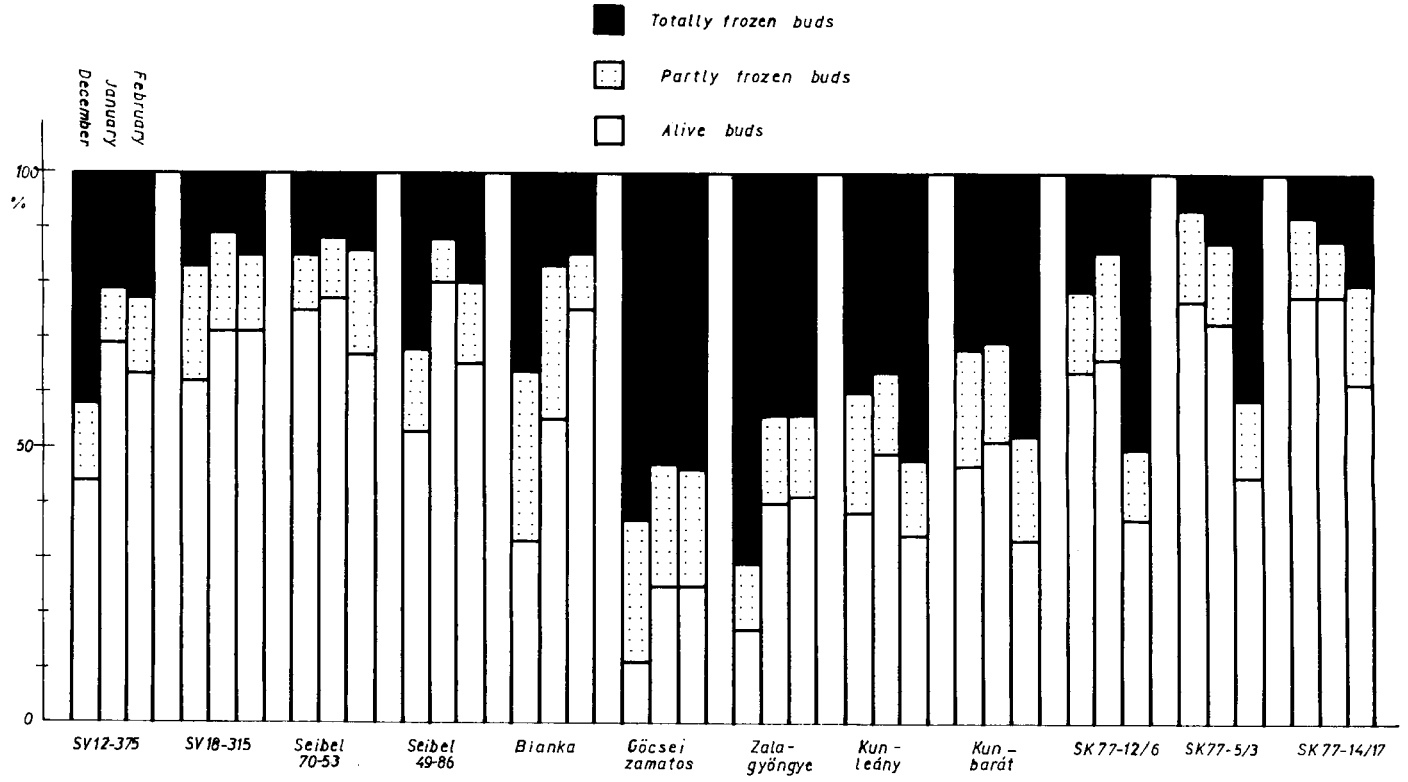


Fig. 4: Results of cold tests with wine grape cultivars based on interspecific hybridization.

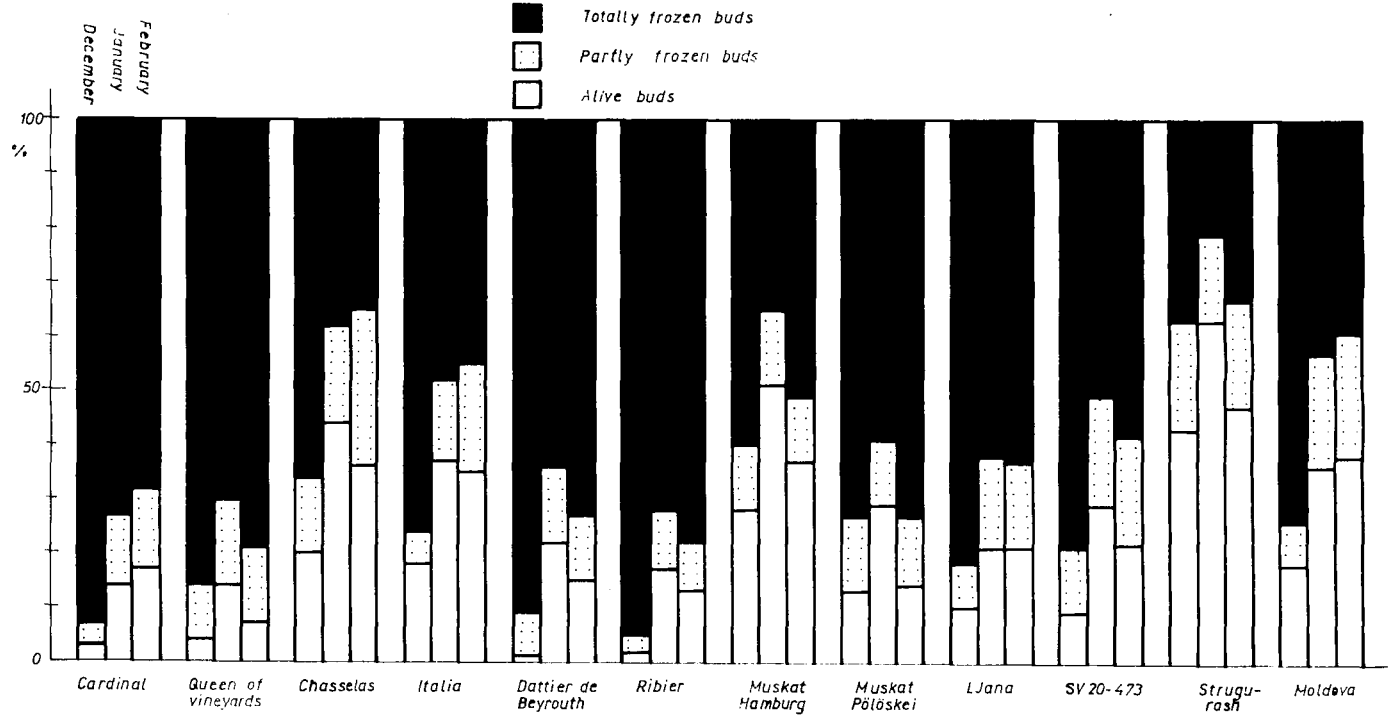


Fig. 5: Results of cold tests with table grape cultivars of different origin.

occidentalis NEGR., subconvarietas *iberica* NÉM., and cultivars native to the southern parts of Yugoslavia (Macedonia, Montenegro, Herzegovina and Istria) which require a high temperature sum to mature and which may be classed in convarietas *pontica* NEGR., subconvarietas *balcanica* NEGR. Of course, this classification does not apply to the cultivar Ugni blanc which has only recently been introduced in southern parts of Yugoslavia. Fig. 3 shows that all of these cultivars had a very low degree of frost resistance. This is quite understandable since they come from regions which are risk-free with respect to the occurrence of low temperatures in winter. These cultivars may rightfully bear a collective name *Vitis mediterranea* ANDRAS., as suggested by ANDRASOVSKY (1926) in his proposal of a polyphyletic classification (as cited by KOZMA 1967).

4. Cultivars based on interspecific hybridization

Fig. 4 shows the results of the cold test for 12 wine cultivars which had been developed on the basis of interspecific hybridization. The first 7 resulted from complex crossings between *V. vinifera* cultivars and American species. The remaining 5 cultivars are interspecific crosses based on *V. amurensis*. These two groups of genotypes were clearly distinctive with respect to resistance to low temperature. The interspecific hybrids in the first group exhibited a particularly high degree of resistance in the middle and at the end of winter. The interspecific hybrids based on *V. amurensis* had a high degree of resistance at the beginning and in the middle of winter but their resistance was diminished at the end of winter. The above data should be taken in consideration when breeding new frost resistant cultivars. It was concluded that most cultivars from both groups of interspecific hybrids may serve as donors of frost resistance. Understandably, best results would be obtained if the inheritance bases of the two groups were combined.

The data presented in the graphs are sufficiently self-explanatory as to render a discussion of individual cultivars unnecessary. Still, a new Hungarian cultivar, Bianka, developed by J. CSIZMAZIA and L. BEREZNAI (CSIZMAZIA 1977), deserves to be mentioned for its high tolerance to low temperature combined with early maturity, high resistance to major fungal diseases and good wine quality (MAJOROS 1983). The cultivar Bianka is evidently superior in a number of important biological characters to the hybrids Seyve Villard and Seibel which have extensively been used in programs of interspecific crossings as donors of resistance to fungal diseases (VERDEREVSKI and VOJTOVICS 1970; CSIZMAZIA 1977; ALLEWELDT 1979; BECKER 1980; BECKER and ZIMMERMANN 1980; DOAZAN 1980; VOJTOVICS 1981). Unfortunately, the cultivar Bianka is prone to berry drop causing yield fluctuations from one year to another. In our new program of breeding resistant cultivars, Bianka has been crossed to several *vinifera x amurensis* genotypes.

5. Table cultivars

It is common knowledge that the table cultivars are sensitive to low temperature. This investigation indicated considerable differences in this character among the tested cultivars (Fig. 5). Of the well-known table cultivars, Chasselas and Muscat Hamburg exhibited the highest level of resistance. The cultivar Italia was less sensitive than Dattier de Beyrouth, Ribier, Cardinal and Queen of Vineyards which in their turn confirmed their high sensitivity to frost.

Of the tested cultivars which are also resistant to fungal diseases, the cultivar Strugurash displayed a surprisingly high level of resistance while Moldova had a relative high resistance. These are two new cultivars developed by means of interspecific hybridization by ZSURAVELY and GUZUN in Kishinev (KORAC 1989). The other interspecific table cultivars (Muscat Poeloeskei, Ljana and Muscat de St. Vallier) were sensitive to low temperature.

Finally, it should be mentioned that the authors are aware that the results presented are based on a single method of testing. Since the resistance to low temperature is a very complex biological character, a more precise definition of cold hardiness in the tested cultivars would require a study of other parameters too, e. g., date of budding in spring, fertility of lateral buds and regeneration capacity following damage incurred by low temperature.

Conclusion

The long-term testing of resistance to low temperature showed that certain cultivars bore in themselves the sign of their ecological-geographical and genetic origins.

1. Most cultivars from Western Europe (convarietas *occidentalis* NEGR., subconvarietas *gallica* NÉM.) exhibited a proportionally high resistance to low temperature. They tended to reach the peak of the resistance in mid winter.

2. The cultivars which were previously grown or are still grown in the continental part of the Balkans (convarietas *pontica* NEGR., subconvarietas *balcanica* NEGR.) featured a low resistance to low temperature and could be grown in that area only if hilling was regularly practised as a means of prevention against low temperature in winter.

3. The cultivars from the warm Mediterranean climate of Southern Europe were sensitive to low temperature. According to their ecological-geographical classification, these cultivars belong to convarietas *occidentalis* NEGR., subconvarietas *iberica* NÉM. and to convarietas *pontica* NEGR., subconvarietas *balcanica* NEGR.

4. The interspecific wine cultivars developed by crossing European grapevines to American species may, in addition to the resistance to fungal diseases, serve as donors of resistance to low temperature. A new Hungarian cultivar, Bianka, deserves to be mentioned for its positive characters. The Euro-American hybrids displayed a high degree of resistance in the middle and at the end of winter. On the other hand, the interspecific hybrids *V. vinifera x V. amurensis* exhibited a high degree of resistance at the beginning and in the middle of winter. It is obvious that a significant progress in breeding for resistance to low temperature could be achieved by combining these two complex inheritance bases.

5. The true table cultivars such as Dattier de Beyrouth, Queen of Vineyard, Cardinal and Ribier were highly sensitive to low temperature whereas the cultivar Italia performed somewhat better. Of the well-known table cultivars, Muscat Hamburg displayed a relatively high degree of resistance. Chasselas, another popular table-wine cultivar, also exhibited a relatively high resistance, especially in the second half of winter.

Of the 5 interspecific table cultivars tested, the new Soviet cultivars, Strugurash and Moldova, had a high level of resistance to low temperature.

Literature

ALLEWELDT, G.; 1979: Die Resistenzzüchtung in der Bundesrepublik Deutschland. XVIe Congr. Intern. O.I.V., Stuttgart.

BECKER, H.; 1980: Results of interspecific hybridization with *Vitis amurensis* and *Vitis vinifera* in Geisenheim. Proc. 3rd Intern. Symp. Grape Breeding, Davis, Ca.

BECKER, N., ZIMMERMANN, HEDI; 1980: Wine quality of newly bred grape varieties resistant to downy mildew. Proc. 3rd Intern. Symp. Grape Breeding, Davis, Ca.

CINDRIC, P.; KORAC, NADA; MEDIC, MIRA; 1986: Ispitivanje belih vinskih sorti rejoniranih u Vojvodini na jednom jednostavnom uzgojnom obliku. Jugoslovensko Vinogradarstvo i Vinarstvo, Beograd, (2-3), 5-14.

-- --; -- -- ; -- -- ; KULJANCIC, I.; 1987: Otpornos na niske temperature nekih vinskih sorti. Savremena Poljoprivreda, Novi Sad, (11-12), 553-563.

CSEPREGI, P.; 1982: A szölö metszese, fitotehnikai müveletei. Budapest.

-- --; ZILAI, J.; 1976: Szölöfajtaink. Budapest.

CSERMNOMOREC, MARIJA, V.; 1976: Opredelenije zimostojkosti vinograd nogo rastenija. Kartja Moldavenjaske. Kisinjev.

CSIZMAZIA, J.; 1977: Peronoszporaresistens szölöfajtak elöálitása és bevezetésük a termesztésbe. Borgazdasàg (2).

DOAZAN, J. P.; 1980: The selection of grapevine genotypes resistant to fungus diseases and their use under field conditions. Proc. 3rd Intern. Symp. Grape Breeding, Davis, Ca.

EIFERT, ANNA; 1975: Einige Aspekte der Frosthärteprüfung bei Modellversuchen in Klimakammern. Vitis **13**, 297-302.

GALET, P.; 1958: Cépages et Vignobles de France, Tome II.

GUZUN, N. I.; CSERNOMOREC, M. V.; CIPKO, M. V.; 1972: Opredelenie morozostojkosti vinograda putem iskustvenogo promorozsivanija. Sadovodstvo, Vinograd. i Vinodelje Moldavii (11).

KONDO, I. N.; 1970: Ustojcivost vinogradnogo rastenija k morozam, zasuhe i pocvenomu zasoleniju. Kartja Moldovenjaske, Kisinjev.

KORAC, NADA; 1989: Ampelografsko ispitivanje stonih sorti vinove loze u fruskogorskom vinogorju. Doktorska disertacija, Novi Sad.

KOZMA, P.; 1967: Szölötermesztés I. Budapest.

LUNTZ, O.; NAGY, GEZANE; 1986: Az 1985 - ös evjárathatása szölötermesztésünkre a fajták viselkedése. Szölötermesztés és Borászat (1-2).

MAJOROS, L.; 1983: Bianca. Kertészet és Szölészet (18).

MARUTJAN, SEDA A.; 1978: Biohemicseskie aspekti formirovania i diagnostiki morozoustoicsivosti vinogradnogo rastenia. Erevan.

MUELLNER, L.; MAYER, G.; 1970: Frostresistenzprüfung bei Reben (*Vitis vinif.*) Mitt. Klosterneuburg **20**, 258-266.

NAGY, GEZANE; 1983: Nehány szölöfajta fagytürésének jellemzése mitokondrium frakcioban lévö transport ATP-az aktivitás mérésevel. Doktori ertekezés, Kecskemét.

NEGRUL, A. M.; 1956: Vinogradarstvo s osnovami ampelografii i selekcii. Moskva.

NÉMETH, M.; 1967, 1970, 1976: Ampelografiai album I, II, III. Budapest.

POGOSJAN, K. S.; 1975: Fiziologicseskie osobenosti morozoustojcivosti vinogradnogo rastenija. Erevan.

REUTER, G.; 1975: Physiologische Kriterien der Klimaresistenz als sortenspezifische Merkmale. Angew. Bot. **49**.

SCHÖFFLING, H.; 1980: Frost resistance of *Vitis vinifera* varieties in the upper Moselle vine-growing area. Proc. 3rd Intern. Symp. Grape Breeding, Davis, Ca.

VALCEV, J. V.; 1988: Selekcia novih sortov vinograda v Narodnoj respublike Bolgarii. Sofia.

VERDEREVSKI, D.; VOJTOVICS, K. A.; 1970: Mildju vinograda. Kishinev.

VOITOVICS, K. A.; 1981: Novie kompleksnoustoicsivie sorta vinograda. Kishinev.

ZILAI, J.; 1981: A szölöfajták értékelése. Szölészet és Borászat (3).

-- --; 1987: A fajtaérték-kutatás modszerei, eredmenyei a szölötermesztésben. Doktori értékezés tézisei, Budapest.

The effect of fertilizers on different wine grape varieties in model container trials

J. André and E. Hajdu

Research Institute for Viticulture and Oenology, H-6001 Kecskemét, Hungary

Summary: Nutrient supply trials were carried out in model container trials with sandy soils adjusted to low and high nutrient levels (P, K, Mg) for 16 varieties with 5 replications as determined from the averages of years and varieties.

The utilization of high nutrient supply in the soil can be summed up as follows:

- Analysis data revealed an increase of P in the leaf and wood but there was no change in the grape. Potassium level increased in the leaf and wood but remained unchanged in the grape. Magnesium level increased in the leaf and wood and also in the grape.
- Of the production indices an increase was observed in the green mass, wood mass, grape number and grape weight but a decrease in mean grape weight and in the sugar and acid content of the must.
- Winterhardiness increased slightly.

It was evident that some varieties differed considerably from the varietal mean in the evaluated parameters.

Key words: fertilizing, soil, nutrient, phosphorus, potassium, magnesium, leaf, wood, berry, yield, must quality, cold hardiness, variety of vine, Hungary.

Trial target

To determine the effect of different P, K, Mg doses at identical N supply on

- the nutrient regime of wine grape varieties
- the development of the vegetative parts
- the quantity and quality of fruit yield
- the winter hardiness of normally developed buds

Material and method

Type of trial:	model trial in containers in plastic tents	
Plastic tent:	unheated	
	covered with plastic film from November till May	
	covered with net from May till November	
Nutrient supply:	storage supply:	prior to planting P (P_2O_5), K (K_2SO_4), Mg ($MgSO_4$) homogenized with the soil
	maintenance supply:	N (NH_4NO_3) in every year
Soil:	sand	
Soil parameters:	pH (KCl) 8.1	
	K_a 25	
	$CaCO_3$ (%) 4-5	
	H% 0.44-0.50	

Available soil nutrient content:

	Origin	After supply	
		low (1)	high (2)
Al P_2O_5 ppm	86	100	200
Al K_2O ppm	70	150	300
KCl Mg ppm	25	80	150

Water supply: irregular (compensating for winter precipitation)
Number of varieties: 16 (11 white wine grape varieties and 5 red wine grape varieties)
Number of replications: 5 plants/variety
Propagation material: standard cutting on its own root
Year of planting: 1982
Location: Kecskemét-Miklóstelep

Analysis results

In leaves: This shows the nutrient requirement of varieties and their nutrient absorption capacity.

Phosphorus

- Varieties of high P requirement and good P absorption: Sztyepnyak, Rheinriesling, Chardonnay, Miklóstelep 7
- Varieties of low P requirement and good P absorption: Kunleány, Zalagyöngye, Steinschiller, Medina

Potassium

- Varieties of high K requirement and good K absorption: Rheinriesling, Chardonnay, Steinschiller, Zengö, Zweigelt, F. Kadarka, Blaufränkisch Tf.
- Varieties of low K requirement and good K absorption: Kunleány, Zalagyöngye, Medina, Ezerfürtü, Sztyepnyak

Magnesium

- Varieties of high Mg requirement and good Mg absorption: Chardonnay, Rheinriesling, Sztyepnyak
- Variety of low Mg requirement and good Mg absorption: RF 48

In fruit nutrient elements are generally not localized.

Phosphorus

The surplus P in berries (and leaves) resulting from high nutrient supply was not significant in most varieties.

- P content increased in the varieties: Chardonnay, Rheinriesling

Potassium

At high nutrient supply the increase in K content found in leaves and berries was not significant in the berries.

- K content increased in the berries of Rheinriesling.

Magnesium

- Mg level in leaves has the same tendency as the Mg level in berries
- No such tendency was found in the varieties: Kecskemét 9, Ezerfürtü, Sztyepnyak, Zengö

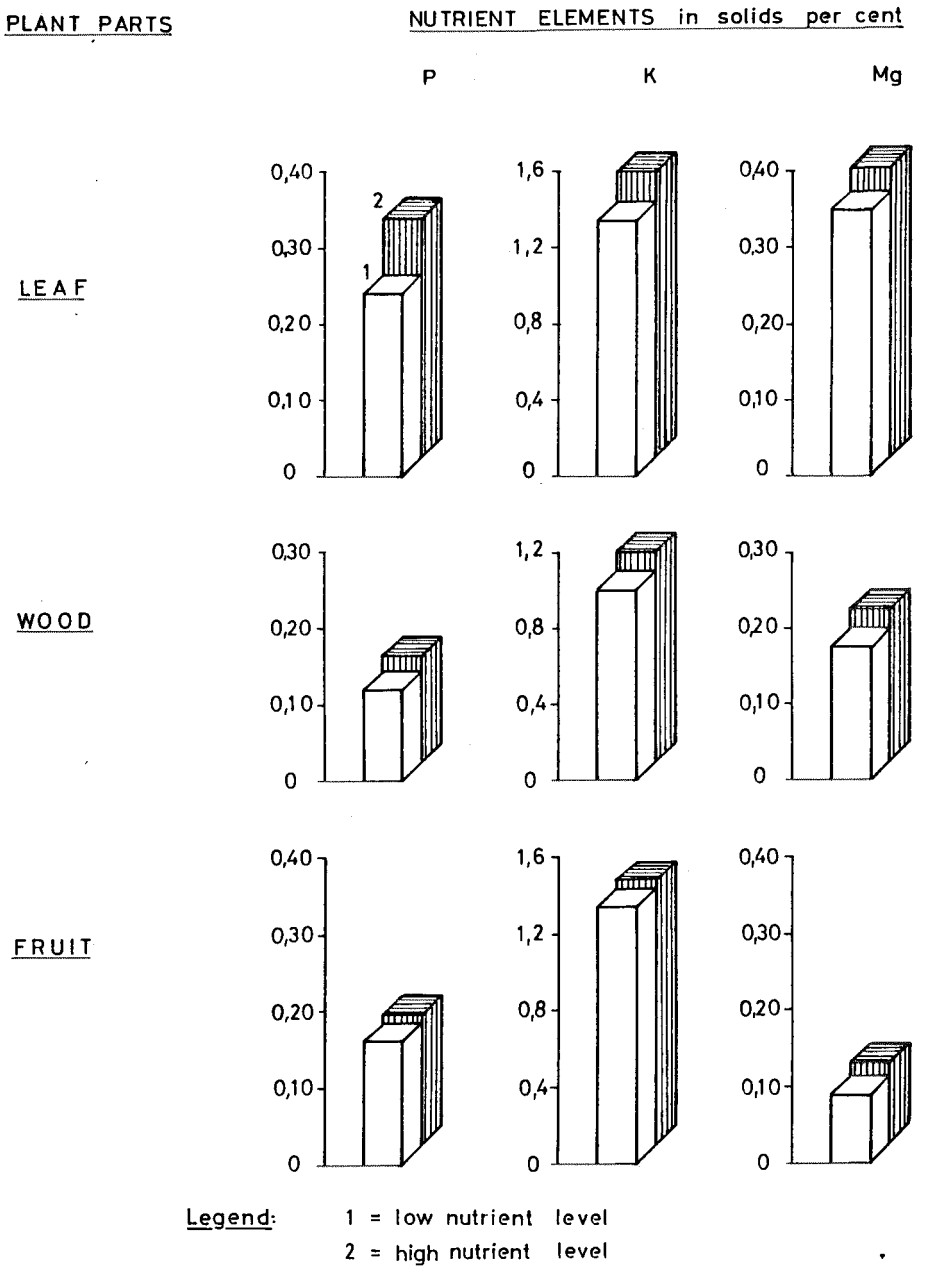


Fig. 1: Analysis data in the mean of 6 years (1982-1987) and 16 varieties.

Analysis data in the mean of 6 years (1982-1987) for 16 varieties. The table presents the difference in P, K, Mg values of plant parts expressed in solids per cent of each variety related to the low nutrient level

VARIETIES / PLANT PARTS	CHARDONNAY	RHEINRIESLING	EZERFÜRTÜ	ZENGŐ	JUBILEUM 75	STEINSCHILLER	KECSKEMÉT-9	ZALAGYÖNGYE	RF 48	KUNLEÁNY	SZTYEPNYAK	BLAUFRÄNKISCH Tf.	ZWEIGELT	F. KADARKA	MEDINA	MIKLÓSTELEP-7
								P								
LEAF	76	48	57	63	29	45	0	36	17	36	53	29	29	29	43	31
WOOD	17	15	8	50	20	15	17	8	9	0	17	25	25	8	8	33
FRUIT	55	43	-7	0	12	6	-24	-13	7	0	-10	6	0	0	-18	12
								K								
LEAF	20	26	23	27	10	18	13	27	20	16	24	23	28	18	37	11
WOOD	-3	22	11	36	11	6	2	23	28	14	17	34	26	26	21	31
FRUIT	4	25	5	4	4	1	-8	5	-8	-13	-10	2	6	12	-5	4
								Mg								
LEAF	22	25	13	42	8	7	10	0	23	11	18	50	3	14	38	35
WOOD	-5	17	-5	33	6	6	16	0	12	-10	-4	36	-9	22	25	5
FRUIT	14	35	4	-9	14	4	1	-6	7	7	-1	25	20	23	8	14

In wood: This allows conclusions as to nutrient translocation and re-utilization.

Phosphorus

- At low nutrient supply there is no significant difference among varieties
- At high nutrient supply differences are considerable and specific for the variety
  20-50 % P increase: Zengö, Jubileum 75, Miklóstelep 7, Blaufränkisch Tf., Zweigelt
  No P increase: Kunleány, Zalagyöngye, F. Kadarka, Medina

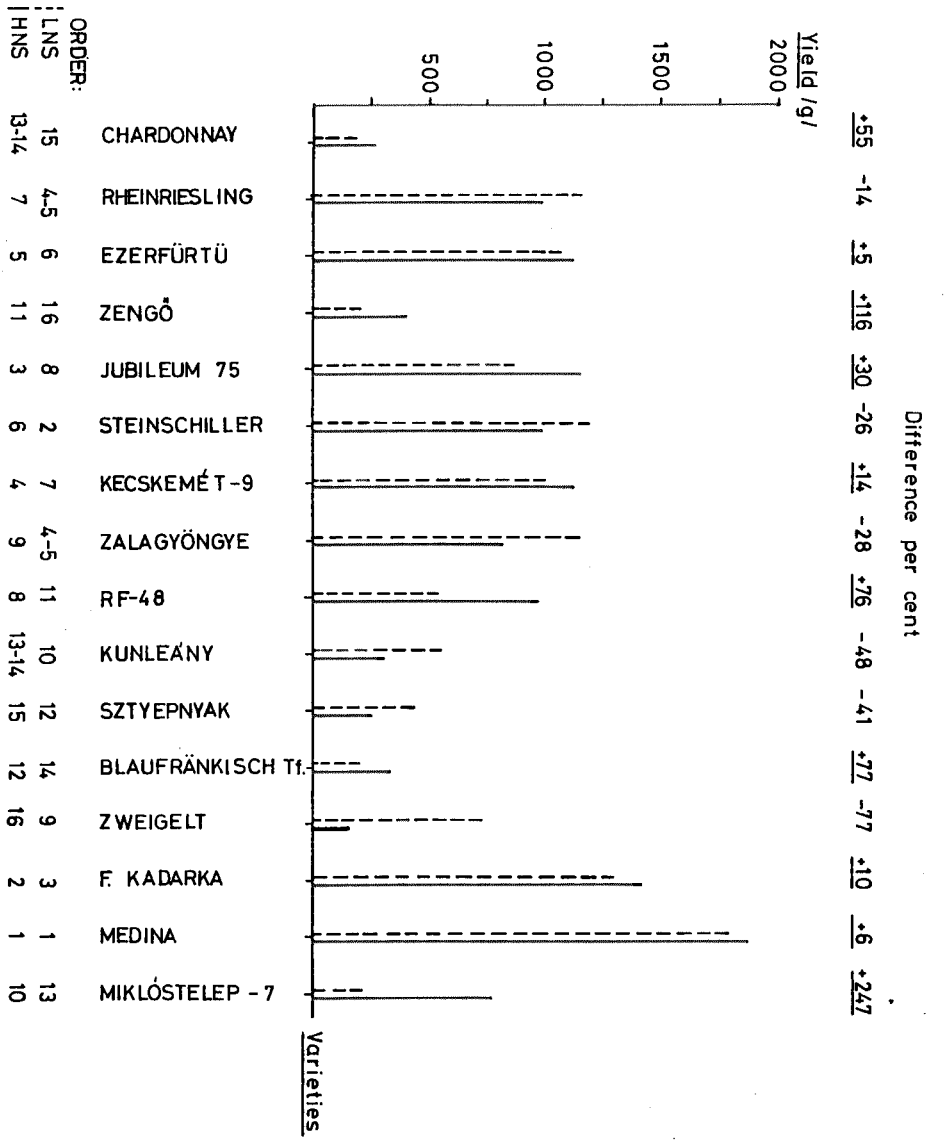


Fig. 2: Cluster yield in the mean of 4 years (1984-1987) at low and high nutrient supply.

Potassium

Even at low nutrient supply the increase of K level is considerable and specific for the variety. In the majority of varieties the K increase in the wood has a tendency similar to that measured in the leaves.

25-35 % K increase: Zengö, Blaufränkisch Tf., RF 48, F. Kadarka, Zweigelt
No K increase: Chardonnay, Kecskemét 9, Steinschiller

Magnesium

At high and low nutrient supply the Mg level in the wood has a tendency similar to that in the leaf with lower absolute values.

- The surplus uptake in leaves cannot be detected in the wood of the varieties: Chardonnay, Sztyepnyak, Kunleány

Measurement results of plant parts

In the mean of 16 varieties, high nutrient availability from the soil in the tested years resulted in the following:

- Increase in green mass, wood mass, cluster number, cluster weight
- Slight increase in winter tolerance of normally developed buds
- Decrease in mean cluster weight, sugar content of the must, acidity of the must

Varieties show considerable difference in means of the characters measured.

Climatic resistance in some interspecific wine grape hybrid families

EDIT HAJDU

Research Institute for Viticulture and Oenology, H-6001 Kecskemét, Hungary

Summary: In the lowland wine regions, which comprise almost half of the total wine production area in Hungary, meteorological observations indicate that severe winter frosts and deficiencies in precipitation occur in every 3rd year. Under these conditions, stable production requires varieties which can tolerate -23 to -25 °C and can be grown at a low yearly precipitation of 350-400 mm.

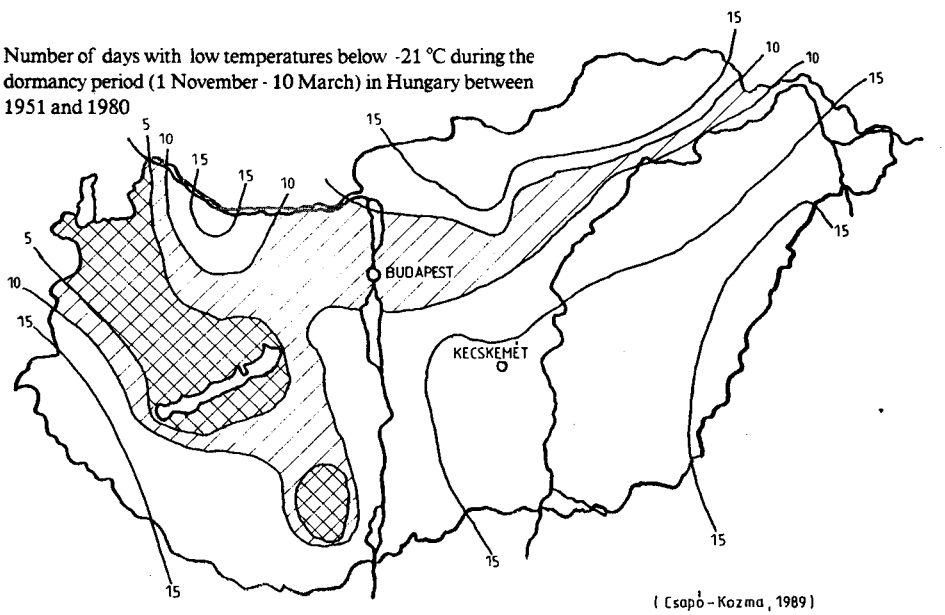
A part of our breeding programme includes breeding of wine grape varieties with climatic resistance. In our crosses interspecific and Eurasian hybrids are used as gene sources. In the progenies of hybrid families some individuals with excellent winterhardiness have segregated. Their distribution and characters are presented.

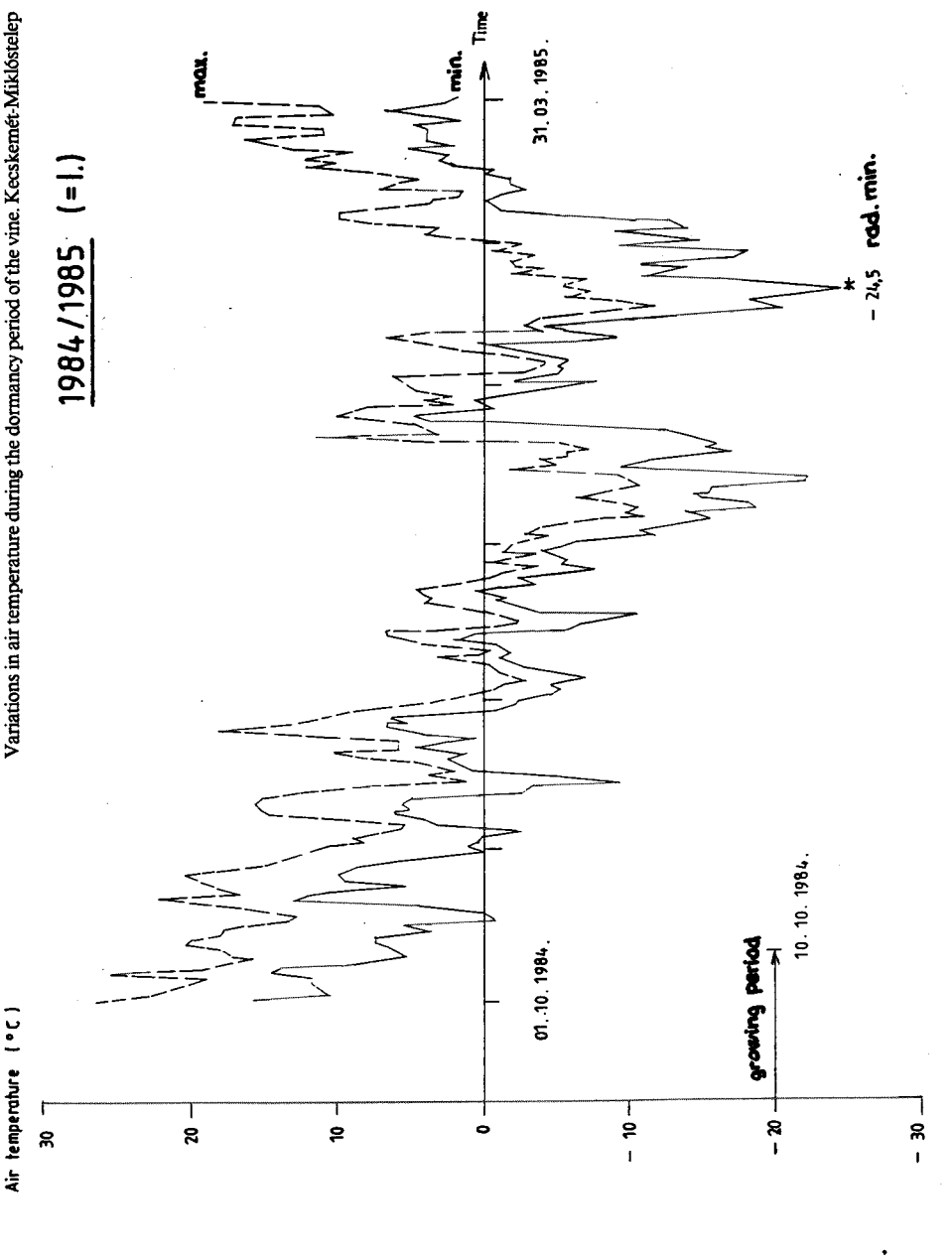
Key words: Hungary, climate, cold, precipitation, winter, dormancy, bud, resistance, selection, hybrid.

Frequency of winters with severe frost in Hungary between 1902 and 1988

Year	Cultivation mode
1928/1929	Low training
1941/1942	" "
1962/1963	" "
1963/1964	" "
1984/1985	High training
1986/1987	" "
1988/1989	" "

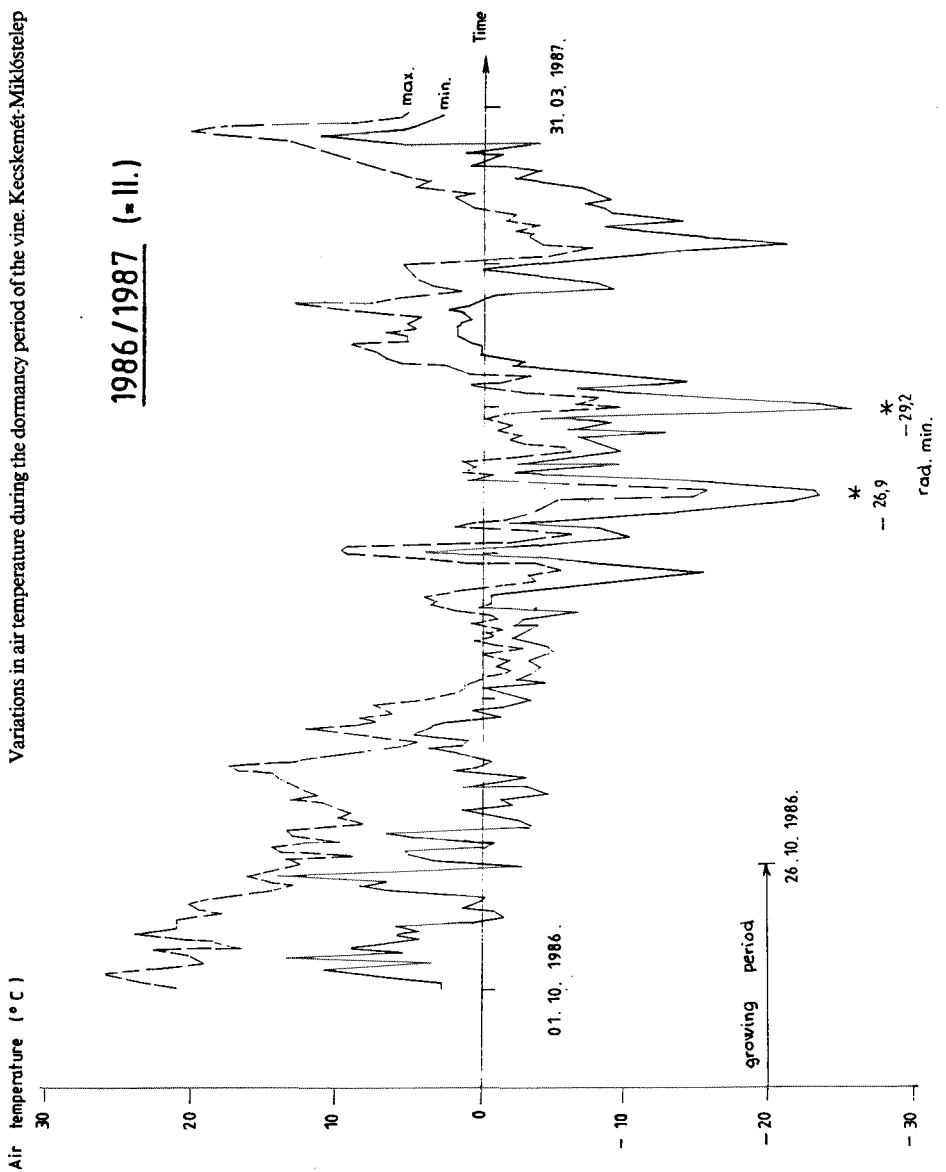
Number of days with low temperatures below -21 °C during the dormancy period (1 November - 10 March) in Hungary between 1951 and 1980



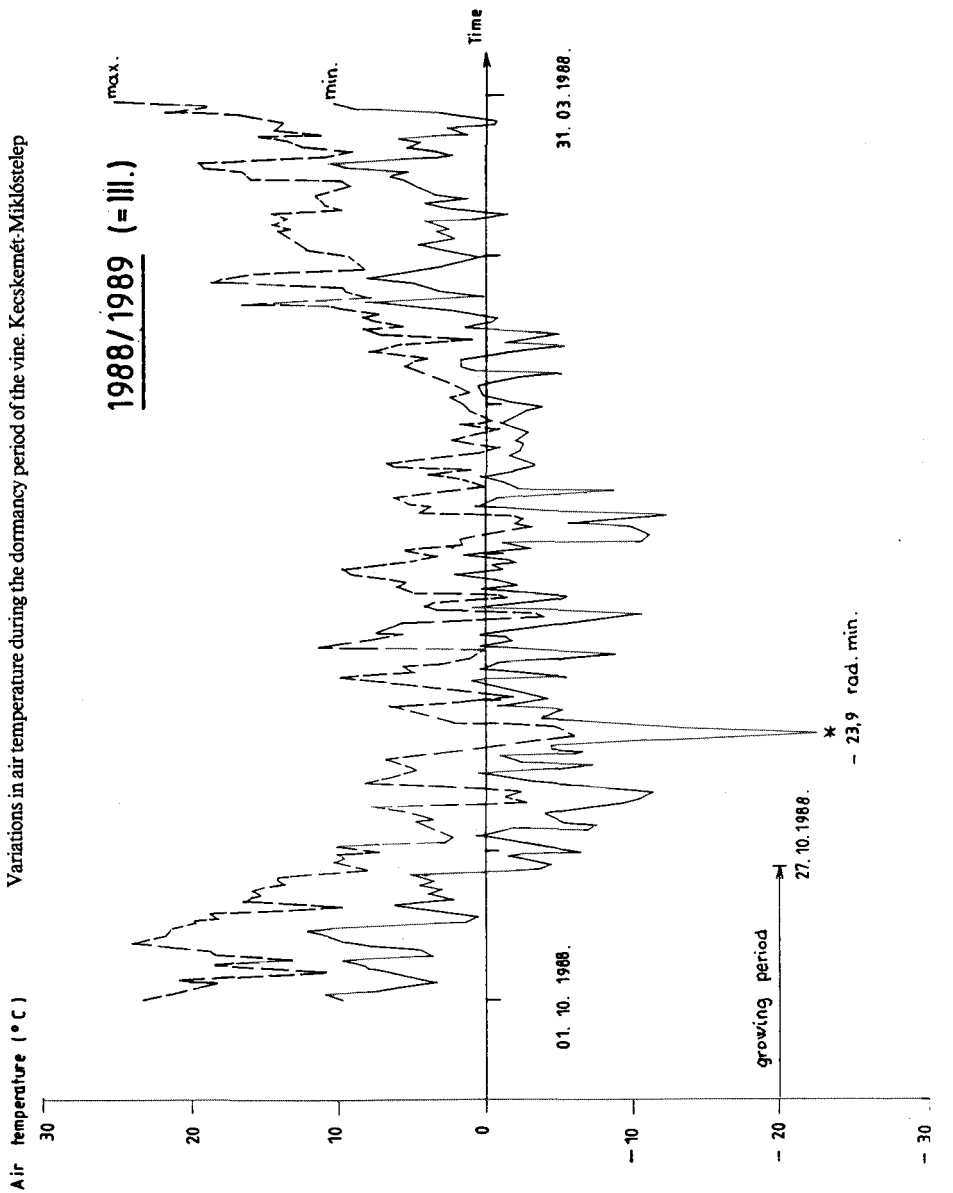


Results

The last three winters with severe frosts (1984/1985, 1986/1987, 1988/1989) exerted high selection pressure on seedlings grown in the field on sandy soils under arid conditions. Selection conditions were excellent for climate tolerance and especially for winter hardiness.

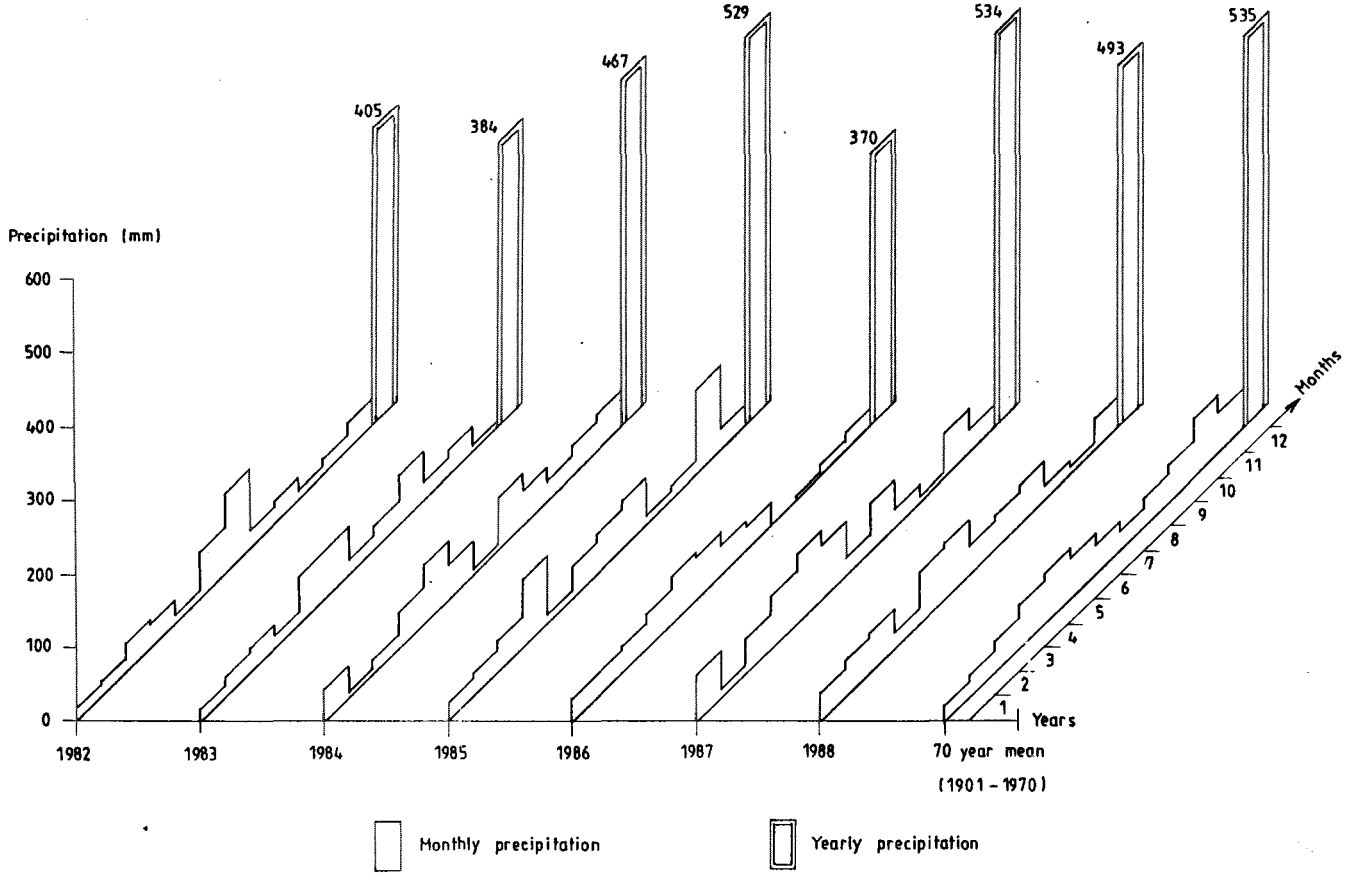


In the winter of 1984/1985 (-23 °C) individuals of good winter tolerance, in the winter of 1986/1987 (-25 °C) individuals of good winter tolerance and relatively good frost tolerance, and at the beginning of winter in 1988/1989 (-24 °C) individuals of good winter tolerance and early dormancy could be selected.



Winter tolerance and relative frost tolerance are well transmitted by the interspecific hybrid varieties RF 48, Zalagyöngye and Kunleány. The interspecific hybrid Kunleány used both as female and male parent in reciprocal crosses transmitted good winter tolerance and also relatively good frost tolerance.

Precipitation distribution per month and year in Kecskemét between 1982 and 1988



Seedling distribution in some hybrid families based on winter hardiness of the main buds in three winters with severe frost
(1984/1985, 1986/1987, 1988/1989 = I., II., III.)

HYBRID CHARACTERISTICS:

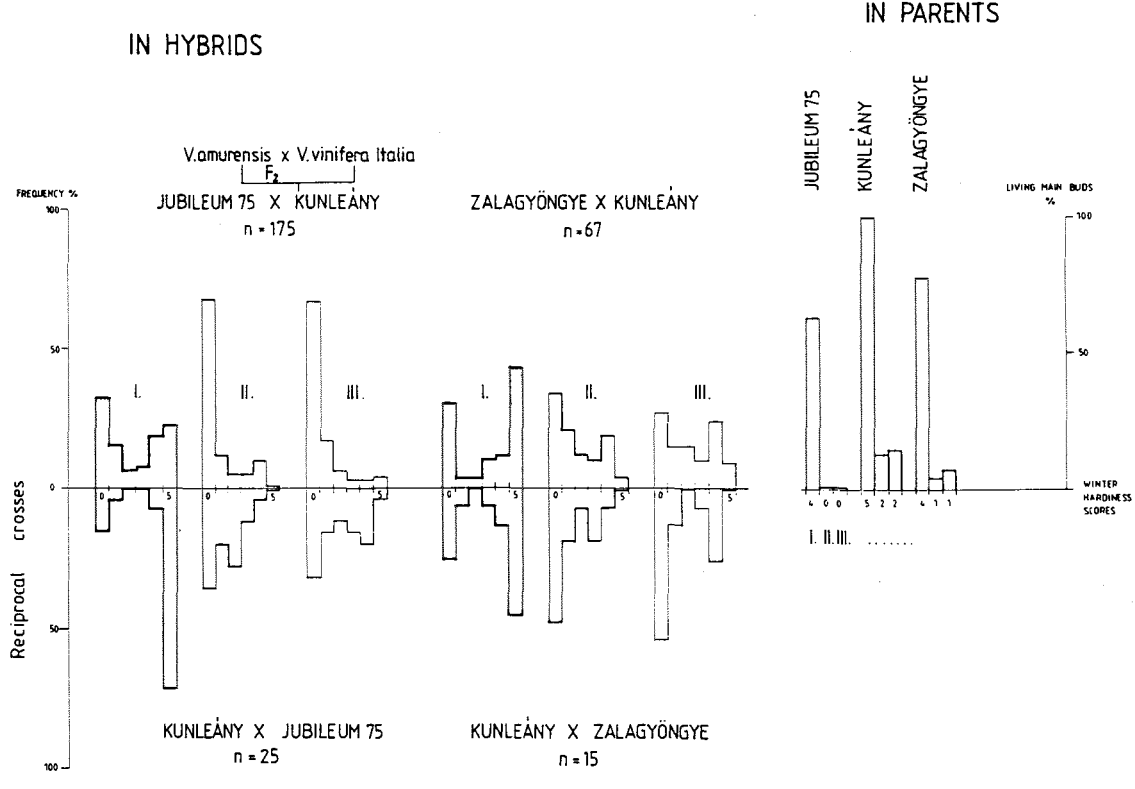
n = seedling number in hybrid families
Year of seedling planting: 1983
Cultivation mode: high training

EVALUATION FOR WINTER TOLERANCE:

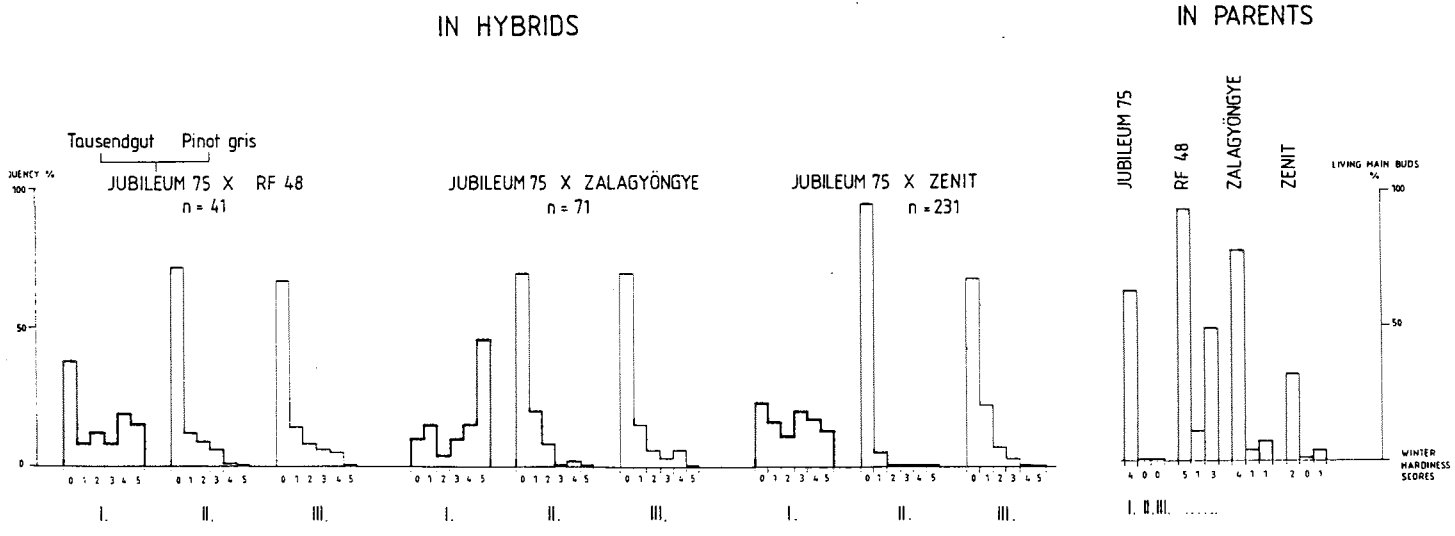
at sprouting: 23. April 1986 (= I.)
6. Mai 1987 (= II.)
19. April 1989 (= III.)

EVALUATION METHOD: scoring

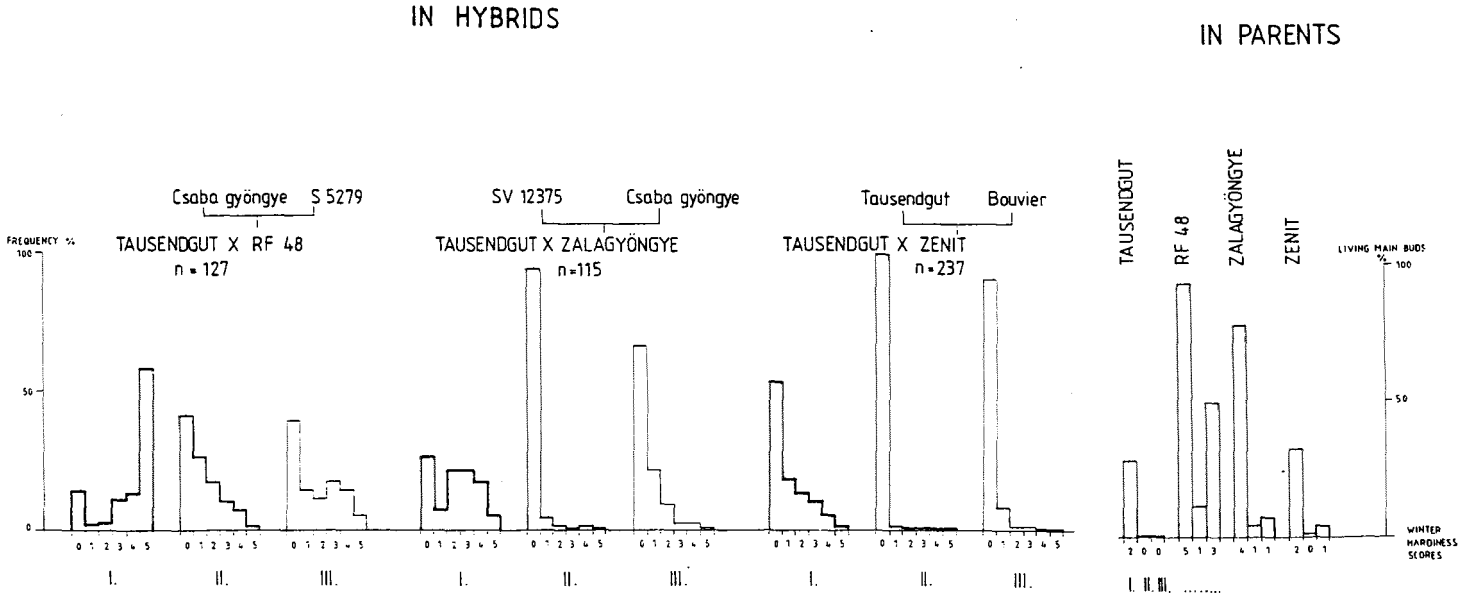
0 = no sprouting
5 = 100% sprouting of main buds



Seedling distribution in some hybrid families based on winter hardiness of the main buds in three winters with severe frost
(1984/1985, 1986/1987, 1988/1989 = I., II., III.)



Seedling distribution in some hybrid families based on winter hardiness of the main buds in three winters with severe frost
(1984/1985, 1986/1987, 1988/1989 = I., II., III.)



Stomatal adaptation of grapevine leaves to water stress

H. DÜRING

Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, F. R. Germany

Summary: Stomata of grapevine leaves respond to the evaporative demand of the atmosphere and to changes of soil water. Leaf epidermis and roots are regarded as sensors of air and soil humidity. Besides a hydraulic communication between soil and leaf, non-hydraulic signals represent a metabolic communication between roots and stomata: stomatal conductance, and thereby the rate of CO_2 assimilation, of turgid vine leaves declined when part of the roots was subjected to progressively drying soil.

Under water stress conditions stomata of leaves reduce transpiration and fully account for putative non-stomatal inhibition of CO_2 assimilation. They enable vine leaves to optimize their CO_2 uptake to water loss ratio (water use efficieny) under sometimes rapidly changing ambient and internal conditions. A close correlation between CO_2 assimilation and stomatal conductance indicates a precise functioning of stomatal action and thereby a high water use efficiency; this correlation coefficient which is generally high in grapevines was demonstrated to increase under water stress conditions. As a screening, the CO_2 assimilation to stomatal conductance ratio and the CO_2 assimilation to transpiration ratio provide valuable information on the water economy of grapevine varieties under drought conditions.

Key words: leaf, stoma, root, photosynthesis, transpiration, water use efficiency, drought, resistance, variety of vine, selection.

In periods of drought, leaves of grapevines are faced with a dilemma: CO_2 assimilation from the atmosphere requires an intensive gas exchange, on the other hand the prevention of excessive water loss demands a reduction of gas exchange. Both, CO_2 uptake and water loss are regulated mainly by turgor-operated valves, the stomata. The ratio of CO_2 assimilation rate of a leaf to its transpiration rate, the water use efficiency (WUE), gives information about the economy of

Table 1: Gas exchange and water use efficiency of field-grown grapevine varieties

	Phoenix	Ga – 47 – 42	Riesling
A, $\mu mol \cdot m^{-2} \cdot s^{-1}$	16,3	11,9	10,8
g_{CO2}, $mmol \cdot m^{-2} \cdot s^{-1}$	126	104	58
E, $mmol \cdot m^{-2} \cdot s^{-1}$	4,9	3,5	1,8
c_i, ppm	220	236	164
A/E	3,3	3,4	6,0
A/g_{CO2}	0,129	0,114	0,186
c_i/c_a at constant Δ_w	0,63	0,67	0,47
WUE	low	low	high

transpirational water loss and, therefore, may help to identify drought tolerant varieties. As is shown in Fig. 1, stomatal conductance of grapevine leaves controles the rate of transpiration and has been reported to fully account for the inhibition of photosynthesis under water stress conditions (DOWNTON *et al.* 1988). Measurements of gas exchange of field-grown cultivars Phoenix, Gf. Ga-47-42 and Riesling (drought tolerant) indicate that at light saturation, ambient CO_2 concentration and favourable air humidity and temperature the rate of photosynthesis (A), stomatal conductance for CO_2 (g_{CO_2}), and transpiration (E) are highest in Phoenix and lowest in Riesling; values of the intercellular CO_2 concentration (c_i) were distinctly lower in Riesling. From these results, the water use efficiency (WUE) can be derived as the A/E, A/g_{CO_2} or c_i/c_a ratio (at constant leaf to air water vapour pressure difference) (Table 1). All three parameters confirm the high WUE of Riesling which can be ascribed to its low stomatal conductance (DÜRING 1987; DÜRING and KLINGENMEYER 1987). Partly closed stomata obviously lower the rate of photosynthesis by decreasing intercellular concentration. But this reduction is relatively small compared to transpiration, thus the WUE of Riesling is increased. Fig. 1 demonstrates that air humidity affects stomatal conductance, an increasing leaf to air water vapour pressure difference leading to a decrease of A, E and g. It is interesting to note that the correlation coefficients Δ_w-g and g-A increase in water stressed vines, in Riesling more than in Silvaner leaves (Table 2). This indicates a higher sensitivity of Riesling leaves to changes in air humidity and also an improved tuning between stomatal conductance and CO_2 assimilation under soil water stress: Stomata operate more precisely (DÜRING 1988).

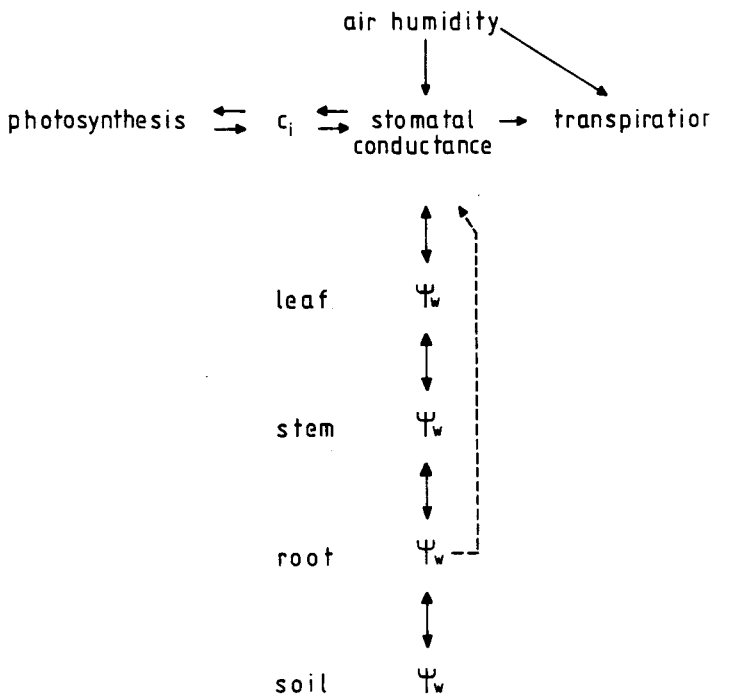


Fig. 1: Stomata of vine leaves play a central role in optimizing gas exchange under changing conditions of ambient soil and air humidity.

The traditional view of the stomatal response to soil drying is that the leaf water potential and turgor decline, thereby promoting stomatal closure. In a root split experiment we have shown that besides hydraulic communications between roots and stomata (Fig. 1) non-hydraulic signals are sent from the roots to the leaves which induce stomatal closure. Although the water potential and turgor of the root split plant indicate a high leaf water status, the stressed part of its roots is suggested to have induced stomatal closure (Fig. 2). These preliminary results indicate that stomatal closure of grapevine leaves can occur independently of any change in leaf turgor but varies as a function of the amount of available soil water. From experiments with other plants it is suggested that roots as sensitive organs measure the available soil water and communicate via chemical signals with the stomata to optimize photosynthesis and transpiration. The ability to sense changes of the available soil water and to induce stomatal closure b e f o r e the leaf water potential declines is assumed to be part of the adaptation mechanisms occurring between drought tolerant rootstocks and scions.

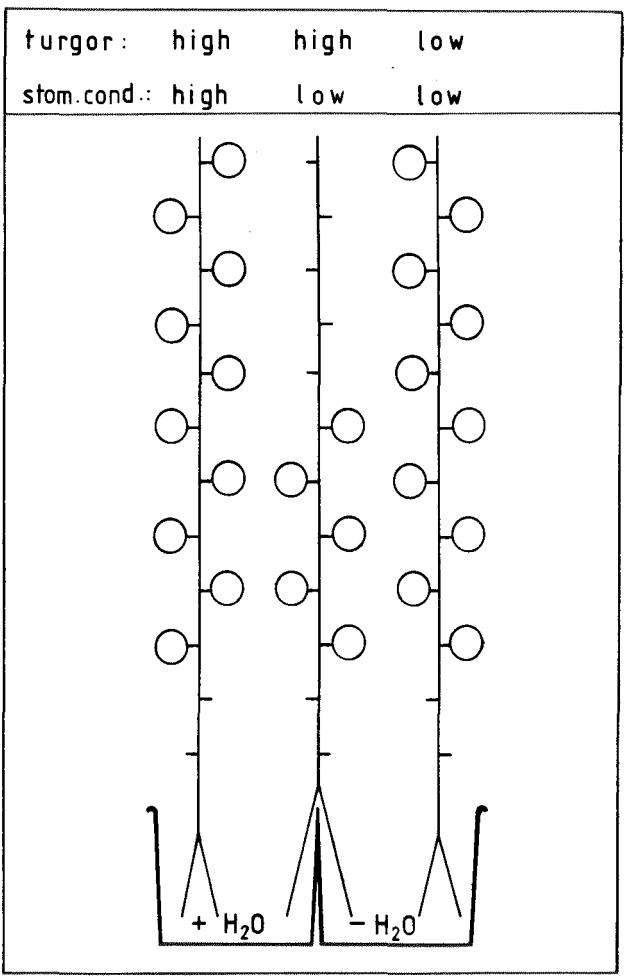


Fig. 2: Root split experiments with 1-year-old Trollinger vines. The root split plant was partly defoliated to compensate its limited water supply.

Table 2: Correlation coefficients of the leaf to air water vapour pressure difference (Δ_w) versus stomatal conductance (g) and of g versus CO_2 assimilation (A) of unstressed and stressed Riesling and Silvaner vines

		correlation coefficient	
variety		air humidity – stom. conductance	stom. conductance – CO_2 assimilation
Riesling	unstressed	−0,39	+0,63
	stressed	−0,82	+0,93
Silvaner	unstressed	−0,40	+0,78
	stressed	−0,72	+0,79

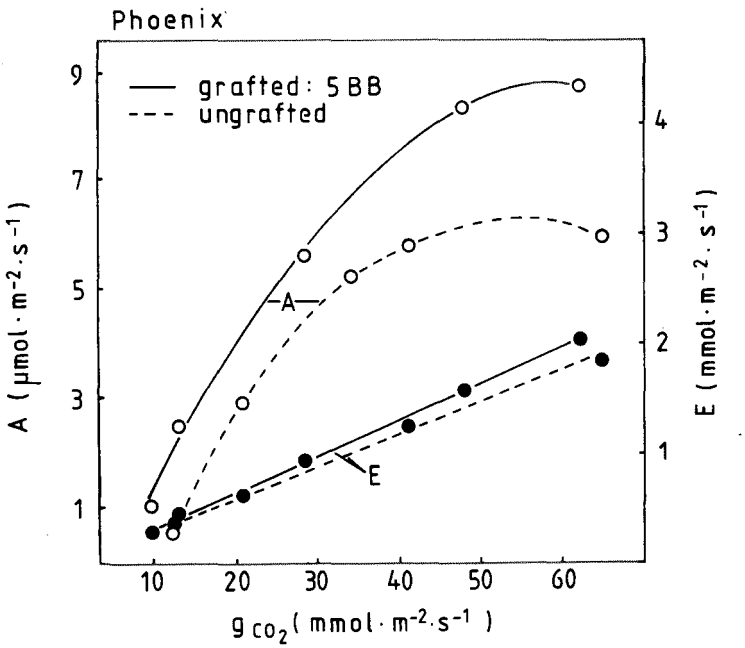


Fig. 3: CO_2 assimilation (A) and transpiration (E) of leaves of ungrafted and grafted Phoenix vines under glasshouse conditions.

Under glasshouse and field conditions several irrigated scion varieties grafted to Kober 5 BB showed higher rates of photosynthesis compared to ungrafted varieties due to a higher carboxylation efficiency (Fig. 3). But only in 1- and 2-year-old vine varieties this higher CO_2 assimilation rate led to increases of the WUE.

In further experiments we will examine the effects of increasing water stress on gas exchange of grafted and ungrafted scions.

Literature

DOWNTON, W. J. S.; LOVEYS, B. R.; GRAND, W. J. R.; 1988: Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis. New Phytol. **110**, 503-509.

DÜRING, H.; 1987: Stomatal responses to alterations of soil and air humidity in grapevines. Vitis **26**, 9-18.

– – ; 1988: CO_2 assimilation and photorespiration of grapevine leaves: Responses to light and drought. Vitis **27**, 199-208.

– – ; KLINGENMEYER, W.; 1987: Stomatal control of water use efficiency in two *Vitis vinifera* cultivars. 3e Symp. Intern. Physiologie Vigne, Bordeaux, 24-27 juin 1986; 179-184.

Influence of drought stress on shoot, leaf growth, leaf water potential, stomatal resistance in wine grape genotypes (*Vitis vinifera* L.) [1]

G. Fanizza and L. Ricciardi

Institute of Plant Breeding, University of Bari, Via Amendola, 165/A, I-70124 Bari, Italy

Summary: Most physiological and morphological tests suggested in the literature for drought resistance are too sophisticated, time-consuming and sometimes unreliable. Quantitative data have indicated that plant growth is very sensitive to water stress. Therefore the elongation rate of shoot and leaf characters (petiole, lamina), which are simple parameters to measure, and leaf water potential and stomatal resistance were determined on 5 sampling dates under water stress and non-stress conditions in different wine grape varieties *(Vitis vinifera)*. The leaf water potential and stomatal resistance show very little variability as well as low correlation in the wine grape varieties considered. On the contrary, the weekly elongation rate of shoot and leaf characters (petiole, lamina) shows high variability and highly significant correlations. Thus some of these morphological characters (lamina and shoot elongation) might be used as test in the early phase (at seedling stage) of a breeding selection program for drought resistance in *Vitis*.

Key words: drought, resistance, shoot, leaf, growth, hydration, analysis, statistics, test, selection, variety of vine, Italy.

Introduction

The literature on drought resistance in plants indicates that almost any parameter of the plant can be changed by water stress since plants are integrated organisms which present different control mechanisms to adjust other processes for counterbalancing the water stress disturbances. In *Vitis*, morphological characters, such as the degree of leaf succulence (Düring and Scienza 1980; Scienza 1983), the stomatal number (Fregoni *et al.* 1978; Düring and Scienza 1980; Zamboni *et al.* 1985), root/shoot ratio (Scienza 1983), and physiological characters, such as leaf water potential and stomatal resistance (Smart 1974; Fregoni *et al.* 1978; Düring and Scienza 1980; Giulivo and Ramina 1981), leaf osmotic potential (Düring 1984), abscisic acid (Loveys and Kriedemann 1973; Fregoni *et al.* 1978; Zamboni *et al.* 1985), have been considered as test methods for drought resistance.

Most of the tests, suggested for annual or perennial species, are too sophisticated and time-consuming. In addition, too few cultivars are used for evaluation of the tests and they are not randomly selected, making these methods unreliable in application. Hsiao and Acevedo (1974) reviewed from literature on the sensitivity of plant processes to water stress; they list cell growth (defined as cell expansion) as the most sensitive parameter to water deficit. Therefore, young leaves and shoots, which present a high metabolic activity for the cell expansion, have been taken into consideration and their growth rate determined as well as the leaf water potential and stomatal resistance in different wine grape cultivars *(V. vinifera)* under water stress and non-stress conditions. This research was carried out to study the variation and covariation among these parameters to determine if some of them (the simplest to measure) are suitable for testing drought resistance in the early phase (at seedling stage) of a grape breeding selection program.

Materials and methods

The study was conducted on 2-year rooted cuttings of 16 wine grape cultivars *(V. vinifera)* of different origin. Each genotype was grown as a single shoot in a large container (1 x 1 x 1 m) used to

[1]) Grant M.P.I. 40 %.

develop drought under more natural conditions; containers were protected from incidental precipitation by a special plastic covering. All varieties were subject to water stressed and non-stressed treatments. The stressed treatment was imposed by withholding irrigation from June 1 throughout the summer. The non-stressed plants were irrigated weekly to field capacity. A completely randomized split plot design (with 4 replications for each variety and treatment) was

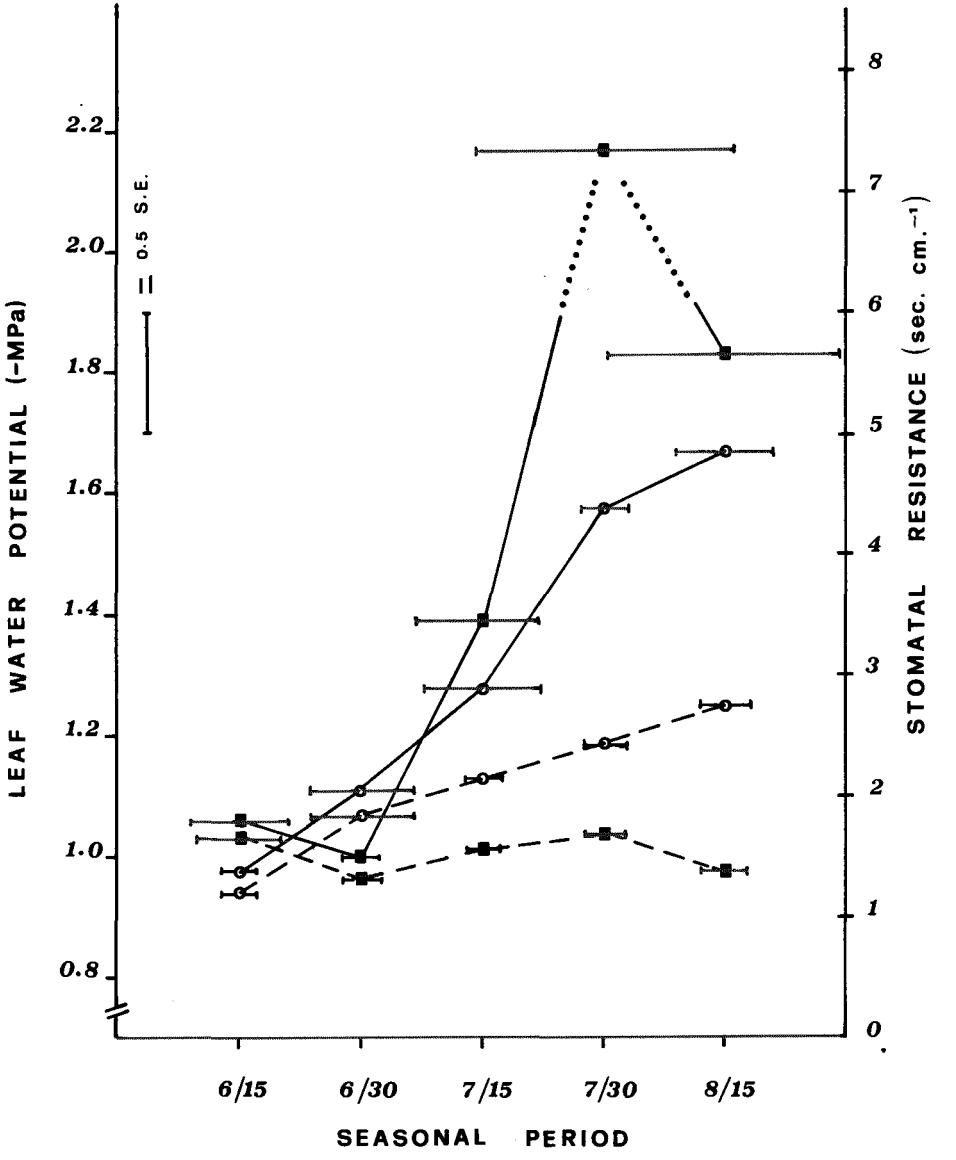


Fig. 1: Leaf water potential (o) and stomatal resistance (■) for midday observations at 5 seasonal sampling dates under water stress (—) and non-stress (– – –) treatments. Values are means of 16 wine grape cultivars. Horizontal bars are S. E.

applied. The leaf water potential (LWP), determinaed by a Scholander pressure chamber, was taken from fully expanded young leaves in the upper third of the shoot; the stomatal resistance (Rs) was measured with a steady state autoporometer on the abaxial surface of the same leaf and

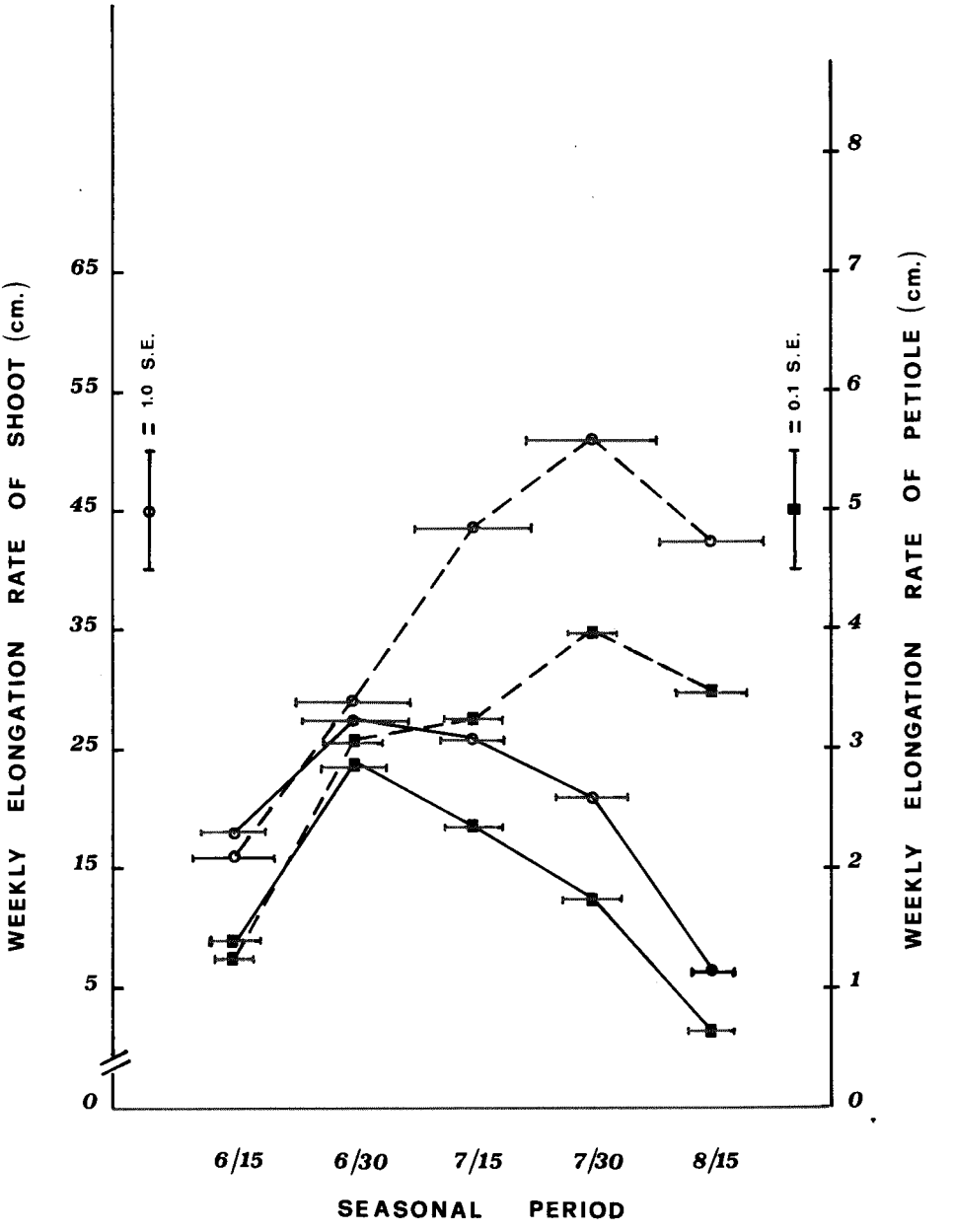


Fig. 2: Growth rate (weekly increase in cm) of shoot (o) and petiole length (■) at 5 seasonal sampling dates under water stress (—) and non-stress (– – –) treatments. Values are means of 16 wine grape cultivars. Horizontal bars are S. E.

repeated on all replications. Both measurements were recorded at midday on 5 sampling dates (June 15 and 30, July 15 and 30, August 15) for both stressed and non-stressed plants.

The variations of the soil water potential were followed through the gravimetric method and through the previously drawn soil moisture release curve for the estimation of the corresponding values of the matrix potential.

The soil water potential of the non-stressed containers remained at about -0.05 MPa. The soil water potential of the stress treatment continued to decline from June 1 without any interruption and reached the value of about -1.0, -1.1 MPa on August 15. Young leaves (the second from the shoot tip) were utilized to measure the weekly rate of growth of the lamina length (ΔL), lamina width (ΔW), petiole length (ΔP) in the 5 mentioned sampling dates; the weekly rate of shoot elongation (ΔS) was also measured. All data were subject to the analysis of variance and Duncan test for the 5 sampling dates, even though the results are reported only for some periods (Tables 1-4). Correlation coefficients between all physiological and morphological parameters were also determined for stressed and non-stressed treatments. The log transformations of LWP data were used for a more homogeneous variance.

Results and discussion

Quantitative data (Hsiao 1973; Hsiao and Acevedo 1974) clearly established that growth is extremely sensitive to water deficit and any reduction in tissue water potential reduces cell expansion in plants. In this work the variation and covariation of the elongation rate of shoots and young leaves, as well as the leaf water potential and stomatal resistance, were studied in several wine grape cultivars grown under water stress and non-stress conditions.

Fig. 1 indicates that the mean leaf water potential of the 16 cultivars increases (more negative) during the seasonal periods considered, in particular under stress condition, where it reaches about -1.7 MPa after 2.5 months from beginning of the stress. The stomatal resistance also tends to increase under stress condition while it shows little seasonal variation under non-stress condition due to the fact that the stomata are not very sensitive to optimal water conditions (Hsiao 1974). Figs. 2 and 3 show that the weekly elongation rate of shoot and leaf characters (petiole, lamina) increases in the first period of stress and then it decreases, while under non-stress condition it increases except in the last observation periods. The graphs show the mean seasonal variation of the cultivars considered for all the physiological and morphological parameters under water stress and non-stress conditions but they do not indicate the difference among varieties. The analysis of variance, reported only for two representative periods, shows no significant differences among varieties and between treatments for all characters at 1 month of stress (Table 1), while significant differences were detected after that period (Table 2) for the same characters, except for the leaf water potential, which presents also low coefficient of variability (about 4 %) both under stress and non-stress conditions. The stomatal resistance (Rs) shows some variability (CV = 10 % S and 6 % NS) but large variation was also detected among replications. In contrast, the weekly growth rate of shoot and leaf characters presents a larger variability, in particular during the last period of stress (Table 2, the CV was over 20 %). As significant differences among varieties were detected in the last periods of stress (Table 2) a Duncan test was performed for these data even though it is only reported for the last date (August 15) (Tables 3-4). Table 3 shows that there is little difference among varieties in the leaf water potential for midday observations under stress and non-stress conditions on the last sampling date but also the same trend appears in the other periods.

As far as the stomatal resistance is concerned, the difference among varieties was not tested (Table 3) because of high variability among replications, which might be due to sampling error attributable to circumstances external to the operator (leaf shading, leaf orientation etc.).

The elongation rate of shoot and leaf characters differs among varieties not only in the last period of stress (Table 4) but also in other periods. Thus the study of the variability of our wine

grape population suggests that the morphological characters are better parameters to discriminate varieties under stress conditions. Correlation coefficients were also computed to study the relationships among the physiological and morphological characters in order to choose those which can be used as an index for drought resistance in *Vitis*. Table 5 shows that there is not a very high correlation (r = 0.35) between leaf water potential and stomatal resistance under water stress and no correlation (r = 0.01) under non-stress conditions. It has been shown (Hsiao and Acevedo 1974; West and Gaff 1976; Syvertsen 1987) that stomata remain unaffected until the leaf water

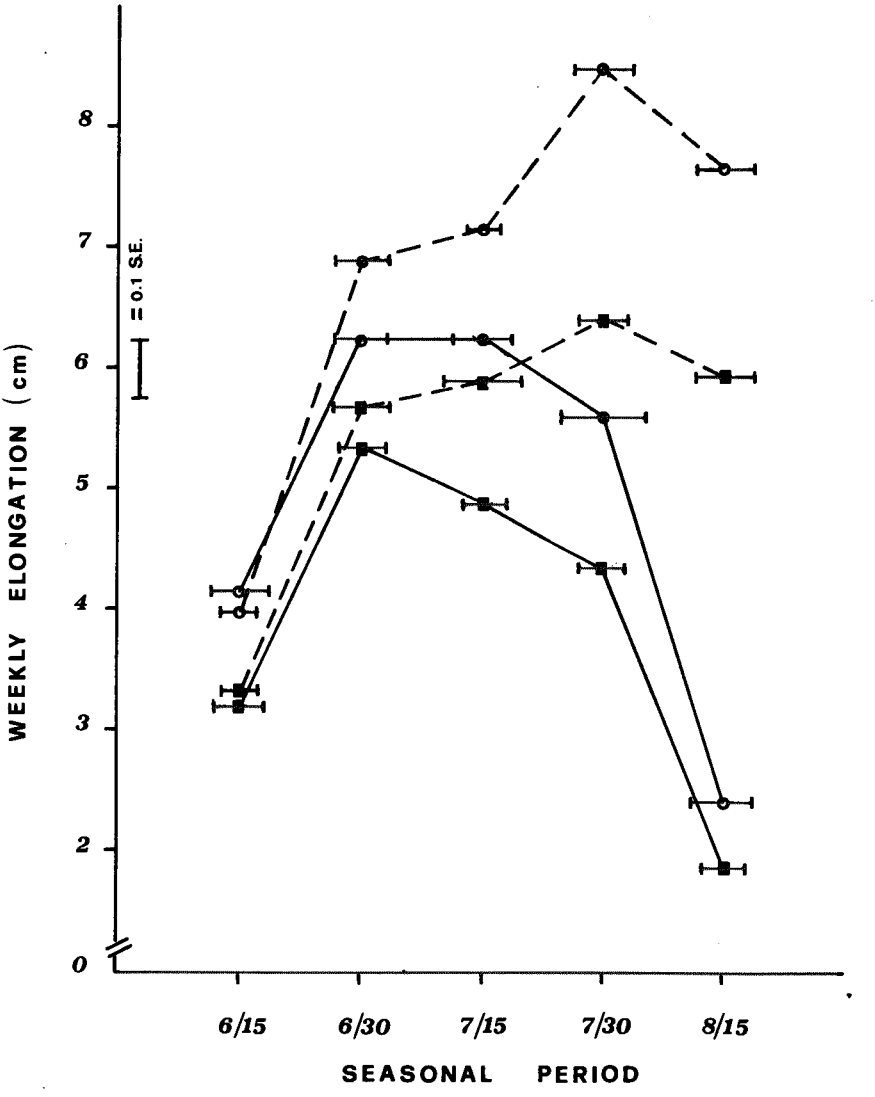


Fig. 3: Growth rate (weekly increase in cm) of lamina length (■) and lamina width (o) at 5 seasonal sampling dates under water stress (—) and non-stress (– – –) treatments. Values are means of 16 wine grape cultivars. Horzontal bars are S. E.

potential drops to a critical value and that the mechanism of stomata closure induced by water deficit is not a simple loss of turgor from the leaf but rather a complex mechanism.

Low correlations were found among the physiological parameters (leaf water potential, stomatal resistance) and morphological ones (elongation rate of shoot and leaf characters) under stress condition, while no correlations were shown under non-stress condition. A lack of correlation between leaf water potential and fruit growth has been found in citrus and other species

Table 1: Descriptive statistics for midday leaf water potential (LWP), stomatal resistance (Rs), and the growth rate (weekly increase) of shoots (ΔS), leaf characters (petiole length (ΔP), lamina length (ΔL), lamina width (ΔW)) at 1 month water stress (S) and non-stress treatments (NS) in 16 wine grapes

Variables	Treatments+	Mean	S E	Range	CV
LWP	S	1.10	0.01	1.05-1.25	4.1
(-MPa)	NS	1.07	0.03	1.05-1.10	2.2
Rs	S	1.4	0.4	1.1- 1.9	6
(sec.cm^{-1})	NS	1.4	0.5	1.1- 1.6	6
ΔS	S	26	1.0	13 -49	17
(cm.)	NS	28	0.8	16 -59	16
ΔP	S	2.8	0.07	1.9- 4.2	13
(cm.)	NS	3.0	0.05	2.0- 5.3	12
ΔL	S	5.3	0.1	4.5- 6.8	11
(cm.)	NS	5.6	0.1	3.7- 8.3	11
ΔW	S	6.5	0.8	5.3- 8.2	10
(cm.)	NS	6.8	1.1	4.0- 9.2	11

+ No significant differences were detected by analysis of variance (F test) for treatments, varieties x treatments, varieties.

Table 2: Descriptive statistics for midday leaf water potential (LWP), stomatal resistance (Rs), and the growth rate (weekly increase) of shoots (ΔS), leaf characters (petiole length (ΔP), lamina length (ΔL), lamina width (ΔW)) at 2.5 months water stress (S) and non-stress treatments (NS) in 16 wine grapes

Variables	Treatments[+]	Mean	S E	Range	CV	V[+]	VxT[+]
LWP (-MPa)	S 128**	1.7	0.07	1.6-1.7	4.0	0.01 n.s	0.1**
	NS	1.25	0.08	1.2-1.3	3.0		
RS ($sec.cm^{-1}$)	S 440**	5.7	1.2	3.4- 8.1	10	0.04 n.s	3.1**
	NS	1.4	0.6	1.0- 1.9	6		
ΔS (cm.)	S 326**	5.6	0.8	2.0-11	23	240**	262**
	NS	42.5	1.0	23 -73	18		
ΔP (cm.)	S 207**	0.6	0.02	0.1- 1.3	29	0.7**	1.3**
	NS	3.5	0.06	2.2- 5.5	11		
ΔL (cm.)	S 414**	1.8	0.1	0.5- 2.8	25	2.4**	3.5**
	NS	5.9	0.1	3.7- 8.2	11		
ΔW (cm.)	S 642**	2.4	0.7	1.3- 3.8	22	3.4**	5.8**
	NS	1.4	0.2	4.7- 9.8	10		

[+] Variances and statistical significance by analysis of variance for treatments, varieties (V), varieties x treatments (VxT) at .01 P level (**).

(ELFVING and KAUFMANN 1972). This might be due to the fact that the physiological or physical mechanisms that control cell growth, water balance and stomata closure in the plants are independent processes. In contrast, very high correlations were shown among the morphological characters. This suggests that some of these parameters might be used as selection indices for drought resistance. In particular, the elongation rate of the lamina or of the shoot, which are simple

Table 3: Cultivar means for midday leaf water potential (LWP) and stomatal resistance (Rs) at 2.5 months water stress (S) and non-stress (NS) treatments

Cultivars	Variables treatments	LWP (-MPa)		Rs ($sec.cm^{-1}$)	
		S	NS	S	NS
Teroldego		1.75a*	1.30a	4.5+	1.4
Sangiovese		1.70a	1.30a	4.5	1.3
French Colomb.		1.70a	1.25ab	6.4	1.1
Peverella		1.70a	1.25ab	5.1	1.9
Montepulciano		1.70a	1.30a	6.7	1.4
Verdeca		1.70a	1.25ab	4.6	1.6
Sangiovese C.		1.70a	1.25ab	7.2	1.6
Aleatico		1.70a	1.25ab	4.6	1.1
Greco		1.65ab	1.25ab	6.7	1.5
Barbera		1.65ab	1.20b	4.1	1.3
Trebbiano		1.65ab	1.20b	4.4	1.1
Malvasia Candia		1.65ab	1.20b	8.1	1.4
Malvasia Nera		1.65ab	1.30a	7.5	1.1
Negro Amaro		1.65ab	1.20b	4.6	1.4
Bombino b.		1.65ab	1.20b	7.8	1.7
Rubired		1.60b	1.25ab	4.1	1.3

* Numbers in the same column followed by the same letter are not different at .01 P level as determined by Duncan test.

+ The significant difference (Duncan test) are not reported because of the high sampling error within each genotype.

parameters to measure, might represent an easy and non destructive test method to be used in the early phase (at seedling stage) of a breeding selection program for drought resistance in *Vitis*. However, further studies are needed to apply this selection test for mature grapevines.

Table 4: Cultivar means for the growth rate (weekly increase) of shoots (Δ S), leaf characters (lamina length (ΔL), lamina width (ΔW), petiole length (Δ P)) under 2.5 months water stress (S) and non-stress (NS) treatments

Cultivars	Variables / Treatments	ΔS		ΔL		ΔW		ΔP	
		S	NS	S	NS	S	NS	S	NS
Teroldego		2.3 e*	38 g	0.5 h	4.6 f	1.3 f	6.1 f	0.1 i	3.1 ef
Sangiovese		8.6 ab	49 c	2.8 a	5.6 e	3.2 b	7.3 d	1.1 a	3.4 cde
French Colom.		2.3 e	55 b	1.3 cd	7.9 a	1.8 e	9.8 a	0.6 cdef	5.2 a
Peverella		2.3 e	47 cd	0.8 g	5.8 de	2.2 d	8.0 c	0.2 hi	3.2 def
Montepulciano		6.3 d	34 g	2.6 c	6.6 c	3.1 b	8.2 c	0.8 bcd	3.7 bc
Verdeca		3.0 e	44 de	1.7 c	6.8 bc	2.1 d	9.2 b	0.4 fghi	3.6 bc
Sangiovese C.		7.0 cd	70 a	1.6 c	7.0 b	1.5 ef	9.0 b	0.5 defg	3.9 b
Aleatico		9.0 ab	41 ef	2.7 a	5.7 de	3.8 a	7.9 c	0.7 cde	3.2 ef
Greco		6.3 d	67 a	1.2 ef	6.0 d	1.7 e	8.9 b	0.5 defg	3.9 b
Barbera		4.0 e	43 def	0.9 fg	5.5 e	1.5 ef	6.4 ef	0.2 hi	3.2 ef
Trebbiano		8.0 bc	39 f	2.6 a	5.6 e	3.7 a	7.4 d	0.8 abc	2.9 fg
Malvasia Candia		3.0 e	26 h	1.1 ef	3.7 g	1.4 f	4.7 h	0.3 ghi	2.6 gh
Malvasia Nera		7.0 cd	34 g	2.0 b	8.2 a	2.7 c	8.9 a	0.8 bcd	5.0 a
Negro Amaro		10.0 a	32 g	2.7 a	5.9 de	3.3 b	6.6 e	1.0 ab	3.6 bc
Bombino		8.0 bc	32 g	2.6 a	5.9 de	3.1 b	7.5 d	1.1 a	4.0 b
Rubired		7.0 cd	32 g	1.5 cd	4.6 f	2.8 c	5.3 g	0.8 bcd	2.4 h

* Numbers in the same column followed by the same letter are not different at .01 P level as determined by Duncan test.

Table 5: Correlation coefficients of leaf water potential (LWP), stomatal resistance (Rs), and weekly growth rate of shoot length (ΔS), lamina length (ΔL), lamina width (ΔW) and petiole length (ΔP) under water stress (above) and non-stress (below) treatments

Variables	Rs	ΔS	ΔL	ΔW	ΔP
LWP	0.35** 0.01	0.31** 0.03	0.36** 0.05	0.32** 0.04	0.33** 0.02
Rs		-0.29** -0.05	-0.33** -0.03	-0.32** -0.04	-0.31** -0.02
ΔS			0.85** 0.76**	0.76** 0.72**	0.73** 0.62**
ΔL				0.93** 0.90**	0.91* 0.87*
ΔW					0.87* 0.86*

* $P \leq 0.05$
** $P \leq 0.01$

Literature cited

DÜRING, H.; 1984: Evidence for osmotic adjustment to drought in grapevines (*Vitis vinifera*). Vitis **23**, 1-10.

---; SCIENZA, A.; 1980: Drought resistance of some *Vitis* species and cultivars. Proc. 3rd Intern. Symp. Grape Breeding, Davis, 1980, 179-190.

ELFVING, D. C.; KAUFMANN, M. R.; 1972: Diurnal and seasonal effects of environment on plant water relations and fruit diameter of citrus. J. Amer. Soc. Hort. Sci. **97**, 566-570.

FREGONI, M.; SCIENZA, A.; MIRAVALLE, R.; 1978: Evaluation précoce de la résistance des porte-greffes à la sécheresse. Génétique et Amélioration de la Vigne, Bordeaux, 287-296. INRA, Paris.

GIULIVO, C.; RAMINA, A.; 1981: Studies on water relations of grapevines *(Vitis vinifera)*. Acta Hort. **119**, 109-121.

HSIAO, T. C.; 1973: Plant responses to water stress. Ann. Rev. Plant Physiol. **24**, 519-570.

-- --; ACEVEDO, E.; 1974: Plant responses to water deficit, water-use efficiency, and drought resistance. Agricult. Meteor. **14**, 59-84.

JOHNSON, H. W.; ROBINSON, H. F.; COMSTOCK, R. E.; 1955: Estimates of genetic and environmental variability in soybean. Agron. J. **47**, 314-318.

LOVEYS, B. R.; KRIEDEMANN, P. E.; 1973: Rapid changes in abscisic acid like inhibitor following alterations in vine leaf water potential. Physiol. Plant. **28**, 476-479.

SCIENZA, A.; 1983: Adattamento genetico della vite allo stress idrico. Vignevini **6**, 27-39.

SMART, R. E.; 1974: Aspects of water relations of the grapevine *(Vitis vinifera)*. Amer. J. Enol. Viticult. **25**, 84-91.

SYVERTSEN, J. P.; 1982: Minimum leaf water potential and stomatal closure in citrus leaves of different age. Ann. Bot. **49**, 827-834.

WEST, D. W.; GAFF, D. F.; 1976: The effect of leaf water potential, leaf temperature and light intensity on leaf diffusion resistance and the transpiration of leaves of *Malus sylvestris*. Physiol. Plant. **38**, 98-104.

ZAMBONI, M.; FREGONI, M.; IACONO, F.; 1986: Comportamento di specie ed ibridi di vite in condizioni di siccità. Vignevini, Suppl. al (12), 119-122.

Stomatal density in various Turkish grape cultivars

A. Eris and A. Soylu

Department of Horticulture, Faculty of Agriculture, Uldag University, P. K. 343, 16180 Bursa, Turkey

Summary: In this study the number of stomata per unit area and dimensions of the stomata were determined in 15 important Turkish grape cultivars which were 20 years old and grafted on Kober 5 BB rootstock. Number of stomata per mm^2 of area varied from 129 ± 18 to 254 ± 10, stomatal length ranged from 22.6 ± 2.6 μm to 28.3 ± 4.3 μm, and stomatal width from 13.6 ± 2.2 μm to 18.6 ± 3.2 μm among cultivars. The cultivars having minimum and maximum number of stomata per unit area were Balbal and Pembe Gemre; Erenköy Beyazi and Müsküle had minimum and maximum stomata size, respectively. Correlations between the number of stomata per unit area and the stomatal length ($r = 0.100$), and width ($r = 0.184$), and between the stomatal length and width ($r = 0.493$) were not significant among cultivars.

On the other hand, Balbal, Hafizali and Yapincak were found to be more tolerant than the other cultivars.

The characteristics of the cultivars in relation to their drought tolerance and stomatal densities were discussed.

Key words: stoma, leaf, transpiration, water, drought, resistance, variety of vine, Turkey.

Introduction

The vineyards of Anatolia are spread over the dry regions in which the summer rainfall is often insufficient. For this reason vine growers make use of the spring and winter rainfall and give more importance to special cultivation practices. Today more than 1000 grape cultivars (possibly some of them are synonymous) are grown in Turkey. These cultivars are different from each other with respect to their qualitative and physiological characteristics. In the adaptation to different ecological conditions (e. g. drought) both rootstocks' and cultivars' own characteristics are important factors to postpone dehydration. Deep root system, thick cutin, and good stomatal control of transpiration as well as the capacity of protoplasm to endure desiccation are the main factors in drought tolerance (Kramer and Kozlowski 1979).

Root system development is dependent on the rootstock variety. Morphological features of the leaves are also important in this respect. According to Kisselew (cited in Levitt 1980), the small-leaf forms of *Scorzonera* survive a greater loss of water then the broad-leaf forms. Runyon (cited in Kramer and Kozlowski 1979) found that the leaves of creosote bush produced during moist weather are large and easily injured by water deficit, but the leaves produced during dry weather can be dried to a saturation deficit of 50 %. Desiccation tolerance of the leaves also shows a great variance between species. Oppenheimer (cited in Kramer and Kozlowski 1979) reported that the leaves of almond could be dried to a saturation deficit of 70 % and olive to 60 % before injury occurred, but fig dried to only 25 %.

The volume/surface ratio of the cells of drought tolerant plants is low. Iljin (cited in Levitt 1980) concluded that this ratio is 1-2 in xerophyllic mosses; near 20 in sensitive cells; 5-10 in cells of intermediate tolerance. Merieux *et al.* (cited in Levitt 1980) found that *Vitis vinifera* plants grown with low moisture have smaller cells and are more drought tolerant.

High osmotic pressure has also been regarded as an important adaptation to water stress (Kramer and Kozlowski 1979).

The size and frequency of stomata have an importance in the physiology of the plants. The relations between stomatal size and frequency and transpiration rate are still uncertain (Vardar 1972). However, plants native to arid regions and regions with long summer droughts have heavily cutinized leaves and very low transpiration rates after the stomata have closed (Kramer and Kozlowski 1979). Stomatal frequency of the drought resistant xerophyte plants is generally high

Table 1: Stomatal frequency of various grape cultivars

Cultivars	Number / mm^2
Kozak Beyazı	201 ± 23
Siyah Razakı	205 ± 25
Çavuş	187 ± 16
Amasya	197 ± 8
Müşküle	140 ± 9
Alphonse[1)]	179 ± 9
Hafızali	192 ± 10
Sultani Çekirdeksiz	251 ± 9
Tarsus Beyazı	153 ± 9
Yapıncak	192 ± 19
Balbal	129 ± 18
Erenköy Beyazı	151 ± 5
Pembe Gemre	254 ± 10
Öküzgözü	159 ± 18
İskenderiye Misketi	184 ± 24
Bıca Razakısı	200 ± 19

1) To be considered only in the calculation of the correlation coefficients

(VARDAR 1972). In addition, some variances have occurred in the structure of stomata of the plants grown under different ecological conditions (MEIDNER and MANSFIELD 1968).

According to SMART (cited in WEAVER 1976), stomata closed at a leaf water potential of -13 bars in vines. In some vine cvs at pre-dawn water potentials down to -8 bars, assimilation and especially transpiration were reduced (DÜRING and KLINGENMEYER 1987). However, stomatal control of transpiration rate varies among the species (KRAMER and KOZLOWSKI 1979). JOHNS and LAZENBY (cited in LEVITT 1980) stated that stomatal control of senescing leaves may be ineffective leading to their death, whereas young leaves are uninjured. Cv. Riesling, considered drought tolerant, has low stomatal conductance resulting in high water use efficiency (ratio CO_2 uptake/H_2O loss) and low hydraulic conductance (DÜRING 1988).

The stomatal frequencies of some grape species, hybrids and cultivars have long been studied (DÜRING 1980; SCIENZA and BOSELLI 1981; DÜZENLI and ERGENOGLU 1988). Many factors can affect the frequency and/or size of stomata such as nutritional conditions (BALO *et al.* 1986), K fertilization (BOSELLI and SCIENZA 1983), N fertilization (RÜHL and IMGRABEN 1985), fungicide applications (SEKERA 1983), vine vigor, leaf position (FORLANI *et al.* 1983), leaf maturity (DÜRING 1980), shading (KRAMER and KOZLOWSKI 1979), rootstocks and training systems (DÜZENLI and ERGENOGLU 1988).

In this study stomata size, density and drought tolerance of some Turkish grape cultivars were determined and discussed.

Materials and methods

The following cultivars were used: Kozak Beyazi, Siyah Razaki, Cavus, Amasya, Müsküle, Hafizali, Sultani Cekirdeksiz (Sultanina), Tarsus Beyazi, Yapincak, Balbal, Erenköy Beyazi, Pembe Gemre, Öküzgözü, Iskenderiye Misketi (Muscat d'Alexandrie) and Buca Razakisi. The cultivars were 20 years old and trained to Guyot and Cordon systems. All vines were grafted on Kober 5 BB rootstock.

Stomatal counts were made on leaves taken from the 3rd-5th nodes after the leaves had reached full maturity. Nail polish, diluted in some degrees with acetone, was spread on the mid portion of upper and lower surface of the leaves and later removed. These moulds were used for countings and measurements. In total, 135 microscopic view fields were considered in the countings. Stomata length and width were measured on 30 randomly selected stomata.

Drought tolerance of the cultivars was determined on plants obtained from rooted cuttings. For this purpose, cuttings of the cultivars were collected just before spring, prepared with one bud and set in boxes containing perlite, then placed in the greenhouse. Temperature was held between 25 and 28 °C. Cuttings leafed out and rooted in this environment and were watered and fertilized with HOAGLAND nutrient solution (HOAGLAND and ARNON 1950). After the shoots had 4-5 leaves, watering was interrupted and the young plants were exposed to water stress. Plants did not receive water during a period of 27 d. At the end of this period, plants were rewatered. The cultivars were then evaluated for injury from water deficit. Wilting of the leaves was recorded since it could be used for indirect estimates of the water status (KRAMER and KOZLOWSKI 1979) and drought tolerance of the vine cvs (DÜRING 1988). In addition, yellowing of the leaves and the plants completely injured from water deficit was also recorded. In total, 30 plants (3 x 10) of each cultivar were used in the experiment.

Results

Stomatal density and size of stomata

No stomata were observed on the upper surface of the leaves. However, stomatal frequency on the lower surface of the leaves revealed great variance among the cultivars (Table 1). Minimum and maximum stomatal frequencies (number/mm^2) were determined in Balbal (129 ± 18) and Pembe Gemre (254 ± 10) cvs. The frequencies of other cultivars were between these two values.

Mean stoma length and width of the cultivars are shown in Table 2. Some differences also existed among the cultivars in this respect. Mean stoma length ranged from 22.6 ± 2.6 to 28.3 ± 4.3 μm, and mean stoma width from 13.6 ± 2.2 to 18.6 ± 3.2 μm. Müsküle and Erenköy Beyazi cvs have maximum and minimum values of both stoma length and width, respectively.

When taking into account the cultivars together, the correlation coefficients between the stomatal frequency and stoma length and width were found to be non-significant ($r = 0.100$ and

Table 2: Mean stoma length and width of various grape cultivars

Cultivars	Stoma length (μm)	Stoma width (μm)
Kozak Beyazı	26.0 ± 3.5	18.4 ± 3.4
Siyah Razakı	27.2 ± 4.7	17.9 ± 3.0
Çavuş	25.7 ± 3.6	17.6 ± 2.6
Amasya	24.1 ± 3.4	17.2 ± 2.3
Müşküle	28.3 ± 4.3	18.6 ± 3.2
Alphonse 1)	24.6 ± 3.3	18.4 ± 2.6
Hafızali	24.4 ± 3.6	16.4 ± 1.6
Sultan Çekirdeksiz	24.4 ± 3.2	17.2 ± 2.0
Tarsus Beyazı	24.5 ± 3.4	15.5 ± 3.1
Yapıncak	27.1 ± 4.1	15.1 ± 4.4
Balbal	23.0 ± 3.3	16.1 ± 2.4
Erenköy Beyazı	22.6 ± 2.6	13.6 ± 2.2
Pembe Gemre	24.2 ± 3.1	16.3 ± 1.8
Öküzgözü	23.8 ± 3.0	16.6 ± 3.1
İskenderiye Misketi	25.0 ± 3.8	17.4 ± 3.3
Buca Razakısı	26.6 ± 4.2	16.4 ± 2.6

1) To be considered only in the calculation of the correlation coefficients

r = 0.184, respectively). However, the correlation coefficient between stoma length and width was relatively high, though not significant (r = 0.493).

Drought tolerance of the cultivars

The percentages of completely injured plants at the end of the 27 d water stress period are shown in Table 3. The cultivars which suffered the most from water deficit were Cavus, Tarsus Beyazi and Amasya. Balbal, Hafizali, Yapincak and Erenköy Beyazi suffered much less injury than the other cultivars. After rewatering, injury in some of the cultivars continued rapidly. For example, total loss in Erenköy Beyazi increased from 16.6 % to 86.6 %. On the other hand, this increase in Yapincak was only 10 % in the last 7 d of the test period (Table 3).

Injury from water deficit began early and increased rapidly in some of the cultivars (e. g. Amasya, Tarsus Beyazi and Cavus). Some of the cultivars could tolerate drought until a certain time and then collapsed rapidly (e. g. Iskenderiye Misketi, Erenköy Beyazi and Buca Razakisi).

Table 3: Percentages of the completely injured plants

Cultivars	Completely Injured Plants (%) At the end of 27 days of unwatering period	At the end of 7 days after rewatering point
Kozak Beyazı	(No data obtained)	
Siyah Razakı	30.0	56.6
Çavuş	96.6	100
Amasya	100	100
Müşküle	36.6	66.6
Hafızali	13.3	53.3
Sultani Çekirdeksiz	76.6	93.3
Tarsus Beyazı	100	100
Yapıncak	16.6	26.6
Balbal	10.0	43.3
Erenköy Beyazı	16.6	86.6
Pembe Gemre	33.3	66.6
Öküzgözü	23.3	66.6
İskenderiye Misketi	40.0	83.3
Buca Razakısı	33.3	70.0

Commencement of the injury was late and the total loss was low in Balbal and Yapincak (Fig.). Siyah Razaki, Pembe Gemre, Hafizali, Öküzgözü and Müsküle were between the two extremes and showed medium degree of injury.

Injury due to water deficit appeared first as yellowing and wilting of the leaves and was followed by death of the shoots. However, yellowing of the leaves was much less distinct in Cavus and Tarsus Beyazi cvs than in the other ones. Drooping leaves were the first sign in Cavus, at the beginning of injury.

Discussion

There are few studies on the stomatal frequency of Turkish grape cultivars. Stomata were found only on the lower surface of the leaves. This was also observed by DÜRING (1980) in some *Vitis* spp. and cvs. So the cultivars studied were shown to be hypostomatal.

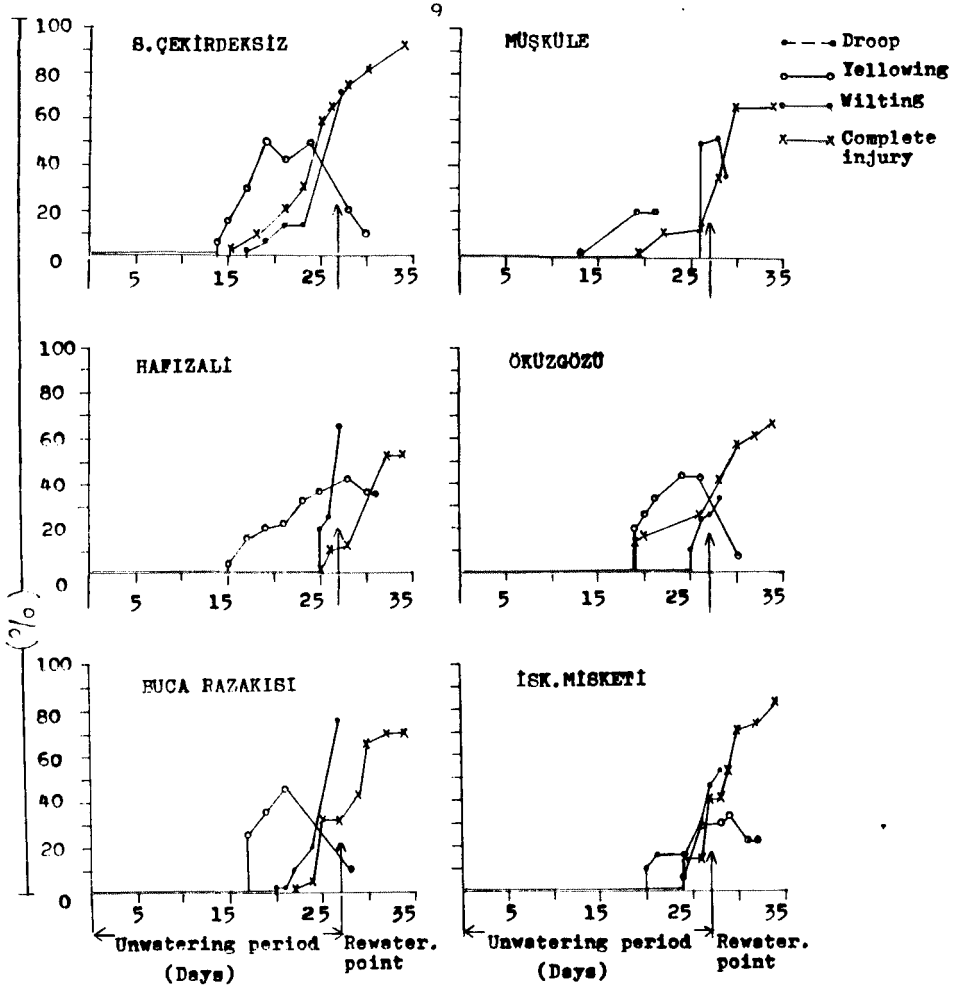
Stomatal frequency of the cultivars varied from 129 ± 18 to 254 ± 10, which was generally between the values found by other researchers in some *Vitis* spp. and cvs (DÜRING 1980; DÜZENLI and ERGENOGLU 1988). The stomatal frequency of Sultani Cekirdeksiz found by DÜZENLI and ERGENOGLU (1988) is very close to our result. But the value found by the same researchers for Tarsus Beyazi (227-233) is different from our finding. This variance can be attributed to

environmental and other factors (Boselli and Scienza 1983; Forlani *et al.* 1983; Rühl and Imgraben 1985; Balo *et al.* 1986; Düzenli and Ergenoglu 1988).

Stomata length and width of the cultivars were found to be different. In general, stomata lengths determined in this study, at least in some cultivars, were close to the value given for *V. vinifera* (29.7 μm) by Kramer and Kozlowski (1979). However, the size of stomata can also be changed by K (Boselli and Scienza 1983), fungidice applications (Sekera 1983), and possibly other factors.

The correlation between the size of stomata (as length and width) and the frequency was not significant. A negative correlation between both characters has been observed in some plant species (Kramer and Kozlowski 1979). The correlation between the length and width of stomata was fairly high (r = 0.493).

There were important differences among the cultivars with respect to their tolerance to water deficit. The following relative order of the cultivars could be given: Cavus, Amasya, Tarsus Beyazi



· The curves of droop, yellowing and wilting of the leaves and completely injured plants. (Continued overleaf.)

and Sultani Cekirdeksiz are least tolerant; Yapincak and Balbal are most tolerant, and other cultivars range between the two extremes. But these moderately tolerant cultivars have also shown different degrees of tolerance.

The relations between stomata frequency and drought tolerance are uncertain. For example, Tarsus Beyazi and Sultani Cekirdeksiz, which are considered to be sensitive cultivars, have 153 ± 9 and 251 ± 9 stomata/mm^2 of area, respectively. The same pattern is also true for the tolerant cvs

10

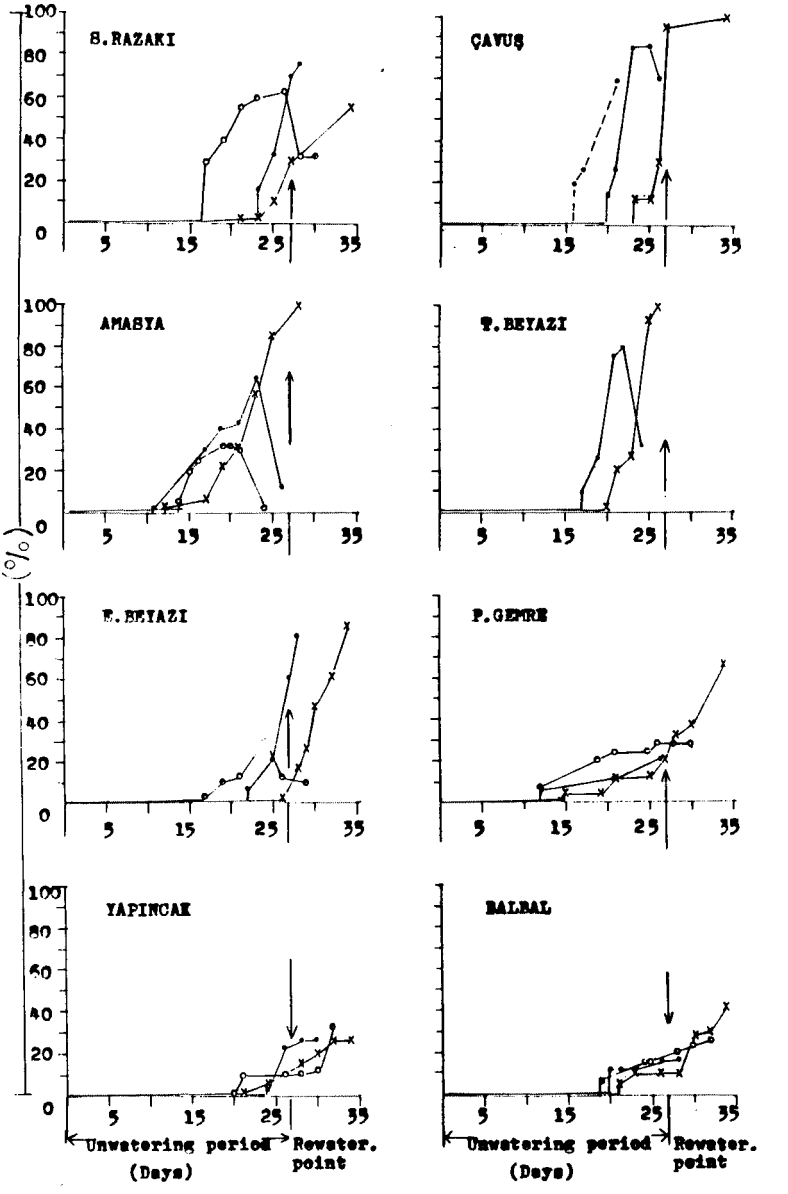


Fig. (continued).

Balbal and Yapincak which have 129 ± 18 and 192 ± 19 stomata/mm^2, respectively. The relations between stomata size and tolerance are also uncertain. Other characteristics of the leaves (e. g. size, firmness, hair density) may play a role in this respect. As a matter of fact, mature leaves of the sensitive cultivars (Cavus, Amasya and Sultani Cekirdeksiz) are large and soft in structure. On the other hand, Balbal and Yapincak also have relatively large and fairly soft leaves. The lower surface of the leaves of Cavus and Tarsus Beyazi is densely covered with hair.

For the reasons mentioned above, it is difficult to state that there is a close relationship between the leaf morphology and drought tolerance of the cultivars investigated. Stomatal closure at certain water potentials can be useful to determine the drought tolerance (KRAMER and KOZLOWSKI 1979). Stomatal conductance and water use efficiency can also be helpful to understand the differences among cultivars (DÜRING 1988). In addition, field studies are needed in this respect.

Literature

BALO, B.; MUSTARDY, L. A.; HLDEG, É.; FALUDI-DANIEL, A.; 1986: Studies on the effect of chilling on the photosynthesis of grapevine. Vitis **25**, 1-7.

BOSELLI, M.; SCIENZA, A.; 1983: Effects of potassium fertilization on density and morphological characteristics of stomata in grapevines. Vignevini **10** (1-2), 27-32. [Abstr.: Vitis **22**, 263.]

DÜRING, H.; 1980: Stomatafrequenz bei Blättern von *Vitis*-Arten und -Sorten. Vitis **19**, 91-98.

– –; 1988: Zur Trockentoleranz des Riesling. Wein-Wiss. **42**, 391-399. [Abstr.: Vitis **27**, 2 C 7].

– –; KLINGENMEYER, W.; 1987: Stomatal control of water use efficiency in two *Vitis vinifera* cultivars. 3e Symp. Intern: Physiologie de la Vigne, Bordeaux, 179-184. [Abstr.: Hort. Abstr. **57**, 7666.]

DÜZENLI, S.; ERGENOGLU, F.; 1988: Studies on the density of stomata of some *Vitis* varieties grafted on different rootstocks trained to various trellis systems. IIIrd Symp. Viticulture of Turkey, 31 May-3 June 1988, Bursa, Turkey.

FORLANI, M.; PASQUARELLA, C.; COPPOLA, V.; 1983: The relationship between stomatal density and vigour in grapevine rootstocks. Riv. Viticolt. Enol. **36**, 117-125. [Abstr.: Hort. Abstr. **53**, 4948.]

HOAGLAND, D. R.; ARNON, D. I.; 1950: The water culture method for growing plants without soil. Calif. Agricult. Exp. Sta. Circ. 347.

KRAMER, P. J.; KOZLOWSKI, T. T.; 1979: Physiology of Woody Plants. Academic Press, New York, San Francisco, London.

LEVITT, J.; 1980: Responses of Plants to Environmental Stress, Vol. II. Water, Radiation, Salt and Other Stress. Academic Press, New York, London, Toronto, Sydney, San Francisco.

MEIDNER, H.; MANSFIELD, T. A.; 1968: Physiology of Stomata. McGraw-Hill, London.

RÜHL, E.; IMGRABEN, H.; 1985: Einfluß der Stickstoffversorgung auf die Stomatazahl und den Blattaufbau von Weinreben (*Vitis vinifera* L.). Wein-Wiss. **40**, 160-171. [Abstr.: Vitis **24**, 4 E 128.]

SCIENZA, A.; BOSELLI, M.; 1981: Fréquence et caractéristiques biométriques des stomates de certains porte-greffes de vigne. Vitis **20**, 281-292.

SEKERA, D.; 1983: The effect of fungicides and fungicide combinations on grape leaf stomata size and aperture. Vinohrad (Bratislava) **21**, 29-31. [Abstr.: Vitis **22**, 410.]

VARDAR, Y.; 1972: Bitki Fizyolojisi Dersleri. I. Ege Ünv. Fen Fak. Kitaplar Ser. 37, Izmir.

WEAVER, R. J.; 1976: Grape Growing. John Wiley and Sons, New York, Chichester, Brisbane, Toronto.

Weather effects of stalk necrosis in *Vitis*

A. LANG [1]) and H. DÜRING [2])

[1]) Physics and Engineering Laboratory, DSIR, Private Bag, Lower Hutt, New Zealand

[2]) Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, BR Deutschland

S u m m a r y : A 5-step physiological explanation is offered for correlations found by THEILER and MÜLLER (1986) between aspects of the weather at flowering and the incidence of stalk necrosis in grape. Four of the steps are well supported in the literature and are discussed briefly.

The other step proposes that the development of xylem in the peduncle is stimulated by floral evapotranspiration. This postulate is tested with measurements of xylem cross-sectional area in clusters taken from vineyards located in diverse regions of New Zealand for which meteorological data were available. Statistical analysis indicates a clear distinction between material from the climatic extremes and a significant ($P = 0.05$) correlation between calculated values of evapotranspiration during flowering and peduncular xylem area.

The beginning of a physiological explanation for the seasonal effects on the incidence of stalk necrosis in grape has stimulated studies which may allow the selection of non-sensitive varieties.

K e y w o r d s : stiellaehme, stalk necrosis, climate, flower, berry, peduncle, rachis, xylem, transpiration.

Introduction

In a recent publication THEILER and MÜLLER (1986) demonstrated strong correlations between the incidence of stalk necrosis (variously known as bunch stem necrosis, shanking, stiellähme, dessèchement de la rafle) in Müller-Thurgau grapes and a number of meteorological variables including daytime temperature, sunshine hours and precipitation. Moreover, they noted that these correlations existed only for weather conditions over the flowering period. They suggested that something happens at this stage of development, the effects of which are apparent only after veraison.

THEILER and MÜLLER's results are provocative in two ways. They raise commercial questions relating to the choice of variety, vineyard siting and canopy management, and they raise physiological questions relating to the nature of the causal link between weather and stalk necrosis. Our paper addresses the second of these aspects. A scheme is proposed which invokes a sequence of causes and effects to explain the correlation. The scheme starts with weather and ends with stalk necrosis (THEILER and MÜLLER's correlative statement) and contains five linking steps. Four of the hypothesised steps are examined by reference to the literature and one by an experiment.

The hypothesis

The hypothesis is presented in summary form in Fig. 1. We shall consider it one step at a time.

Step (i) - weather : flower evapotranspiration

Here we propose that flower evapotranspiration is affected by the prevailing weather. Although plants undoubtedly exert a strong control over evapotranspiration, this is only part of the story and few would seriously dispute that water losses from an exposed plant organ are strongly influenced by factors such as those identified by THEILER and MÜLLER (1986) viz. temperature, insolation and rainfall. Models which describe how meteorological variables drive evapotranspiration under a range of identified conditions are well established and their use is commonplace in scientific research and irrigation management systems.

Step (ii) - flower evapotranspiration : peduncle xylem area

In this step we propose that xylem area development in the peduncle is in proportion to the mean evapotranspiration rate of the inflorescence during the flowering period. We suggest that the xylem's carrying capacity (area) is stimulated in some way by demand during the tissue differentiation phase such that sap pressure gradient is stabilised. The flow : area link is consistent with the observation that in a very wide range of plants and organs xylem sap flow is related to the cross-sectional area of the xylem tissues (SIAU 1971; ZIMMERMANN and BROWN 1971). We examine this relationship in the second part of our paper with an experiment.

Step (iii) - peduncle xylem area : xylem/phloem sap flow ratio

As with xylem areas and flows discussed above, translocation flow bears a close relation to the area of cross section of the phloem. Indeed the relationship, usually expressed as specific mass transfer or flux density (EVANS and DUNSTONE 1970; CANNY 1973), is most remarkably constant across a wide range of size scales, plant organs and genera. Now it is axiomatic that something which influences one variable will also influence the ratio which this variable bears to another. In the present situation, all else being equal, it follows that xylem area change may be assumed to result in a changed ratio of xylem to phloem tissues and this may fairly be taken to indicate a change in the ratio of their sap flows.

Step (iv) - xylem/phloem sap flow ratio : Ca/K content of cluster

Potassium has for a long time been known to be strongly phloem mobile forming the dominant cationic species in extracted sieve tube sap (ZIEGLER 1975). Calcium on the other hand is traditionally thought of as phloem immobile (MARSCHNER 1983). Both ions are of course freely mobile in the xylem sap. The generally low levels of Ca and high levels of K in fruits are thought to arise through a common pattern of growth in which the xylem, while making a significant early contribution to growth, is largely replaced by a dominant phloem contribution later on (SIMON 1978). This state of affairs has led to the idea that some classes of mineral imbalance disorders may result from imbalance between phloem and xylem supplies (FERGUSON and WATKINS 1989).

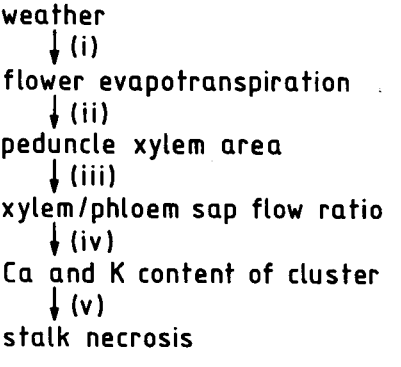


Fig. 1: A sequence of five steps by which it is proposed the weather at flowering affects the incidence of stalk necrosis. Each step is in the form of a subsidiary hypothesis, viz. weather effects flower evapotranspiration, which affects peduncle xylem area, and so on.

Step (v) - Ca and K in cluster : stalk necrosis

Stalk necrosis in grape is thought to be a mineral imbalance disorder, although agreement on which minerals are involved does not seem to have been reached. This confusion is not unexpected if we allow that a non pathogenic or physiological necrosis could well have a number of different causes. One possibility is that stalk necrosis occurs as a result of an imbalance between K and Ca. For example, FEUCHT *et al.* (1975) showed that stalk necrosis in grape can be associated with high K/Ca ratios in the peduncles (but compare CHRISTENSEN and BOGGERO (1985) who found otherwise).

The experiment

An experiment was performed to examine the postulate of step (ii) that development of peduncular xylem is stimulated by evapotranspiration rate.

Materials and methods

Material

Riesling was chosen for the experiment because it was widely available and because it is known to be susceptible to stalk necrosis. THEILER and COOMBE (1985) noted that development of xylem tissues in grape peduncles occurs exclusively over a brief early period of about 2 weeks duration, from just before to just after first bloom (this finding agrees with our own observations). In the present study therefore, clusters were collected in January and February 1986, 4-6 weeks after flowering, to allow ample time for xylem development to have been completed.

Collections were made from four different vineyards in New Zealand chosen to represent as wide a range of climate types as possible and for which detailed meteorological records would be

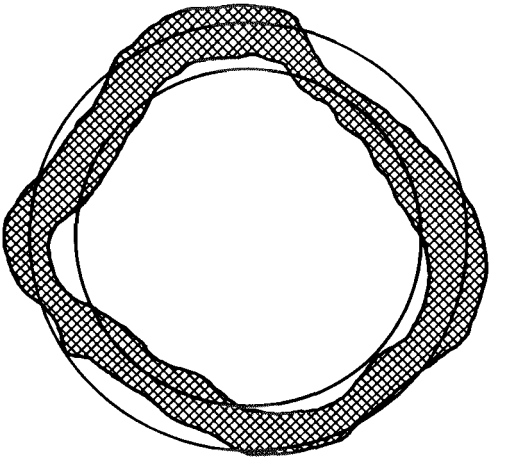


Fig. 2: A diagram to illustrate the 'circles' method used to measure xylem cross-sectional area in the grape peduncle. The irregular xylem image is shown hatched and circles which best match the inner and outer limits are superimposed. Circles were selected by eye from a set of standards.

available. In the North Island the vineyards were in Martinborough (Wairarapa), Mangere (Auckland), Te Kauwhata (North Waikato), and in the South Island, Queenstown (Central Otago).

Measurement of xylem areas

Transverse sections of peduncle tissue were cut by hand from regions just proximal to each of the first three nodes within each cluster. A thin and complete section was chosen to represent each region and placed on a marked slide. This gave three sections per cluster for area measurement. The number of berries downstream of each region was recorded. The sections were stained in phloroglucinol and HCl (Purvis *et al.* 1964) to colour the lignified xylem tissues a distinct red.

Because of the large number of sections to be examined (more than 250) a quick method for estimating xylem cross-sectional area was required. Using a projection microscope in a darkened room, the magnified images were focused onto a sheet of paper on which circles of known radii had been inscribed. The pair of circles which best matched the inside and outside boundaries of the xylem were chosen by eye making allowance for irregularities in circularity (see Fig. 2). Xylem area was then computed as the difference between the areas of the two circles divided by the enlargement factor. The 'circles' method had been previously checked against a tedious but more certain one. This involved tracing around the irregular inner and outer boundaries of the xylem image when projected onto a sheet of plain paper, cutting this out with scissors and weighing. Measurements obtained using the 'circles' method bore a close linear relation to measurements obtained from the same slides using the 'scissors' method showing no significant systematic divergence from a 1 : 1 line. Moreover, no 'circles' estimate of area departed by more than 12 % from the 1 : 1 line. With variability between specimens rather larger than this, the method was judged satisfactory.

Evapotranspiration estimates

Mean daily evapotranspiration (mm d^{-1}) was calculated for each vineyard from local meteorological data. In each case means cover a 9-d period spanning the date of first flower. Estimates of potential evapotranspiration based upon meteorological variables were adjusted to allow for precipitation on the basis that actual evapotranspiration was depressed below potential evapotranspiration when soil water content was less than half field capacity (Priestley and Taylor 1972).

Results

Not unexpectedly, measurements of xylem area at different points in the same cluster were found to vary considerably. It was thought that the area at a point might depend on the number of berries downstream. In order to remove this source of variability in the main experiment, a separate study was made of the relationship between xylem area and the number of berries downstream. Clusters used for this came from a single vineyard. A close linear relation ($r = 0.86$, $n = 54$)* was found between the cross-sectional area of xylem at any point in the peduncle and the number of berries downstream (see Fig. 3). So that xylem area measurements from different sized clusters and different nodes were comparable, area data were therefore normalised by dividing by the number of berries downstream to give the variable 'xylem cross-sectional area per berry'. This allowed an assessment of the extent of xylem area development in clusters collected from the four different vineyards.

Statistical analyses were carried out on log transformed values to correct a slightly asymmetrical distribution. Mean xylem areas per berry for each vineyard are plotted against evapotranspiration estimates in Fig. 4. Error bars represent the sample estimate of the population mean ($P = 0.05$). The vineyards fall into three distinct groupings ($P = 0.001$ or better). Linear regression gives a regression coefficient $r = 0.978$ which for $n = 4$ indicates a significant ($P = 0.05$) correlation.

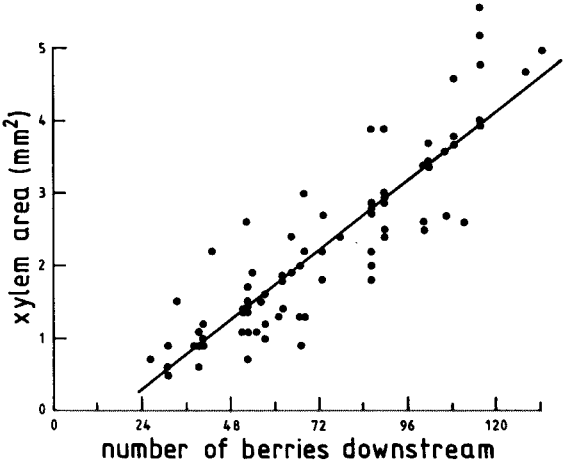


Fig. 3: The plot of xylem cross-sectional area vs the number of berries downstream. Linear regression ($r = 0.86$, $n = 54$) indicates that the two variables are strongly correlated. The remaining variability does not appear to be systematic and is attributed to the natural variability of material and to measurement error.

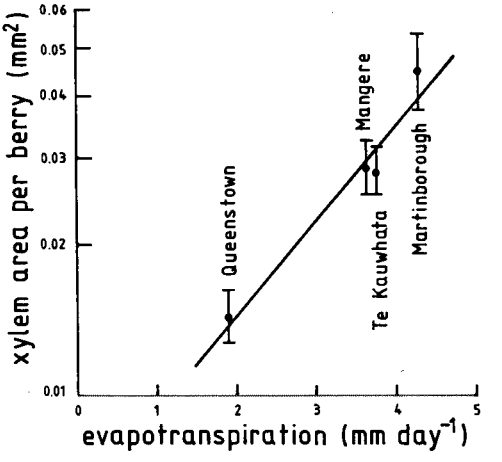


Fig. 4: The plot of xylem cross-sectional area per berry vs a calculated mean daily evapotranspiration at the time of flowering. Error bars represent the sample estimate of the population mean ($P = 0.05$).

Discussion

The results show a clear distinction between vineyards in the cross-sectional area of peduncular xylem in Riesling grapes and this distinction correlates significantly with meteorological factors in line with step (ii) our our hypothesis. That is, the experiment supports the postulate that xylem area development in grape peduncles is in proportion to the mean evapotranspiration rate during the flowering period.

The remaining steps of the hypothesis (i, iii, iv, v) find good support in the literature as has been shown. We therefore conclude that the hypothesis offers a satisfactory explanation of the effects of weather upon stalk necrosis in grape. This opens the way for breeding screens and management practices to be evolved which minimise or eliminate the problem.

In view of the importance of stalk necrosis to the grape industry, further research along the lines of that reported here would be worthwile. A number of aspects could be looked at in greater detail. Step (ii) of the hypothesis should be examined in an experiment involving more vineyards and which made comparisons with measured rather than with calculated values of floral evapotranspiration. The encouraging results presented here would also justify a more extensive study of the whole hypothesis. This study might include varieties which covered a range of susceptibilities to stalk necrosis and with an attempt made to assess the incidence of stalk necrosis. The intermediate variables are also amenable to measurement. These include the Ca/K composition of the fruit and the xylem : phloem sap flow ratio (LANG 1989).

Acknowledgements

We are grateful for the patient work of Mr. E. Kendrick of The Physics and Engineering Laboratory, DSIR who carried out the extensive hand sectioning and xylem area measurement required in this study.

References

CANNY, M. J.; 1973: Phloem Translocation. Cambridge University Press, London, New York.

CHRISTENSEN, L. P.; BOGGERO, J. D.; 1985: A study of mineral nutrition relationships of waterberry in Thompson seedless. Amer. J. Enol. Viticult. **36**, 57-64.

EVANS, L. T.; DUNSTONE, R. L.; 1970: Some physiological aspects of evolution in wheat. Austral. J. Biol. Sci. **23**, 725-741.

FERGUSON, I. B.; WATKINS, C. B.; 1989: Bitter pit in apple fruit. Hort. Rev. **11**, 289-355.

FEUCHT, W.; FORCHE, E.; PORSTENDORFER, J.; 1975: Ermittlung der Kalium- und Calciumverteilung in Traubenachsen von *Vitis vinifera* mit Hilfe der Röntgenstrahlenmikroanalyse am Rasterelektronenmikroskop. Vitis **14**, 190-197.

LANG, A.; 1989: Xylem, phloem and transpiration flows in developing apple fruits. (In press.)

MARSCHNER, H.; 1983: General introduction to the mineral nutrition of plants. In: LÄUCHLI, A.; BIELESKI, R. L. (Eds.): Encyclopedia of Plant Physiology, New Ser., Vol **12**, Inorganic Plant Nutrition, 5-60. Springer-Verlag, Berlin.

PRIESTLEY, C. H. B.; TAYLOR, R. J.; 1972: On the assessment of surface heat flux and evaporation using large scale parameters. Monthly Weather Rev. **100**, 81-92.

PURVIS, M. J.; COLLIER, D. C.; WALLS, D.; 1964: Laboratory Techniques in Botany. Butterworths, London.

SIAU, J. F.; 1971: Flow in Wood. Syracuse University Press, New York.

SIMON, E. W.; 1978: The symptoms of calcium deficiency in plants. New Phytol. **80**, 1-15.

THEILER, R.; COOMBE, B. G.; 1985: Influence of berry growth and growth regulators on the development of grape peduncles in *Vitis vinifera* L. Vitis **24**, 1-11.

– – ; MÜLLER, H.; 1986: Beziehungen zwischen Klimafaktoren und dem Stiellähmebefall bei Riesling x Sylvaner. Vitis **25**, 8-20.

ZIEGLER, H.; 1975: Nature of transported substances. In: ZIMMERMANN, M. H.; MILBURN, J. A. (Eds.): Encyclopedia of Plant Physiology, New Ser., Vol. 1, 59-100. Springer-Verlag, Berlin, Heidelberg, New York.

ZIMMERMANN, M. H.; BROWN, C. L.; 1971: Trees Structure and Function. Springer-Verlag, Berlin, Heidelberg, New York.

Section 5: Tissue and cell culture

Applications of tissue culture to the genetic improvement of grapevines

M. G. Mullins

Department of Viticulture and Enology, University of California, Davis, California 95616, USA

Summary: The grapevine was among the first plants to be cultured *in vitro* (1944). Regeneration by somatic embryogenesis and organogenesis was reported in the 1970s and plantlet production from cell suspensions or callus is now a routine procedure in many laboratories. Methods for isolating grapevine protoplasts have yet to be achieved. The fragmented apex technique, involving high-frequency adventitious bud formation, is a novel and efficient method for rapid multiplication of grapevines but culture of anthers and pollen has been generally unsuccessful. Micropropagation procedures for *vinifera* grapes, *Vitis* species and interspecific hybrids, including rootstocks, are all available. Seedless-seedless hybridization, involving embryo rescue in crosses with stenospermocarpic female parents, is of major significance in breeding seedless table grapes.

There has been substantial progress in protoplast cell, tissue and organ culture of grapevines, but this technology is still less well developed than with some other fruit crops (notably citrus and apples). So far, tissue culture has little impact on genetic improvement. Exploitation of somaclonal variation for clonal selection is an attractive option for premium wine cultivars. There is evidence of somaclonal variation *in vitro* but the usefulness of this random genetic variation in viticulture is still uncertain. To date, results of field trials with vines from somatic embryos have been disappointing. The grapevine is proving to be a difficult subject for *Agrobacterium*-mediated genetic transformation (*A. tumefaciens* and *A. rhizogenes*) and microprojectile technology is another option which is being investigated.

Key words: tissue culture, somaclonal variation, protoplast technology, genetic engineering, biotechnology, breeding, genetics, review.

Introduction

The wine industry is characterized by extreme conservatism in the cultivars used for winemaking. Most of the world's 9 million ha of vineyards are planted with traditional cultivars which have been perpetuated for centuries by vegetative propagation. The well-known cultivars of French viticulture, such as Cabernet Sauvignon, Pinot noir and Chardonnay, are all of Roman or pre-Roman origin (Levadoux 1956; Rives 1971; Bouquet 1982 a). The histories of the traditional cultivars of other European grape-growing countries are equally as long.

Quality in wine has become closely associated with the winemaking characteristics of a relatively short list of traditional cultivars. Further, the growing of these cultivars has become fixed by custom, or by law under the system of Appellation d'Origine Contrôlée in France and by similar legislation in Italy and Spain. The traditional cultivars of Europe are also predominant in viticulture in the new worlds of North and South America, Australia and South Africa.

So far, wine grape breeding has made little impact at the level of the scion. The breeding of new wine grapes is feasible in technical terms, but new cultivars with unfamiliar names and wines with unfamiliar flavors face a battle for acceptance in the market place. As a consequence of these special circumstances, clonal selection, the exploitation of variation within traditional cultivars, has become a widely-used procedure for the improvement of wine grapes.

Rootstock breeding has made a substantial contribution to viticulture. The phylloxera resistant rootstocks bred in the late 19th century (e. g. SO 4, St. George, A x R #1) represent the first and most successful example of biological control of an insect pest. In the last 40 years, several new rootstock cultivars have been bred which confer resistance to nematodes or to unfavorable soil conditions. Table grape and raisin production is not subject to the constraints which affect wine production. Consequently, plant breeding has made a more significant contribution than with wine grapes. Many new table grape cultivars have been released in recent years, particularly of seedless grapes.

The advent of tissue culture and genetic engineering, and the application of this technology to crop improvement, has much significance for viticulture. There are two main areas of interest (i) procedures which improve the efficiency of conventional breeding and (ii) applications of cell and tissue culture which augment genetic variation within existing genotypes, i. e. clonal variation. The former has a major role in the breeding of rootstocks, table grapes and raisin grapes. The latter is of particular importance for premium wine grapes because of the 'genetic straightjacket' within which this form of viticulture is constrained by tradition, legislation and the market place.

Progress in grapevine tissue culture

A prerequisite for the application of tissue culture to grapevine improvement is the availability of highly efficient methods for plant regeneration or plant propagation *in vitro*. Substantial progress has been made in recent years but tissue culture of grapes has had a long history.

The grapevine was among the first plants to be cultured *in vitro* (Morel 1944). Proliferation of callus and formation of adventitious roots were the subjects of several reports during the 1950s and 1960s, but the grapevine proved to be recalcitrant with respect to regeneration *in vitro*. Somatic embryogenesis was first reported by Mullins and Srinivasan in 1976, and organogenesis was reported in the same year by both Favre (1976) and Hirabayashi *et al.* (1976). These developments were pre-dated by the first report on cultivation of grapevine protoplasts (Skene 1974). Since then, there have been several publications on methodological factors affecting isolation, survival and division of grapevine protoplasts (Skene 1975; Brezeanu *et al.* 1982; Hasler *et al.* 1982, 1983; Bessis *et al.* 1985; Lebrun 1985; Wright 1985; De Filippis and Ziegler 1985; Yamakawa *et al.* 1985; Barbier and Bessis 1988), but plant regeneration has yet to be reported.

There have been approximately 50 publications on micropropagation of grapevines since the original report of Jona and Webb (1978), i. e. reports on methods for induction of axillary shoot proliferation and subsequent formation of adventitious roots by microcuttings.

A novel method for rapid multiplication *in vitro* using fragmented shoot apices was developed by Barlass and Skene (1978) and has since been much refined (Barlass and Skene 1980 a, 1980 b; Barlass *et al.* 1981). In this procedure adventitious buds are formed with very high frequency in the tissues produced by cultured leaf primordia. Recently, it has been shown that numerous adventitious buds can be induced on hypocotyl explants of somatic embryos of grape cultivars (Vilaplana and Mullins 1989).

There is a single report from China of haploid plantlet production in grapevines (Zou and Li 1981), but attempts elsewhere to obtain haploids by culture of anthers and pollen of *Vitis vinifera* have been unsuccessful. In many grapevine genotypes the connective of anthers is a highly regenerative tissue and it gives rise to somatic embryos with high frequency (Rajasekaran and Mullins 1979, 1983). Callus produced by cultured anthers may contain haploid metaphases or nuclei in which the DNA content is consistent with the haploid condition (1C-2C), but derivatives of these cells do not seem to participate in embryo formation; plants from anther callus are diploid and heterozygous (Rajasekaran and Mullins 1983). Classical androgenesis involving internal divisions in pollen grains and extrusion of embryogenic callus, as seen in many Solanaceae and Cruciferae, has not been observed in grapevines. At the level of the intact plant, mixoploidy was observed in twinned seeds by Bouquet (1982 b), but no haploid individuals were recovered.

Application of the embryo rescue technique to stenospermocarpic grapes has enabled 'seedless-seedless' hybridizations. With this technique 'seedless' genotypes can be used as both male and female parents because zygotic embryos are rescued before they abort. This greatly increases the frequency of seedless progeny (Spiegel-Roy *et al.* 1975, 1986; Ramming and Emershad 1982; Cain *et al.* 1983; Goldy and Amborn 1987; Goldy *et al.* 1988).

Practical applications: overview

So far, seedless-seedless hybridization has had the greatest impact of any aseptic method on grapevine improvement, and it represents a major improvement in the methodology for breeding seedless table grapes. Haploids, and homozygous diploids derived from them, would be particularly useful for grapevine breeding and for genetic studies. However, it is now 18 years since the first experiments on cultivation *in vitro* of grapevine anthers (MULLINS 1971) and haploids are still unavailable. In fact, evidence for the existence of grapevine haploids is equivocal and there is a suggestion that haploidy may be a lethal condition in the clonal cultivars of *Vitis vinifera* L. (RAJASEKARAN and MULLINS 1983).

The main application of micropropagation has been in the production of pathogen free stock. Tissue culture was first used for virus elimination in the 1960s (GALZY 1964) and it is now a standard procedure in clean stock programs. Recently, the fragmented apex technique has been used to produce grapevines which are free from infection by viroids (DURAN-VILA *et al.* 1988). The relative ease with which nodal explants of grapevine cultivars can be induced to proliferate axillary buds has led to the use of micropropagation as a vehicle for mutation breeding (REISCH *et al.* 1985; BARLASS 1986; KIM *et al.* 1986). However, the usefulness of induced mutation for grape cultivar improvement has yet to be established.

In terms of potential applications there is much interest in the possibility that tissue culture procedures may be used to create or amplify genetic variation within commercially important cultivars of wine grapes and, thereby, provide new raw material for clonal selection. These potential applications are founded on the processes of somatic embryogenesis, organogenesis, and on the exploitation of both random genetic variation and directed genetic change.

Somaclonal variation in wine grapes - reality or illusion ?

In many species, plants regenerated from callus, cells or protoplasts exhibit considerable variation in morphological and physiological attributes (LARKIN and SCOWCROFT 1981; REISCH 1983; EVANS *et al.* 1984). This variability arises from gross changes in chromosome numbers, or structure, or from more subtle changes in the nuclear DNA which occur during the tissue culture process. The random spontaneous genetic variation which arises during plantlet formation *in vitro* is termed 'somaclonal' variation. Somaclonal variants of sugar cane, potato, rice, wheat, barley and rape have been discovered which possess disease resistance and several other agronomically interesting characters (SEMAL 1986). There has been enthusiastic speculation on the potential of somaclonal variation in perennial plant breeding (DE WALD and MOORE 1987), but experience so far has been disappointing. In viticulture, however, there is still great interest in somaclonal variation because it could provide a means of augmenting clonal variation (MULLINS 1985; MAURO *et al.* 1986).

In addition to variation that arises as a consequence of the tissue culture procedures, there are other potential sources of genetic variation in long-established cultivars of vegetatively propagated plants which are dependent upon tissue culture for their expression. Many fruit cultivars arose as somatic mutations are chimeric in structure, and rearrangements in chimeric structure occur during plant regeneration *in vitro* (MCPHEETERS and SKIRVIN 1983; SKENE and BARLASS 1983). In addition, ancient clones such as the traditional cultivars of grapevines are likely to have accumulated a considerable load of mutations over the centuries and cell culture methods may provide the means by which this normally covert variation can be expressed.

Grapevines of most major cultivars and many hybrids have now been regenerated *in vitro* by somatic embryogenesis using nucellar tissues of unfertilized ovules (MULLINS and SRINIVASAN 1976) or the vegetative tissues of anthers (RAJASEKARAN and MULLINS 1983). Hundreds, if not thousands, of grapevines have been produced from somatic embryos by researchers in several

countries. Evidence of somaclonal variation has come primarily from research on genotypes which are highly regenerative *in vitro*, for example, Gloryvine, a *Vitis vinifera x Vitis rupestris* hybrid. Gloryvines raised from somatic embryos often exhibit abnormalities such as dwarfism and albinism. Leaf shape is normally a highly stable character in grapes and it is the basis of ampelography (GALET 1979), but plants produced *in vitro* often show marked variations in leaf shape, including differences in petiolar sinuses and lobation. These differences tend to be transient and may be similar in nature to the temporary variations which occur in thermotherapy (VALAT and RIVES 1973) or after micropropagation *in vitro* (CHANCELLIER and COSSIO 1988). In addition, Gloryvines raised from somatic embryos show variation in sex expression (RAJASEKARAN and MULLINS 1983), indicating, perhaps, change in a single gene (DOAZAN and RIVES 1967; NEGI and OLMO 1971; ANTCLIFF 1980). Gloryvine is a male genotype, but 3 vines among the 125 which were planted in a field trial have proved to be hermaphrodite and they produced fruit for each of 5 years of the life of the trial.

In research on selection for salinity tolerance in *Vitis rupestris* SCHEELE cv. St. George (LEBRUN *et al.* 1985), cell lines were selected which grew in suspension cultures containing up to 150 mM NaCl. These apparently salt tolerant cell suspensions gave rise to somatic embryos, but the embryos became necrotic and died in the presence of 50 mM NaCl once radicle elongation had commended. From these results it appears that somaclonal variation in NaCl tolerance is manifested by cell suspensions of St. George but that tolerance at cellular level and in immature embryos is not closely correlated with tolerance in fully differentiated embryos and in intact plants.

In 1977, 12 vines of the original 'somatic Cabernet Sauvignon' (MULLINS and SRINIVASAN 1976) were planted at the Viticulture Research Station, Griffith, New South Wales, Australia. This planting was a curiosity rather than an experiment but it has provided some interesting observations. Initially, the somatic vines were highly variable in growth and cropping, as are most newly established grapevines, but they have become more uniform with the passage of time. A similar loss of variability with increasing age has been found in other grapevines regenerated from cells, for example, reversion to the ampelographically accepted leaf shape of the cultivar. The original 12 somatic Cabernet Sauvignon are now vigorous, well-established vines and are characteristic of their cultivar, but in other respects they are unremarkable.

A more extensive field trial was planted at Griffith in 1983 to compare the growth and cropping of Cabernet Sauvignon vines from somatic embryos and hardwood cuttings. Griffith is located in a hot, inland region of irrigated viticulture (Murrumbidgee Irrigation Area). The region is isolated and is phylloxera free. Grapevines are grown on their own roots. In this trial the somatic vines were juvenile at the time of planting and they have proved to be very slow to come into bearing. In the 1988 season, yield, fruitfulness (bunches/shoot), bunch size and trunk diameter were all substantially less than that of conventionally propagated vines. These results are difficult to interpret because it is not clear if the poor performance of somatic vines relative to the controls is related to juvenility effects or to an inherent inferiority. This can be overcome by re-propagating cuttings from bearing mother plants of the two types – somatic and conventional – and by establishing a new trial. It will be some years before the viticultural value of somatic embryogenesis can be determined.

So far, studies on somaclonal variation in wine grapes have given inconsequential, equivocal or disappointing results, but it is premature to conclude that this source of variation has nothing to offer to grapevine improvement because the mode of plant regeneration *in vitro*, somatic embryogenesis, may be unsuitable for the proper expression of somaclonal variation. In citrus it is now clear that somatic embryogenesis produces uniform propagules, including plants regnerated from protoplasts (VARDI *et al.* 1982; KOBAYASHI 1987). In many species, variability is most pronounced in populations derived from callus by organogenesis (VASIL 1983). In the case of grapevines, somatic embryo formation is preceded by a callus and cell suspension phase, but it seems that callus formation may be only one of the predisposing factors to the occurrence of

random genetic variation *in vitro*. Organogenesis from callus has been demonstrated in grapes but it occurs with low frequency (FAVRE 1976; HIRABAYASHI *et al.* 1976; RAJASEKARAN and MULLINS 1981; MORIGUCHI *et al.* 1988). Refinement of these procedures to produce, on a routine basis, large populations of plants of the leading cultivars may provide access to levels of somaclonal variation that are useful for selection purposes.

The variation which arises in tissue culture, or which is induced by mutagens, is essentially random in nature and its successful exploitation is dependent upon the availability of rapid, accurate screening procedures. The development of these methods is relatively straightforward for characters such as disease resistance. Micropathogenicity tests are already available for selection *in vitro* for resistance to downy mildew (*Plasmopara viticola*: MOREL 1948; LEE and WICKS 1982) and powdery mildew (*Uncinula necator*: KLEMPKA *et al.* 1984) and selection at the level of phytoalexin production is an interesting possibility (STEIN and HOOS 1984). It must be emphasized, however, that selection among somaclones for qualitative characters such as wine quality will remain as difficult and as time-consuming as conventional clonal selection with conventionally propagated grapevines.

Protoplast technoloy

The role of protoplast technology in plant improvement is to increase genetic variation. First, plants regenerated from protoplasts may exhibit somaclonal variation for agronomically useful characters. Second, by fusion of protoplasts it is possible to effect organelle transfer (chloroplasts and mitochondria) and gene transfer between sexually incompatible parents. Finally, protoplasts are useful in biotechnology for genetic transformation by direct uptake of foreign DNA or through procedures such as electroporation. However, the first step in applying protoplast technology to grapevine improvement is the availability of methods for plant regeneration from protoplasts. As indicated above, this has yet to be achieved but some progress has been made with isolation techniques.

Meanwhile, substantial advances have been made with other woody perennial fruit plants. Intergeneric hybrids have been produced by fusion of protoplasts from the sexually incompatible species *Citrus sinensis* and *Severinia disticha* (GROSSER *et al.* 1988 b) and from fusion of protoplasts of pear and cherry (*Pyrus communis* var. *pyrasta* and *Prunus avium x P. pseudocerasus*: OCHATT *et al.* 1988 b). In addition, plants have been produced from fusion of protoplasts of sexually compatible species (*Citrus sinensis x Poncirus trifoliata*: GROSSER *et al.* 1988 a). Plant production from protoplasts of individual species of *Citrus, Prunus* and *Pyrus* is now a routine procedure (VARDI *et al.* 1982; OCHATT and POWER 1988 a, 1988 b, 1986; OCHATT *et al.* 1988 a). Thus far, callus has been produced by grape protoplasts (SKENE 1975), but organ and plant regeneration has proved to be elusive. It is probable that lack of success with grapevine protoplasts reflects a lack of research, and a lack of researchers, in the field of cell biology of grapevines, and it is predictable that this technical blockage will be overcome in the near future. Judgment on the usefulness of protoplast technology for grape improvement must be reserved for the time being.

Genetic engineering

A prospect of biotechnology is that it may be possible to insert foreign genes into the genomes of traditional cultivars such as Cabernet Sauvignon and Chardonnay without altering the cultivars concerned in any of their other characteristics – including wine quality. Of special interest is the conferring of resistance to virus disease by incorporation of viral coat protein genes (ABEL *et al.* 1986; BEACHY *et al.* 1987; CUOZZO *et al.* 1988) or by expression of virus satellite RNA (HARRISON *et al.* 1987). Another interesting possibility is the conferring of resistance to lepidopteran pests by

incorporation into the grapevine genome of genes encoding production of *Bacillus thuringensis* toxin (BARTON *et al.* 1987).

The current situation with the application of biotechnology to grapevine improvement is similar to that with protoplasts. Much has been written on the potential of biotechnology for genetic improvement of woody plants but the first step, the production of genetically transformed grapevines which express a marker gene, has yet to be reported. Genetically transformed grapevine roots have been obtained after inoculation of whole plants (cv. Grenache) grown *in vitro* (GUELLEC *et al.* 1988) with *Agrobacterium rhizogenes* containing two independent plasmids (i) the wild-type Ri-plasmid (pRi 15834) and (ii) a Tri-derived plasmid which carries the NPT II gene (neomycin phosphotransferase II) and the nopaline synthase gene. Expression of the NPT II gene confers kanamycin resistance to transformed plant cells. Recently, cell suspensions of Cabernet Sauvignon have been transformed by co-cultivation with *Agrobacterium* strains confirming resistance to kanamycin (BARIBAULT *et al.* 1989).

In some Australian work, plants exhibiting nopaline production and chimerism for kanamycin resistance were produced after co-cultivation of shoot apical fragments of grapevine cultivars with *Agrobacterium tumefaciens* containing the plasmid PGV3850::1103 neo (BENNETT 1988). However, the presence of foreign DNA could not be confirmed and attempts to purify these chimeric grapevines were unsuccessful. Microprojectile technology (MCCABE *et al.* 1988) is another approach to grapevine transformation which is being actively pursued. Microscopic particles of tungsten coated with DNA are literally 'shot' into the nuclei of meristems or regeneratively competent callus. The DNA concerned carries a marker gene, β-glucuronidase (GUS), which enables transformed cells to be identified by a color reaction (blue) when treated with the appropriate substrate.

So far, there have been encouraging preliminary results in several laboratories, both with *Agrobacterium*-mediated transformation and with particle acceleration, but no genetically transformed grapevines have emerged. This is in contrast to other horticultural crops such as pear (BROWNING *et al.* 1985), apple and strawberry (JAMES 1987), and walnut (DANDEKAR *et al.* 1988; MCGRANAHAN *et al.* 1988) where genetically transformed plants expressing marker genes have been reported. In the case of walnut, somatic embryogenesis was the means by which *Agrobacterium*-mediated transformation was achieved, and this route may yet be successful for grapevines. It is frustrating that the grapevine should prove to be such recalcitrant material for transformation when rapid advances are being made with other woody perennials. Sustained investment in research is needed if the exciting possibilities of biotechnology are to become realities in viticulture.

Literature cited

ABEL, P. P.; NELSON, R. S.; DE, B.; HOFFMANN, N.; ROGERS, S. G.; FRALEY, R. T.; BEACHY, R. N.; 1986: Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science **232**, 738-743.

ANTCLIFF, A. J.; 1980: Inheritance of sex in *Vitis*. Ann. Amelior. Plantes **30**, 113-122.

BARBIER, M.; BESSIS, R.; 1988: Effets de différents facteurs contribuant à l'amélioration de l'isolement de protoplastes à partir de feuilles de vigne (*Vitis vinifera* L.). Bull. Soc. Bot. France Lett. Bot. **135**, 251-261.

BARIBAULT, T. J.; SKENE, K. G. M.; SCOTT, N. S.; 1989: Genetic transformation of grapevine cells. Plant Cell Rep. **8**, 137-140.

BARLASS, M.; 1986: Development of methods applicable to the selection of mutants from *in vitro* culture of grapevines. In: Nuclear Technology and *In Vitro* Culture for Plant Improvement, 259-265. IAEA, Vienna.

-- -- ; SKENE, K. G. M.; 1978: *In vitro* propagation of grapevine (*Vitis vinifera*) from fragmented shoot apices. Vitis **17**, 335-340.

-- -- ; -- -- ; 1980 a; Studies on the fragmented shoot apex of grapevine. I. The regenerative capacity of leaf primordial fragments *in vitro*. J. Exp. Bot. **31**, 483-488.

– – ; – – ; 1980 b: Studies on the fragmented shoot apex of grapevine. II. Factors affecting growth and differentiation *in vitro*. J. Exp. Bot. **31**, 489-495.

– – ; – – ; CLINGELEFFER, P. R.; 1981: Studies on the fragmented shoot apex of grapevine. III. A scanning electron microscope study of adventitious bud formation *in vitro*. J. Exp. Bot. **32**, 1079-1083.

BARTON, K. A.; WHITELEY, H. R.; YANG, N. S.; 1987: *Bacillus thuringensis* delta-endotoxin expressed in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. Plant Physiol. **85**, 1103-1109.

BEACHY, R. N.; ABEL, P. P.; NELSON, R. S.; ROGERS, S. G.; FRALEY, R. T.; 1987: Transgenic plants that express the coat protein gene of TMV are resistant to infection by TMV. In: Molecular Strategies for Crop Protection, 205-213. Alan R. Liss, Inc., New York.

BENNETT, R. K.; 1988: Studies on the Development of Systems for the Genetic Transformation of Crop Plants. MScAgr Thesis, Univ. of Sydney, Australia.

BESSIS, R.; BARBIER, M.; FAVRE, E.; 1985: Methodological study of producing protoplasts of the vine [Abstr.]. Coll. Amélioration de la Vigne et Culture *in vitro*; Moët-Hennessy, Paris, April 1986, 196.

BOUQUET, A.; 1982 a: Origine et évolution de l'encépagement français à travers les siècles. Progr. Agric. Vitic. **99**, 110-121.

– – ; 1982 b: Premières observations sur le déterminisme génétique de la polyembryonie spontanée chez un hybride interspécifique *Vitis vinifera x V. riparia*. Vitis **21**, 33-39.

BREZEANU, A.; IORDAN, M.; ROSU, A.; 1982: Protoplast isolation by enzymatic procedures from *Nicotiana tabacum* L., *Daucus carota* ssp. *sativus* (HOFFM.) ARCANGELI, *Vitis vinifera* L. and *Citrullus lanatus* (THUMB.) MANSF. Rev. Roum. Biol. Veg. **27**, 133-139.

BROWNING, G.; JONES, O. P.; MURPHY, H. E.; HOPGOOD, M. E.; 1985: Transformation of rootstocks and scion varieties of pear by Agrobacteria. Rep. E. Malling Res. Sta. for 1984, 118-119.

CAIN, D. W.; EMERSHAD, R. L.; TARAILO, R. E.; 1983: *In ovulo* embryo culture and seedling development of seeded and seedless grapes. Vitis **22**, 9-14.

CHANCELLIER, S.; COSSIO, F.; 1988: Risultati di osservazione su un clone di 'Corvina Veronese' (*Vitis vinifera* L.) moltiplicato attraverso la coltura *in vitro*. Riv. Viticolt. Enol. **41**, 110-117.

CUOZZO, M.; O'CONNELL, K. M.; KANIEWSKI, W.; FANG, R. X.; CHUA, N. H.; TUMER, N.; 1988: Viral protection in transgenic tobacco plants expressing the cucumber mosaic virus coat protein or its antisense RNA. BioTechnology **6**, 549-557.

DANDEKAR, A. M.; MARTIN, L. A.; MCGRANAHAN, G.; 1988: Genetic transformation and foreign gene expression in walnut tissue. J. Amer. Soc. Hort. Sci. **113**, 945-949.

DE FILIPPIS, L. F.; ZIEGLER, H.; 1985: The physiology of grapevine (*Vitis vinifera* L.) protoplasts isolated from green and senescing leaves. Biochem. Physiol. Pflanzen **180**, 645-653.

DE WALD, S. G.; MOORE, G. A.; 1987: Somaclonal variation as a tool for the improvement of perennial fruit crops. Fruit Var. J. **41**, 54-57.

DOAZAN, J. P.; RIVES, M.; 1967: Sur le déterminisme génétique du sexe dans le genre *Vitis*. Ann. Amélior. Plantes **17**, 105-111.

DURAN-VILA, N.; JUAREZ, J.; ARREGUI, J. M.; 1988: Production of viroid-free grapevines by shoot tip culture. Amer. J. Enol. Viticult. **39**, 217-220.

EVANS, D. A.; SHARP, W. R.; MEDINA-FILHO, H. P.; 1984: Somaclonal and gametoclonal variation. Amer. J. Bot. **71**, 759-774.

FAVRE, J. M.; 1976: Influence de l'état physiologique de la plante et de la nature de l'organe prélevé sur l'obtention des néoformations caulinaires de la vigne. Congr. Nat. Soc. Savantes Lille **101**, 465-474.

GALET, P. (translated by L. T. MORTON); 1979: A Practical Ampelography. Grapevine Identification. Comstock Publ. Assoc. Cornell University Press, Ithaca, New York.

GALZY, R.; 1964: Technique de la thermothérapie des viroses de la vigne. Ann. Epiphyt. **15**, 245-256.

GOLDY, R. G.; AMBORN, U.; 1987: *In vitro* culturability of ovules from 10 seedless grape clones. HortScience **22**, 952.

– – ; EMERSHAD, R.; RAMMING, D.; CHAPARRO, J.; 1988: Embryo culture as a means of introgressing seedlessness from *Vitis vinifera* to *Vitis rotundifolia*. HortScience **23**, 886-889.

GROSSER, J. W.; GMITTER, F. G.; CHANDLER, J. L.; 1988 a: Intergeneric somatic hybrid plants of *Citrus sinensis* cv. Hamlin and *Poncirus trifoliata* cv. Flying Dragon. Plant Cell Rep. **7**, 5-8.

– – ; – – ; – – ; 1988 b: Intergeneric somatic hybrid plants from sexually incompatible woody species: *Citrus sinensis* and *Severinia disticha*. Theor. Appl. Gen. **75**, 397-401.

GUELLEC, V.; DAVID, C.; PETIT, A.; TEMPE, J.; BRANCHARD, M.; 1988: *Agrobacterium*-mediated transformation of grapevine (*Vitis vinifera* L.) [Abstr.]. Coll. Les Stratégies de Sélection Face aux Technologies Modernes; Moët-Hennessy, Versailles, September 1988, 41.

HARRISON, B. D.; MAYO, M. A.; BAULCOMBE, D. C.; 1987: Virus resistance in transgenic plants that express cucumber mosaic virus satellite RNA. Nature **328**, 799-802.

HASLER, M.; RUFFNER, H. P.; RAST, D. M.; 1982: High-yield isolation of grape leaf protoplasts as an instrument in physiological research. Experientia **38**, 564-565.

-- -- ; -- -- ; -- -- ; 1983: Ultrastructure of grape leaf protoplasts in comparison with the source tissue. Vitis **22**, 193-201.

HIRABAYASHI, T.; KOZAKI, I.; AKIHAMA, T.; 1976: *In vitro* differentiation of shoot from anther callus in *Vitis*. HortScience **11**, 511-512.

JAMES, D. J.; 1987: Cell and tissue culture technolgoy for the genetic manipulation of temperate fruit trees. Biotechnol. Gen. Eng. Rev. **5**, 33-79.

JONA, R.; WEBB, K. J.; 1978: Callus and axillary bud culture of *Vitis vinifera* 'Sylvaner Riesling'. Sci. Hort. **9**, 55-60.

KIM, S. K.; REISCH, B. I.; ALDWINCKLE, H. S.; 1986: *In vitro* grape shoot tip mutagenesis. Vignevini **13** Suppl. al (12), 26-27.

KLEMPKA, K. C.; MEREDITH, C. P.; SALL, M. A.; 1984: Dual culture of grape powdery mildew (*Uncinula necator* BURR.) on its host (*Vitis vinifera* L.). Amer. J. Enol. Viticult. **35**, 170-174.

KOBAYASHI, S.; 1987: Uniformity of plants regenerated from orange (*Citrus sinensis* OSB.) protoplasts. Theor. Appl. Gen. **74**, 10-14.

LARKIN, P. J.; SCOWCROFT, W. R.; 1981: Somaclonal variation: a novel source of variability from cell cultures for plant improvement. Theor. Appl. Gen. **60**, 197-214.

LEBRUN, L.; 1985: Isolation and culture of grapevine protoplasts [Abstr.]. Coll. Amélioration de la Vigne et Culture *in vitro*; Moët-Hennessy, Paris, April 1985, 215.

-- -- ; RAJASEKARAN, K.; MULLINS, M. G.; 1985: Selection *in vitro* for NaCl-tolerance in *Vitis rupestris* SCHEELE. Ann. Bot. **56**, 733-739.

LEE, T. C.; WICKS, T.; 1982: Dual culture of *Plasmopara viticola* in grapevine and its application to systemic fungicide evaluation. Plant Dis. **66**, 308-310.

LEVADOUX, L.; 1956: Les populations sauvages et cultivées de *Vitis vinifera*. Ann. Amélior. Plantes **6**, 59-118.

MAURO, M. C. L.; NEF, C.; AMBID, C.; FALLOT, J.; 1986: Utilisation de l'embryogenèse somatique en vue d'augmenter la variabilité chez *Vitis vinifera* var. Cabernet-Sauvignon. Vignevini **13** Suppl. al (12), 27-29.

MCCABE, D. E.; SWAIN, W. F.; MARTINELLI, B. J.; CHRISTOU, P.; 1988: Stable transformation of soybean (*Glycine max*) by particle acceleration. BioTechnology **6**, 923-926.

MCGRANAHAN, G. H.; LESLIE, C. A.; URATSU, S. L.; MARTIN, L. A.; DANDEKAR, A. M.; 1988: *Agrobacterium*-mediated transformation of walnut somatic embryos and regeneration of transgenic plants. BioTechnology **6**, 800-804.

MCPHEETERS, K.; SKIRVIN, R. M.; 1983: Histogenic layer manipulation in chimeral 'Thornless Evergreen' trailing blackberry. Euphytica **32**, 351-360.

MOREL, G.; 1944: Sur le développement de tissus de vigne cultivés *in vitro*. C. R. Séances Soc. Biol. **138**, 62.

-- -- ; 1948: Cultures associées de tissus végétaux et de parasites obligatoires. Ann. Epiphyt. **14**, 78-84.

MORIGUCHI, T.; KOZAKI, I.; MATSUTA, N.; YAMAKI, S.; 1988: Plant regeneration from grape callus stored under a combination of low temperature and silicone treatment. Plant Cell Tissue Organ Culture **15**, 67.

MULLINS, M. G.; 1971: Induction of haploidy in woody perennial fruit plants. CSIRO Div. Hort. Res. Rep. 1969-1971, 26-27.

-- -- ; 1985: Amplification of clonal variation in the grapevine: progress and prospects. Coll. Amélioration de la Vigne et Culture *in vitro*; Moët-Hennessy, Paris, April 1985, 63-73.

-- -- ; SRINIVASAN, C.; 1976: Somatic embryos and plantlets from an ancient clone of the grapevine (cv. Cabernet-Sauvignon) by apomixis *in vitro*. J. Exp. Bot. **27**, 1022-1030.

NEGI, S. S.; OLMO, H. P.; 1971: Conversion and determination of sex in *Vitis vinifera* L. Vitis **9**, 265-279.

OCHATT, S. J.; CHAND, P. K.; RECH, E. L.; DAVEY, M. R.; POWER, J. B.; 1988 a: Electroporation-mediated improvement of plant regeneration from Colt cherry (*Prunus avium x Prunus cerasus*) protoplasts. Plant Sci. **54**, 165-169.

– –; PATAT-OCHATT, E. M.; RECH, E. L.; DAVEY, M. R.; POWER, J. B.; 1988 b: Somatic hybridization of pear and cherry to obtain new rootstocks [Abstr.]. Coll. Les Stratégies de Sélection Face aux Technologies Modernes; Moët-Hennessy, Versailles, September 1988, 25.

– –; POWER, J. B.; 1988 a: Rhizogenesis in callus from Conference pear (*Pyrus communis L.*) protoplasts. Plant Cell Tissue Organ Culture **13**, 159.

– –; – –; 1988 b: Plant regeneration from mesophyll protoplasts of Williams Bon Chretien (syn. Bartlett) pear (*Pyrus communis* L.). Plant Cell Rep. **7**, 587-589.

RAJASEKARAN, K.; MULLINS, M. G.; 1979: Embryos and plantlets from cultured anthers of hybrid grapevines. J. Exp. Bot. **30**, 399-407.

– –; – –; 1981: Organogenesis in internode explants of grapevines. Vitis **20**, 218-227.

– –; – –; 1983: The origin of embryos and plantlets from cultured anthers of hybrid grapevines. Amer J. Enol. Viticult. **34**, 108-113.

RAMMING, D. W.; EMERSHAD, R. L.; 1982: *In ovulo* embryo culture of seeded and seedless *Vitis vinifera* L. HortScience **17**, 487.

REISCH, B. I.; 1983: Genetic variability in regenerated plants. In: EVANS, D. A.; SHARP, W. R.; AMMIRATO, P. V.; YAMADA, Y. (Eds.): Handbook of Plant Cell Culture, **1**, Techniques for Propagation and Breeding, 748-769. MacMillan Publishing Co.

– –; ALDWINCKLE, H. A.; ROBERTS, M. H.; KIM, S. K.; 1985: I. Embryogenesis from petiole cultures of 'Horizon' grapes. II. *In vitro* grape shoot tip mutagenesis. Coll. Amélioration de la Vigne et Culture *in vitro*; Moët-Hennessy, Paris, April 1985, 161-170.

RIVES, M.; 1971: L'ampélographie dans l'histoire. In: RIBÉREAU-GAYON, J.; PEYNAUD, E. (Eds.): Traité d'Ampélologie: Sciences et Techniques de la Vigne, 145-147. Dunod, Paris.

SEMAL, J. (Ed.); 1986: Somaclonal Variations and Crop Improvement. Martinus Nijhof Publishers, Dordrecht, Boston, Lancaster.

SKENE, K. G. M.; 1974: Culture of protoplasts from grapevine pericarp callus. Austral. J. Plant Physiol. **1**, 371-376.

– –; 1975: Production of callus from protoplasts of cultured grape pericarp. Vitis **14**, 177-180.

– –; BARLASS, M.; 1983: Studies of the fragmented shoot apex of grapevine. IV. Separation of phenotypes in a periclinal chimera *in vitro*. J. Exp. Bot. **34**, 1271-1280.

SPIEGEL-ROY, P.; SAHAR, N.; BARON, I.; LAVI, U.; 1975: *In vitro* culture and plant formation from grape cultivars with abortive ovules and seeds. J. Amer. Soc. Hort. Sci. **110**, 109-112.

– –; – –; – –; – –; 1986: Ovule and seed culture from early ripening seedless and seeded grape cultivars. Vignevini **13** Suppl. al (12), 34-35.

STEIN, U.; HOOS, G.; 1984: Induktions- und Nachweismethoden für Stilbene bei Vitaceen. Vitis **23**, 179-194.

VALAT, C.; RIVES, M.; 1973: Information and comments on variation induced by thermotherapy. Riv. Pathol. Veg. **4**, 291-293.

VARDI, A.; SPIEGEL-ROY, P.; GALUN, E.; 1982: Plant regeneration from citrus protoplasts: variability in methodological requirements among cultivars and species. Theor. Appl. Gen. **62**, 171-176.

VASIL, I. K.; 1983: Regeneration of plants from single cells of cereals and grasses. In: LURQUIN, P. F.; KLEINFOFS, A. (Eds.): Genetic Engineering in Eucaryotes. 233-252. Plenum Press, New York.

VILAPLANA, M.; MULLINS, M. G.; 1989: Regeneration of grapevines (*Vitis* spp.) *in vitro*: Formation of adventitious buds on hypocotyls and cotyledons of somatic embryos. J. Plant Physiol. **134**, 413-419.

WRIGHT, D. C.; 1985: Factors affecting isolation of protoplasts from leaves of grape (*Vitis vinifera*). Plant Cell Tissue Organ Culture **4**, 95-100.

YAMAKAWA, T.; ONOMICHI, K.; KODAMA, T.; MINODA, Y.; 1985: Application of feeder layer method for improved colony formation of grape cells and protoplasts at low density. Agricult. Biol. Chem. **49**, 3583-3585.

ZOU, C.; LI, P.; 1981: Induction of pollen plants of grape (*Vitis vinifera* L.). Acta Bot. Sinica **23**, 79-81.

Progress toward the production of transgenic grapevines by *Agrobacterium*-mediated transformation

C. P. MEREDITH [1]), S. M. COLBY [1]), J. A. STAMP [3]), A.M. DANDEKAR [2]) and E. B. BANDMAN [1])

[1]) Department of Viticulture and Enology, University of California, Davis, California 95616, USA

[2]) Department of Pomology, University of California, Davis, California 95616, USA

[3]) Agritope Inc., 15425 S.W. Koll Parkway, Beaverton, Oregon 97006, USA

Summary: Grape possesses the basic prerequisites for *Agrobacterium*-mediated transformation – it is a host for *Agrobacterium* and plant regeneration can be induced from cultured grape explants. Leaf explants were cocultivated with disarmed *Agrobacterium* vectors carrying kanamycin resistance and GUS genes and cultured on shoot-inducing medium containing kanamycin. After 21 d, intense and sharply-defined blue regions were observed, including some blue organized meristematic structures, consistent with plant-driven GUS gene expression. No GUS activity was detected in control explants. Among single leaf tips excised from over 200 regenerated shoots, one was GUS positive. The recovery of transgenic shoots might be improved by increasing the frequency or modifying the site of transformation and/or regeneration.

Key words: transgenic grapevine, Agrobacterium, crown gall, tissue culture, kanamycin resistance, β-glucuronidase, gene expression, genetic engineering, genetics.

Introduction

Of the several different transformation strategies that have now been successfully employed to produce transgenic plants, the most common is *Agrobacterium*-mediated transformation (KLEE et al. 1987). This approach exploits the natural ability of this bacterium to introduce genes into plant cells. *Agrobacterium* strains from which the disease-causing properties are removed ('disarmed') can be endowed with new genes of academic or agricultural interest from virtually any other organism. When such a strain is allowed to infect a plant cell, the new gene is introduced and integrated into the plant's own genetic material and becomes a permanent part of the genotype of the recipient plant cell. Because the descendents of such a transformed cell inherit the new gene, a plant regenerated from transformed cells will be transgenic: it will carry the new gene in every cell.

The basic requirement for successful *Agrobacterium*-mediated transformation is an explant that is both transformable and regenerable. The plant species must be a host for *Agrobacterium* and the particular explant tissue used must be susceptible to infection. The recovery of a transgenic plant is dependent on a means to regenerate an entire plant from the explant. These requirements have been fulfilled in systems as diverse as tomato leaf disks from which adventitious shoots develop (HORSCH *et al.* 1985) and walnut somatic embryos that produce secondary embryos on their surface (MCGRANAHAN *et al.* 1988). Grape (*Vitis vinifera* L.) is a well-known host for *Agrobacterium* and, in fact, crown gall disease is a serious viticultural problem (PEARSON and GOHEEN 1988). Entire plants can also be regenerated from a variety of grape explant types via either adventitious shoots or somatic embryos (MONETTE 1988). Because the basic requirement is met, it is reasonable to expect that *Agrobacterium*-mediated transformation can be successfully adapted to grape.

The AGROBACTERIUM strains associated with grapevines in nature are predominantly of the biovar 3 type (PEARSON and GOHEEN 1988). However, the strains generally used as plant gene vectors are biovar 1, a distinctly different group with different properties (KNAUF *et al.* 1982). To establish whether the biovar 1 strains in general use might be employed as gene vectors for grape, we investigated the ability of several biovar 1 strains to incite galls on *in vitro*-grown plants of

Results of a typical cocultivation experiment with 'Thompson Seedless' leaf explants. Data was collected after 15 d of incubation, at which time all tissue was sacrificed for analysis and none was allowed to develop further

	Number of explants	Percentage
Total	193	
Producing adventitious buds and shoots	120	62
Containing GUS-positive regions	183	95
GUS expression in meristematic structures	14	7
Structures partially GUS-positive	7	4
Structures entirely GUS-positive	7	4

several *vinifera* cultivars. Although differences were observed among strains, galls were produced on all cultivars and the presence and expression of introduced genes could be detected in gall tissue, thus demonstrating that biovar 1 *Agrobacterium* strains could infect and transform grape cells (MARTIN 1987).

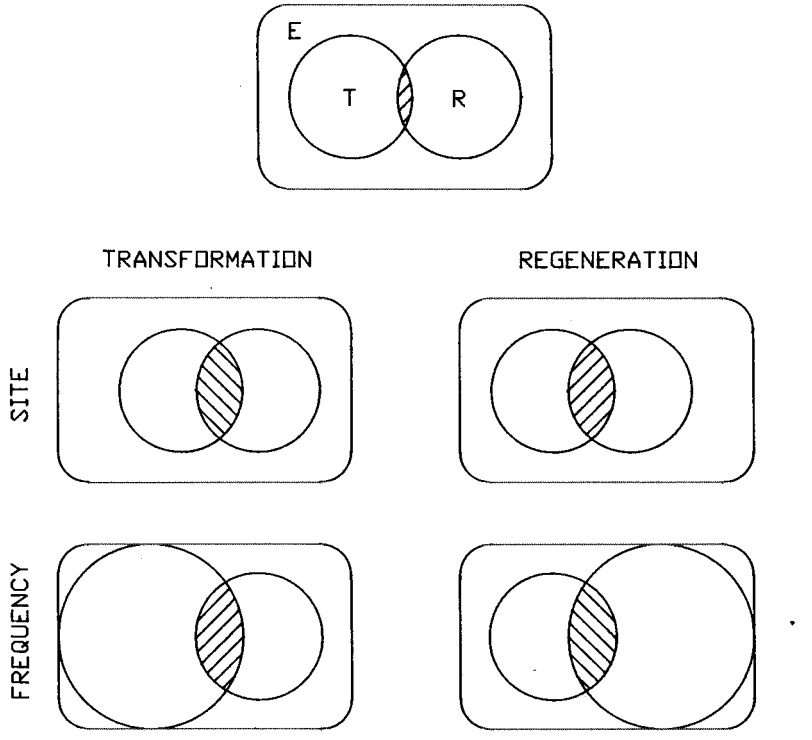
Materials and methods

Employing disarmed *Agrobacterium* vectors carrying chimeric genes encoding aminoglycoside phosphotransferase (kanamycin resistance) and β-glucuronidase (GUS), an enzyme whose activity can be detectecd as a blue color in a histochemical assay (JEFFERSON *et al.* 1987), we conducted a series of studies with leaf explants from which the development of adventitious shoots can be induced. Leaves of *V. vinifera* cvs Thompson Seedless (syn. Sultanina) and French Colombard were obtained from *in vitro* nodal cultures and either cultured intact or bisected perpendicular to the midrib. Under the appropriate conditions, this explant type will produce adventitious shoots on the petiolar stub and at the basal end of severed veins (STAMP *et al.* 1989). Leaf explants were cocultivated with *Agrobacterium* and then incubated on shoot-inducing medium containing carbenicillin and kanamycin. At periodic intervals, explants were sacrificed and sectioned for histochemical analysis of GUS activity. Other explants were incubated until adventitious shoots developed. A single leaf-tip was removed from each adventitious shoot for GUS assay and the shoots were also excised and tested for their ability to root in kanamycin-containing medium.

Results and discussion

Analysis of GUS expression by both stereo- and compound microscopy at 4 d post cocultivation revealed diffuse pale blue patches on the lamina and petiole regions and within the leaf veins, suggestive of *Agrobacterium*-driven GUS gene expression. After 11 d, however, intense and sharply defined blue-staining regions could be seen with the aid of a stereomicroscope. Closer examination of these regions with a compound microscope revealed both individual cells and groups of cells containing dark blue cytoplasm, consistent with plant-driven GUS expression in clones of transformed plant cells. Many of these regions were internal but some occurred on the explant surface. Infrequently, organized meristematic structures associated with the early stages of shoot formation were observed that were either partially or entirely intensely blue-staining, suggesting that, in these explants, cells that can be transformed (or their descendants) can also participate in regeneration (Table). No GUS activity was detected in control explants that had not been cocultivated with *Agrobacterium*.

Explants that were allowed to continue until adventitious shoots formed (28-56 d) exhibited bleaching typical of kanamycin toxicity. Islands of green tissue and, less commonly, green shoots were observed on some cocultivated explants. Histochemical analysis of leaf tips from over 200 of these green shoots revealed one GUS-positive leaf tip. The putatively transgenic shoot from which this leaf tip was obtained was destroyed in a laboratory accident before a Southern analysis could be performed.



Improving the recovery of transgenic plants. The relative number of cells in the explant (E) that participate in both transformation (T) and regeneration (R) is indicated by the shaded area. Increasing the frequency or changing the site of either transformation or regeneration will increase the size of the shaded area.

Although the approach employed here possessed the two requisite components – an explant that is both transformable and regenerable – it has not yet resulted in the production of transgenic plants at an acceptable frequency. There are a number of avenues by which the results might be improved.

A transgenic plant is the result of the coincidence of transformation and regeneration in the same cells (Fig.). The larger the number of transformed cells that also participate in regeneration, the higher the probability of recovering a transgenic plant. Both transformation and regeneration are phenomena that are amenable to manipulation. Critical parameters of both processes are frequency and site. An increase in frequency or a change in the site of either transformation or regeneration may increase the size of the coincident cell population (Fig.). The site and/or frequency of transformation might be improved by choice of *Agrobacterium* strain, cocultivation conditions, or explant type. The site and/or frequency of regeneration might be modified by choice of explant or culture conditions. A histological study of adventitious shoot development from leaf explants has revealed that the coincidence of transformation and regneration is likely very small under our conditions (COLBY, S. M.; JUNCOSA, A.; STAMP, J. A.; MEREDITH, C. P.; in preparation) and we are currently investigating several approaches by which we hope to improve our success.

Literature cited

HORSCH, R. B.; FRY, J. E.; HOFFMANN, N.; EICHHOLTZ, D.; ROGERS, S. G.; FRALEY, R. T.; 1985: A simple and general method for transferring genes into plants. Science **227**, 1229-1231.

JEFFERSON, R. A.; KAVANAGH, T. A.; BEVAN, M. W.; 1987: GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. **6**, 3901-3907.

KLEE, H.; HORSCH, R.; ROGERS, S.; 1987: *Agrobacterium*-mediated plant transformation and its further applications to plant biology. Ann. Rev. Plant Physiol. **38**, 467-486.

KNAUF, V. C.; PANAGOPOULOS, C. G.; NESTER, E. W.; 1982: Genetic factors controlling the host range of *Agrobacterium tumefaciens*. Phytopathology **72**, 1545-1549.

MARTIN, L. A.; 1987: Genetic Transformation and Foreign Gene Expression in Tissue of Different Woody Species. Master of Science Thesis, Univ. of California, Davis, USA.

MCGRANAHAN, G. H.; LESLIE, C. A.; URATSU, S. L.; MARTIN, L. A.; DANDEKAR, A. M.; 1988: *Agrobacterium*-mediated transformation of walnut somatic embryos and regeneration of transgenic plants. Bio-Technology **6**, 800-804.

MONETTE, P. L.; 1988: Grapevine. In BAJAI, Y. P. S. (Ed.): Biotechnology in Agriculture and Forestry, Vol. **6**, Crops II, 3-37. Springer-Verlag, Berlin.

PEARSON, R. C.; GOHEEN, A. C. (Eds.); 1988: Grape Disease Compendium. American Phytopathological Society, St. Paul, Minnesota, USA.

STAMP, J. A.; COLBY, S. M.; MEREDITH, C. P.; 1989: Direct shoot organogenesis and plant regeneration from leaves of grape (*Vitis vinifera* L.). Plant Cell Tissue Organ Culture (submitted).

Grapevine root transformation with *Agrobacterium rhizogenes* [1])

I. Gribaudo and A. Schubert

Centro Miglioramente Genetico Vite, C.N.R., Via P. Giuria, 15, I-10126 Torino, Italy

Summary: Grapevine shoots were obtained from tissue cultures of cvs Barbera, Moscato bianco and Nebbiolo; their stems were wounded and inoculated with the following strains of *Agrobacterium rhizogenes*: A4, 8196, NCPP 2659, and 15834. Root production at the inoculation site was obtained in about 25 % of the stems of all cultivars with all the bacterial strains, with the exception of NCPP 2659, which gave a lower root proliferation rate. Cultures of roots obtained after inoculation were established and their growth was enhanced by some media and by addition of cytokinins and auxins to the medium. Root cultures obtained after inoculation with the strain 8196 were genetically transformed, as shown by opine production. In contrast, opines were seldom detected in the root cultures obtained with the other bacterial strains. Light microscope observation showed that the cortex of transformed roots has more cell layers and a larger average cell size than in normal roots.

Key words: Agrobacterium rhizogenes, gene transfer, genetic engineering, genetics, tissue culture, light, growth regulator, root, growth, opine, histology.

Introduction

Grapevine breeding and selection have been undertaken utilizing scientific methods for more than one century, and most of the efforts of breeders have been directed toward the attainment of resistance to damaging pests and diseases or to the improvement of fruit quality and ripening time. Most of these problems, however, have been only partly overcome by traditional breeding methods, because of the difficulty in introducing only a single selected gene into a genome, without introducing other unwanted genetic characters.

The transfer of genetic material between different cells, using various kinds of vectors, is now an established technique for some plants (Armitage *et al.* 1988) and offers interesting perspectives for other less studied plants like grapevine. These methods are particularly interesting for their ability to transfer only the sequences which encode for a particular character. *Agrobacterium rhizogenes* is a commonly employed vector which has been tested on several species; expression of its Ri (root-inducing) T-DNA leads to root proliferation from the inoculation site. Such roots can be grown *in vitro* and whole plants can be regenerated from transformed root cultures (Birot *et al.* 1987; Zambryski *et al.* 1989). However, very little information is available on the possibility of utilizing *A. rhizogenes* on grapevine (Hemstad and Reisch 1985). In this work, we have tested methods to obtain and grow cultures of grapevine roots transformed with *A. rhizogenes*.

Material and methods

Bacterial strains

The *A. rhizogenes* strains used were: 1855, in a preliminary experiment, A4, 15834, 8196, and NCPP 2659. Bacteria were grown at 28 °C in Petri dishes containing the following medium (YMB): K_2HPO_4 0.5 g • l^{-1}; $MgSO_4 \cdot 7H_2O$ 0.2 g • l^{-1}; NaCl 0.1 g • l^{-1}; mannitol 1 %; yeast extract 0.04 %; agar 18 g • l^{-1}.

Cultures were stored at 4 °C for 1-2 months. Grapevine shoots were inoculated with *Agrobacterium* cultures grown for 36-48 h on fresh culture medium.

[1]) Contribution no. 210 of Centro Miglioramento Vite, C.N.R., Torino.

Plant material

In vitro rooted stock plants of *Vitis vinifera* cv. Nebbiolo (clone 111), Moscato bianco (clone 4), and Barbera (clone 84) were cultured on a hormone-free JONA and WEBB (1978) medium modified by increasing $FeSO_4$ + NA_2EDTA to 200 μM and thiamine HCl to 15 μM. The pH was adjusted to 5.6 before autoclaving at 120 °C for 10 min.

All the grapevine clones originated from clonal selection performed by this Centre.

Inoculation

5-6 cm long grapevine shoots were obtained by cutting the upper part of the *in vitro* stock plants. The shoots were wounded with a scalpel, partially scraping an internode in the median part of the stem, and inoculated with the bacteria. The shoots were then planted in the hormone-free medium described above. The control shoots were wounded but not inoculated.

In a preliminary experiment, shoots of Nebbiolo were inoculated with *A. rhizogenes* strain 1855. In a subsequent factorial experiment, shoots of Nebbiolo, Moscato and Barbera were inoculated separately with the *A. rhizogenes* strains A4, 8196, NCPP 2659 and 15834. The inoculation was replicated on a minimum of 11 shoots to a maximum of 80 for each treatment. The inoculated plants were kept in a growth chamber at 24 °C, with 250 μmol • s^{-1} • m^{-2} photon flux from fluorescent lamps and 16/8 h photoperiod.

Results were statistically analyzed by using chi square test.

Root cultures

Experiments on root cultures were performed using roots obtained from Nebbiolo shoots inoculated with strain A4.

In an initial experiment, 1 cm root pieces with tips were cultured on three different media, as following:

- medium A: as described by BECARD and FORTIN (1988) (medium MW), with 10 g • l^{-1} agar and 30 g • l^{-1} sucrose;
- medium B: MURASHIGE and SKOOG (1962) medium with half strength salts, 10 g • l^{-1} agar and 15 g • l^{-1} sucrose;
- medium C: MURASHIGE and SKOOG medium modified as suggested by MUGNIER and MOSSE (1987), with 30 g • l^{-1} sucrose bot no agar: a thin layer of medium was poured in each Petri dish.

The pH of all media was adjusted to 5.6 before autoclaving at 120 °C for 15 min. Root cultures were incubated at 24 °C; half of the cultures were kept under continuous dark conditions and another half under a 16/8 h photoperiod as described above. After 2 weeks culture, the growth of each root apex was measured.

In a subsequent experiment, roots were cultured in continuous darkness on medium B containing (GUELLEC, personal communication) 25 mg • l^{-1} of filter-sterilized Claforan (sodium cefotaxime; Roussel Maestretti, Milano); after 1 month, 1-2 cm long root fragments containing root apexes were transferred onto medium B without Claforan and with the following hormones added:

- medium IB: 0.1 μM BAP + 0.15 μM IBA;
- medium IA: 0.1 μM BAP + 0.15 μM IAA;
- medium NA: 0.1 μM BAP + 0.15 μM NAA.

In this case, it was not possible to measure root length because of excessive ramification: thus average root fresh weight was recorded after 4 weeks culture.

Results were statistically analyzed by ANOVA.

Table 1: Percentage of stems with callus and roots and average root number, 18 d after inoculation with *Agrobacterium rhizogenes* strain 1855 on the internode of Nebbiolo grape

	+ 1855	CONTROL	EFFECT
STEMS WITH			
CALLUS (%)	91.3	19.2	* *
ROOTS (%)	69.6	0	* *
AVERAGE ROOT NO.	2.2	0	

Opine analysis by paper electrophoresis

Root extracts were prepared according to PETIT *et al.* (1986) and spotted onto Whatman no. 3MM paper. After electrophoresis (100 V • cm^{-1} for 10 min) in formic acid-acetic acid-water buffer (30/60/910 v/v/v)the dried paper was treated with either silver nitrate reagent or Pauly reagent.

Histological observations

Roots growing out of the inoculation points were collected, embedded in O.C.T. compound (Miles Scient.; Naperville, IL) and sectioned using a cryostatic microtome.

Control roots were taken from the base of non-inoculated shoots, rooted on hormone-free medium; they were treated as above described.

Results

Plant reaction to inoculation

Shoots of Nebbiolo inoculated with *A. rhizogenes* strain 1855 produced slight amounts of callus and roots in the inoculation site, as reported in Table 1.

In the factorial experiment, production of small quantities of callus was more frequent in inoculated plants, independent of grapevine cultivar. No roots were produced in the control plants. Concerning the effects of *Agrobacterium* strain, an average of 21-27 % of the treated shoots produced roots from the inoculation point, with the exception of shoots inoculated with the strain NCPP 2659 (10 %). There was no considerable difference among cultivars, even if some combinations (i.e. Moscato and Barbera + 8196; Nebbiolo + 15834 and Moscato + A4) showed a greater response to inoculation (see Table 2).

Table 2: Comparative results of Barbera, Nebbiolo and Moscato stem inoculations with *Agrobacterium rhizogenes* strains NCPP 2659, A4, 15834, and 8196, 1 month after inoculation. For totals column and rows, values followed by a common letter do not differ significantly at P = 0.05 (small letters) or P = 0.01 (capital letters)

			BARB.	NEBB.	MOSC.	TOTAL
Agrobacterium rhizogenes strain	NCPP 2659	STEMS WITH CALLUS (%)	36	100	35	60 Aab
		ROOTS (%)	9	20	0	10 ABa
		root no.	1	1.7	-	
		root lenght (mm)	20	5.8	-	
	A4	STEMS WITH CALLUS (%)	17	59	50	53 Aa
		ROOTS (%)	0	25	42	24 Aa
		root no.	-	1.6	2.8	
		root lenght (mm)	-	12.4	6.1	
	15834	STEMS WITH CALLUS (%)	72	87	67	75 Ab
		ROOTS (%)	18	40	20	27 Aa
		root no.	2.5	2.2	2	
		root lenght (mm)	50.6	14.2	72.5	
	8196	STEMS WITH CALLUS(%)	36	58	92	59 Aab
		ROOTS (%)	54	8	75	21 Aa
		root no.	3.7	1.5	2	
		root lenght (mm)	7.7	22.7	5.8	
NOT INOCUL.		STEMS WITH CALLUS (%)	27	31	7	23 Bc
		ROOTS (%)	0	0	0	0 Bb
TOTAL		STEMS WITH CALLUS (%)	37 A	60 B	49 AB	54
		ROOTS (%)	16 A	16 A	25 A	18

Table 3: The effect of different media and photoperiods on Nebbiolo root growth (average increase in length as cm). The roots were obtained from stems inoculated with *Agrobacterium rhizogenes* strain A4. Medium A: BECARD and FORTIN (1988); medium B: MURASHIGE and SKOOG (1962), half-strength salts; medium C: liquid full strength MURASHIGE and SKOOG

MEDIUM	24 hrs dark 0 hrs light	8 hrs dark 16 hrs light	
A	15.1 ± 2.8	10.7 ± 2.0	EFFECT
B	6.4 ± 1.4	4.3 ± 7.5	MEDIUM *
C	5.6 ± 0.9	2.5 ± 0.6	
	EFFECT ILLUMINATION *		

Roots usually proliferated where callus was present, but in a few cases the internode formed roots but no callus: this may indicate that the growth of the two tissues is independent.

Rhizogenesis was relatively slow: for instance, after inoculation with the strain A4 only 9.2 % of Nebbiolo shoots produced roots from the inoculated internodes after 18 d, but the percentage increased to 27.3 % after 28 d. If the roots were cut off, the inoculated internodes produced new roots.

Root growth from the wounded and inoculated internode is independent of shoot basal rooting: treated shoots rooted in the basal part of the stem, placed in culture medium, as the control shoots did.

Root cultures

Roots produced by Nebbiolo internodes wounded and inoculated with *A. rhizogenes* strain A4 were cultured on different media. The basal medium described by BECARD and FORTIN (1988) was more effective in promoting root growth than the other media, as reported in Table 3. Roots grew better in continuous darkness.

Addition of BAP (0.1 μM) and auxins (IAA, IBA or NAA 0.15 μM) to the culture medium enhanced root growth, this effect being unrelated to the auxin type (see Table 4).

Opine analysis

Paper electrophoretic analysis revealed that not all the cultured roots synthesized opines. Roots obtained by grapevine internodes inoculated with *A. rhizogenes* strains A4 and 15834 revealed the presence of opines in 20 and 30 % of the samples, respectively, while 83 % of the root

Table 4: Root growth after 1 month of culture on media containing 0.1 mg • l^{-1} BAP and 0.15 mg • l^{-1} of different auxins. Roots were obtained from Nebbiolo shoots inoculated with *Agrobacterium rhizogenes* strain A 4

AUXIN (mg/l)	ROOT FRESH WEIGHT (mg)	EFFECT
IBA 0.15	200 ± 39	N.S.
NAA 0.15	189 ± 58	
IAA 0.15	173 ± 42	

samples obtained from inoculations with strain 8196 had opines. None of the roots grown following inoculation with strain NCPP 2659 produced opines.

It must be noted that among the faster growing root lines (obtained by stem inoculation of strains A4 and 15834) some produced opines and some did not.

Histological observations

Compared to normal roots, roots produced by the inoculated stems showed a strong increase in the cell layer number and in the cell size of the cortical parenchyma. Also, the intercellular spaces appeared larger than in the normal roots. In contrast, no remarkable difference could be noted in the epidermis and the underlying cells.

Discussion

The method described proved to be effective in transforming roots of all the three grapevine cultivars tested. The roots originating from the inoculation site have particular morphological features, and they can be best grown in presence of BAP and auxins, in continuous darkness.

Not all these roots proved to be transformed when the electrophoretic analysis of opines was performed. Fast growing root lines were obtained by stem inoculation of *A. rhizogenes* strains A4 and 15834; surprisingly, some of those root lines did not produce opines. However, it seems unlikely that in this case roots were not transformed, as preliminary tests showed that non-inoculated roots grew very poorly. The fast growing roots which did not produce opines could be the results of an incomplete transfer of the *Agrobacterium* plasmid. Agropine-type strains like A4 and 15834 have two separated T-regions (T_L and T_R): during transformation plant cells may receive either the T_L-DNA (which induces root proliferation) or the T_R-DNA (coding opine synthesis) or both (Melchers and Hooykaas 1987). More detailed analyses are needed to ascertain the nature of these root lines, and DNA analysis is planned.

Roots originating from inoculations with *A. rhizogenes* strain 8196 are mostly transformed, but they do not grow in the tested culture conditions. They usually darken and die in a short span of time or grow very slowly. It is possible that this problem may be overcome by changing the concentration of growth regulators in the medium.

The production of non-transformed roots from the inoculation point could depend on the wound made before inoculation. If being too deep, the wound could mimic the effects of apical cutting, i. e. root production at the new shoot base.

In conclusion, this method is useful to obtain transformed grapevine roots. Further investigations will be carried out in order to test the response of a larger number of grapevine varieties and to improve root growth in culture.

These investigations will be the first steps toward controlled transformation of grapevine cultivars. However, transgenic plants cannot be obtained unless an effective regenerating system from grapevine roots or callus or cell suspensions is developed. Many efforts are now directed toward this target.

Acknowledgements

Authors wish to thank Prof. JACQUES TEMPÉ for providing the A4, 8196, 15834, and NCPP 2659 *A. rhizogenes* strains and the electrophoretic equipment; Prof. PAOLO COSTANTINO for the strain 1855; JACQUES TEMPÉ, ANNIK PETIT, PAOLA BONFANTE-FASOLO and VERONIQUE GUELLEC for their precious suggestions; CLAUDIA CAMINO for the technical help.

References

ARMITAGE, P.; WALDEN, R.; DRAPER, J.; 1988: Vectors for the transformation of plant cells using *Agrobacterium*. In: DRAPER, J.; SCOTT, R.; ARMITAGE, P.; WALDEN, R. (Eds.): Plant Genetic Transformation and Gene Expression. Blackwell Sci. Publ.

BECARD, G.; FORTIN, J. A.; 1988: Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. New Phytol. **108**, 211-218.

BIROT, A. M.; BOUCHEZ, D.; CASSE-DELBART, F.; DURAND-TARDIF, M.; JOUANIN, L.; PAUTOT, V.; ROBAGLIA, C.; TEPFER, D.; TEPFER, M.; TOURNEUR, J.; VILAINE, F.; 1987: Studies and uses of the Ri plasmids of *Agrobacterium rhizogenes*. Plant Physiol. Biochem. **25**, 323-335.

HEMSTAD, P. R.; REISCH, B. I.; 1985: *In vitro* production of galls induced by *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* on *Vitis* and *Rubus*. J. Plant Physiol. **120**, 9-17.

JONA, R.; WEBB, J. K.; 1978: Callus and axillary-bud culture of *Vitis vinifera* 'Sylvaner Riesling'. Sci. Hort. **9**, 55-60.

MELCHERS, L. S.; HOOYKAAS, P. J. J.; 1987: Virulence of *Agrobacterium*. Oxford Surv. Plant Mol. Cell. Biol. **4**, 167-220.

MUGNIER, J.; 1987: Infection by *Polimixa betae* and *Plasmodiophora brassicae* of roots containing root-inducing transferred DNA of *Agrobacterium rhizogenes*. Phytopathology 77, 539-542.

-- -- ; MOSSE, B.; 1987: Vesicular-arbuscular mycorrhizal infection in transformed root-inducing T-DNA roots grown axenically. Phytopathology 77, 1045-1050.

MURASHIGE, T.; SKOOG, F.; 1962: A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. **15**, 473-497.

PETIT, A.; BERKALOFF, A.; TEMPÉ, J.; 1986: Multiple transformation of plant cells by *Agrobacterium* may be responsible for the complex organization of T-DNA in crown gall and hairy root. Mol. Gen. Genet. **202**, 388-393.

ZAMBRYSKI, P.; TEMPÉ, J.; SCHELL, J.; 1989: Transfer and function of T-DNA genes from *Agrobacterium* Ti and Ri plasmids in plants. Cell **56**, 193-201.

High frequency regeneration from grapevine petioles: Extension to new genotypes

B. I. Reisch, M. H. Martens and Z. M. Cheng

Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456, USA

Summary: Auxins were found to enhance shoot organogenesis when used in a high cytokinin medium at low levels. This enhancement was genotype specific. In the presence of 10.0 μM BA, Vanessa regenerated best with the inclusion of 4.0 μM IAA-dl-aspartate, while Catawba regenerated best with 2.0 μM IAA-glycine in the medium. With Ravat 51, 14-15 % of explants regenerated in the presence of either 2.0 μM IAA-glycine or 4.0 μM IAA-dl-aspartate, whereas there was no regeneration in the absence of auxin. Tests with 0.0-8.0 μM TDZ as a cytokinin source indicated that petiole explants of Vanessa regenerate best (10 %) at 4.0 μM TDZ. Our results clearly show that high cytokinin media can be easily extended to petiole explants of other genotypes to facilitate shoot organogenesis and that low levels of auxins, especially IAA conjugates can enhance the level of regeneration.

Key words: tissue culture, petiole, genotype, growth regulator, organogenesis, regeneration.

Introduction

Improved ability to produce plants from tissue cultures of *Vitis* would expedite efforts in genetic engineering, *in vitro* mutant isolation, rapid multiplication of elite stocks, and the production of haploids and then homozygous diploids.

In grapes, varying degrees of organogenesis and somatic embryogenesis have been obtained. Shoot organogenesis and embryogenesis has been obtained from vegetative tissues, such as shoot apices (Barlass and Skene 1978, 1980 a, 1980 b), leaf callus (Favre 1977; Hirabayashi 1985; Stamp and Meredith 1988), and internode callus (Krul and Worley 1977; Rajasekaran and Mullins 1981). Yet most of the available techniques are limited by low frequency of regeneration, genotype and explant specifity, and a process involving two or three steps.

The objective of our research was to extend the regeneration system developed for *Vitis x labruscana* Catawba (Cheng and Reisch 1989) to other genotypes. We also planned to examine auxin and cytokinin effects on regeneration from petiole tissues.

Materials and methods

The grape cultivars Catawba, Vanessa and Ravat 51 (Vignoles) were established *in vitro* from vines grown at the New York State Agricultural Experiment Station, Geneva, NY. All explants for regeneration experiments were derived from *in vitro* shoot cultures.

Axillary buds from the basal part of growing primary shoots were isolated and washed for 30 min in tap water with Micro detergent (International Products Corp., Trenton, NJ, USA). After woody parts and outer layers of bud scales were removed, buds were submerged in 70 % ethanol for 2-3 min and then were transferred to 1.85 % sodium hypochlorite for 15 min, followed by 3-5 washes in sterile distilled water. Apex tissues, 2-4 mm long, were isolated from buds and inoculated for shoot multiplication on MS basal medium (Murashige and Skoog 1962) supplemented with 4.0 μM BA. Conditions for further growth of shoot tip cultures were described earlier (Reisch 1986).The micropropagated shoots were later cultured in MS medium or C_2d (Chee and Pool 1987) with 2.0 or 4.0 μM BA prior to excision of explants. All media were solidified with agar (Difco bacto) at 7.0 g/l.

The basic medium for regeneration experiments was NN69 (NITSCH and NITSCH 1969). This medium was supplemented with various plant growth regulators or other components. Vitamin concentrations were those of MURASHIGE and SKOOG (1962). Myo-inositol (100 mg/l), sucrose (20 g/l), BA, IBA, TDZ and amino acid conjugates of IAA were added to the media prior to autoclaving.

Petioles from shoot cultures were isolated with great care to exclude nearby axillary buds. Each treatment included 6-12 Petri dishes (25 x 100 mm) with 10 explants each.

The cultures for regeneration were grown in the dark (in cardboard boxes) in a culture room at 24-26 °C. After 6 weeks of culture, cultures were transferred to BA containing media under a 16 : 8 light : dark photoperiod to induce further shoot growth.

Table 1: Regeneration from petioles of Vanessa and Catawba after 8 weeks on media with 10.0 µM BA plus the indicated auxin (60-120 petioles/tmt)

VARIETY	AUXIN SOURCE	% REGENERATION
Vanessa	none	3.3
Vanessa	4.0 µM IAA-dl- asp	9.0
Vanessa	2.0 µM IAA-gly	2.5
Vanessa	0.5 µM IBA	8.3
Vanessa	0.5 µM NAA	0.0
Catawba	none	5.0
Catawba	4.0 µM IAA-dl-asp	7.0
Catawba	2.0 µM IAA-gly	10.8
Catawba	0.5 µM IBA	7.5
Catawba	0.5 µM NAA	0.0

Table 2: Regeneration from Ravat 51 petioles after 6 weeks (40-90 petioles/tmt)

BA (µM)	AUXIN (µM)	% REGENERATION
10.0	0.0	0.0
10.0	4.0 IAA-dl-ala	5.9
10.0	2.0 IAA-gly	15.0
10.0	20.0 IAA-gly	2.9
10.0	4.0 IAA-dl-asp	14.4
10.0	80.0 IAA-dl-asp	5.0

Table 3: Regeneration from Vanessa petioles after 8 weeks (65-100 petioles/tmt)

TDZ (µM)	% REGENERATION
0.0	0.0
2.0	4.6
4.0	10.0
6.0	6.1
8.0	7.3

Results and discussion

Auxin effects on grapevine regeneration from petiole cultures

Earlier results with Catawba suggested that regeneration could be greatly improved with the addition of autoclaved IBA to the culture medium. In this first experiment, the effects of IBA as well as other auxin sources were examined. Petioles were excised from *in vitro* shoots of both Vanessa and Catawba. Cultures were incubated in the dark for 6 weeks, 12 plates, 10 petioles/plate, on five media: 1. 10 μM BA, 2. 10 μM BA + 4.0 μM IAA-dl-aspartate, 3. 10 μM BA + 2.0 μM IAA-glycine, 4. 10 μM BA + 0.5 μM IBA (autoclaved), and 5. 10 μM BA + 0.5 μM NAA.

Results obtained do not re-confirm the large increase seen in Catawba regeneration on IBA containing medium (Table 1). Yet they do suggest that IBA can increase the rate of regeneration. The glycine conjugate of IAA seems to be the most effective auxin source for Catawba, while the aspartate conjugate is the most effective for Vanessa regeneration. The use of NAA at the level tested prevented regeneration of both cultivars.

A second experiment concentrated on defining the optimum levels of IAA conjugates in the medium. Conjugates of IAA are reported to stabilize the levels of IAA in tissue culture media and provide for a slower, steadier release of auxin over a longer period of time. Preliminary results in our lab have shown that these conjugates are extremely active in grape tissue cultures. Using petioles from *in vitro* shoots of Ravat 51, three conjugates of IAA were tested in combination with 10 μM BA at the following levels: 10 μM BA (control), 10 μM BA + 4.0 μM IAA-dl-alanine, 10 μM BA + 2.0 μM IAA-glycine, 10 μM BA + 20.0 μM IAA-glycine, 10 μM BA + 4.0 μM IAA-dl-aspartate, and 10 μM BA + 80.0 μM IAA-dl-aspartate.

All conjugates tested effectively improved regeneration over the control (Table 2). The highest levels of regeneration with Ravat 51 petioles were achieved on media with either 2.0 μM of the glycine conjugate or 4.0 μM of the aspartate conjugate. In the latter case, the effective concentration is 2.0 μM since only the 'l' form of the conjugate can be naturally hydrolysed *in vitro*.

Cytokinin effects on grapevine regeneration from petiole cultures

Preliminary experiments with thidiazuron (TDZ) in the range of 0.0-2.0 μM showed that TDZ was an effective cytokinin-like compound when used with grape petiole cultures. We then sought to determine its optimum effective level and to test it at very high levels. Earlier results suggested that it would be effective alone or in combination with BA. This present experiment tested TDZ in the absence of other growth regulators. Petioles were derived from Vanessa shoots grown *in vitro* and cultured in the dark at the indicated concentrations of TDZ for 6 weeks followed by transfer to the light (16 h photoperiod).

Without TDZ, no callus growth or regeneration was observed (Table 3). All levels of TDZ tested (2.0-8.0 μM) produced shoots from the cut ends of the petioles. The optimum level of TDZ was determined to be 4.0 μM, a level which is twice as high as any previously tested level of TDZ in our lab.

In the present experiments, two new genotypes, Vanessa and Ravat 51, have been shown to be regenerable via the petiole regeneration system. The frequency of regeneration is lower than that reported for Catawba, yet it was comparable to the frequency for Catawba in the one experiment (Table 1) in which Catawba was run side by side with Vanessa. For unknown reasons, the level of regeneration may vary considerably from experiment to experiment. This may be due to explant physiological status on the shoot proliferation medium, node position of the petiole, and other *in vitro* factors which may affect the hormone or nutritional status of petioles prior to explanting to regeneration medium.

Acknowledgements

This research was supported by the New York State Grape Production Research Fund and Grant No. US 1181-86 from BARD - The United States - Israel Binational Agricultural Research & Development Fund. We would like to express our gratitude for the personal support provided by JOHN DYSON. Support from Hatch Project 32487 is also acknowledged.

Literature cited

BARLASS, M.; SKENE, K. G. M.; 1978: *In vitro* propagation of grapevine (*Vitis vinifera* L.) from fragmented shoot apices. Vitis **17**, 335-340.

-- -- ; -- -- ; 1980 a: Studies on the fragmented shoot apex of grapevine. I. The regenerative capacity of leaf primordial fragments *in vitro*. J. Exp. Bot. **31**, 483-488.

-- -- ; -- -- ; 1980 b: Studies on the fragmented shoot apex of grapevine. II. Factors affecting growth and differentiation *in vitro*. J. Exp. Bot. **31**, 489-495.

CHEÉ, R.; POOL, R. M.; 1987: Improved inorganic media constituents for *in vitro* shoot multiplication of *Vitis*. Sci. Hort. **32**, 85-95.

CHENG, Z. M.; REISCH, B. I.; 1989: Shoot regeneration from petioles and leaves of *Vitis x labruscana* cv. Catawba. Plant Cell Reports **8** (7) [in press].

FAVRE, J. M.; 1977: Premiers résultats concernant l'obtention *in vitro* de néoformations caulinaires chez la vigne. Ann. Amélior. Plantes **27**, 151-169.

HIRABAYASHI, T.; 1985: Somatic embryogenesis from leaf tissues of grape. Coll. Amélioration de la Vigne et Culture *in Vitro*, 23-24 April, Moët-Hennessy, Paris, France, 75-82.

KRUL, W. R.; WORLEY, J. F.; 1977: Formation of adventitious embryos in callus cultures of Seyval, a French hybrid grape. J. Amer. Soc. Hort. Sci. **102**, 360-363.

MURASHIGE, T.; SKOOG, F.; 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 473-479.

NITSCH, J. P.; NITSCH, C.; 1969: Haploid plants from pollen grains. Science **163**, 85-87.

RAJASEKARAN, K.; MULLINS, M. G.; 1981: Organogenesis in internode explants of grapevines. Vitis **20**, 218-227.

REISCH, B. I.; 1986: Influence of genotype and cytokinins on *in vitro* shoot proliferation of grapes. J. Amer. Soc. Hort. Sci. **111**, 138-141.

STAMP, J. A.; MEREDITH, C. P.; 1988: Somatic embryogenesis from leaves and anthers of grapevine. Sci. Hort. **35**, 235-250.

Isolation and culture of grapevine protoplasts

G. Reustle and G. Alleweldt

Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, F. R. Germany

Summary: Methods were established for isolation of protoplasts from different organs and tissues of grapevine plants grown *in vitro*. Cell division could not be induced in protoplasts from leaves, shoot tips and petioles of cv. Optima, whereas stem and root protoplasts showed division activity. Protoplasts derived from stems continued developing and formed microcalli and calli. In experiments using stem protoplasts of several varieties, root and stem protoplasts divided in all cases; stem protoplasts of 4 varieties (Riesling, Kerner, Optima, Vidal) could be regenerated to callus. In leaf protoplasts, cell division could be induced only in case of cvs Vidal and Rupestris du Lot, however without formation of callus.

Key words: Protoplast, isolation, variety of vine, leaf, shoot, root, in vitro culture, methodology, cell division, cell wall formation, yield, viability, callus regeneration.

Introduction

For some objectives of plant improvement, tissue culture, e. g. meristems and embryos, are used with the advantage of the ease to regenerate whole plants.

Protoplasts are also the object of intensive investigations. They are useful for basic studies on plant physiology and in particular, they offer new alternatives in plant breeding. They are helpful for effective selection of somaclonal variation at the cellular level, for somatic hybridization and for genetic transformation via direct DNA uptake.

A prerequisite for the use of protoplast systems is the regeneration to plants (Krul 1989). Regeneration from protoplasts is possible for many plant species and in the case of potato, protoplast techniques e. g. fusion, are well established and already integrated in breeding programs (Puite *et al.* 1988).

In spite of many investigations with grapevine protoplasts (Shimizu 1985; Wright 1985; Barbier and Bessis 1987 a, 1987 b), plant regeneration has not been reported and only few scientists have obtained callus from protoplasts of grapevine tissue (Skene 1975; Brezeanu and Rosu 1984; Yamakawa *et al.* 1985; Lee and Wetzstein 1988). The presented results describe the isolation procedure for grapevine protoplasts and the effect of the donor material on cell division and callus formation of protoplasts.

Material and methods

The protoplast isolation method was developed using leaf material of *in vitro* grown grapevines, cv. Optima. *In vitro* cultures were established on LS-medium (Linsmaier and Skoog 1965) supplemented with 0.01 ppm NAA and 0.03 ppm BAP. Culture conditions were 14 h photoperiod, 100 μE • m^{-2} • s^{-1} light intensity and 24-26 °C.

In some experiments, donor plants were cultured in medium with reduced ammonium concentration (150 mg/l) and without hormons. Furthermore, donor plants were incubated at reduced growth conditions (10 μE • m^{-2} • s^{-1}, 8 °C) for 2-6 weeks prior to isolation.

In a further attempt, leaves were preconditioned according to Haberlach *et al.* (1985) in order to induce cell division of protoplasts.

Abbreviations: NAA, 1-naphtaleneacetic acid; BA, 6-benzyladenine; 2,4-D, 2,4-dichlorophenoxyacetic acid; MES, 2 (N-morpholino)ethanesulfonic acid: BSA, bovine serum albumin: PVP, polyvinylpyrrolidone.

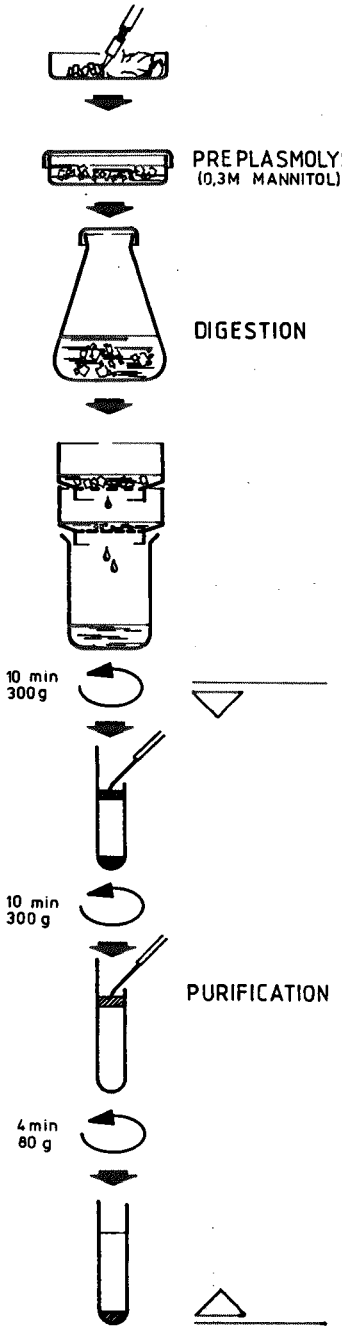


Fig.1: Isolation procedure.

Isolation

Prior to isolation procedure (Fig. 1), donor plants were cultured in darkness for 24 h.

Plant material was cut into small pieces and incubated for 15 min in 0.3 M mannitol for preplasmolysis. Afterwards, the material was transferred to an enzyme solution for digestion. The solution contained a combination of three different cellulases: cellulase *Aspergillus niger* (0.2-0.8 %), cellulase *Penicillium funiculosum* (0.2-0.8 %) and cellulase *Trichoderma viride* (0.4-1.6 %) (De Filippis and Ziegler 1985) and Macerozyme R-10 (0.1-0.5 %). The mixture was supplemented by BSA (0.5 %), MES-KOH (20 mM), $CaCl_2$ (1 mM), VKM-salts (1/10 strength), according to Binding and Nehls (1977) and 0.54 M sucrose or 0.6 M mannitol. Cellulase concentration and duration of digestion depended on the used material.

After digestion, the protoplast suspension was sieved (100 μm and 50 μm) to eliminate large undigested pieces. To purify the solution from cell debris and broken cells two flotation steps (300 *g* for 10 min) in 0.6 M sucrose followed by a sedimentation step (80 *g* for 4 min) in wash-solution (VKM-salts, 18.7 g/l NaCl) were carried out. Finally the protoplast pellet was resuspended in VKM-culture medium (Binding and Nehls 1977).

Cell density was adjusted to 2-5 • 10^5 protoplasts/ml medium. Yield was estimated in a counting chamber, viability and cell wall formation were detected using fluorescein diacetate and calcofluor white, respectively.

Culture

Protoplasts were cultured in darkness at 26 °C. The culture medium was supplemented with different substances (BSA, PVP-40, amino acids) and 1 ppm 2,4-D and 0.5 ppm BAP. Treatments of subculture were carried out, depending on the development of protoplasts and the intensity of browning of the medium.

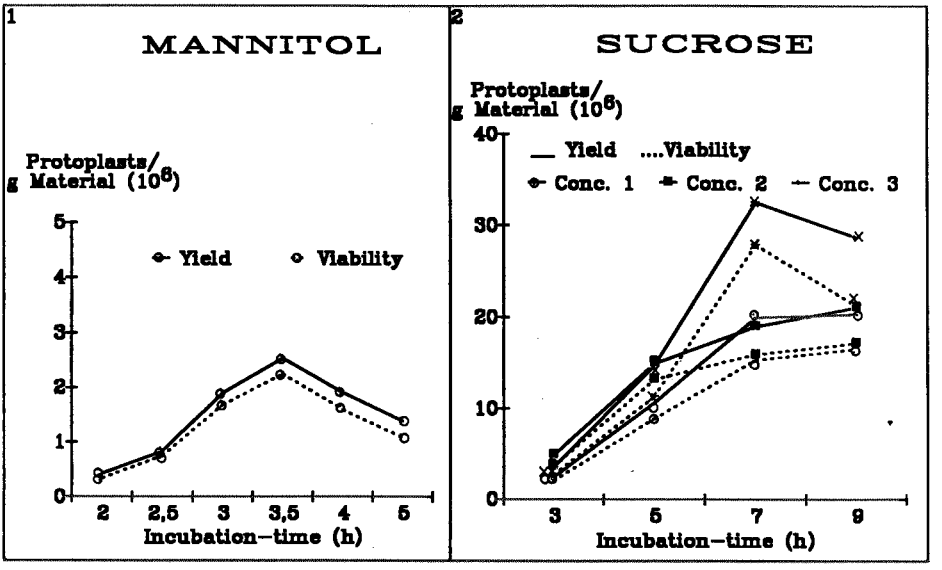


Fig. 2: Effect of osmoticum, enzyme concentration and incubation time on yield and viability of leaf protoplasts (enzyme concentration see Table 1).

Table 1: Effect of incubation time and enzyme concentration on viability and cell wall formation (CWF) of leaf protoplasts after 14 d of culture

Duration of Isolation	Conc.1 Viability %	Conc.1 CWF* %	Conc.2 Viability %	Conc.2 CWF %	Conc.3 Viability %	Conc.3 CWF %
3 h	37,4	17,4	68,9	28,3	40,9	10,9
5 h	31,7	13,5	33,1	18,9	33,9	13,9
7 h	21,0	9,2	10,5	3,4	19,7	7,9
9 h	13,8	7,9	8,4	5,0	9,7	3,8

	Conc.1	Conc.2	Conc.3
cellulase (total concentration)	0,8 %	1,6 %	3,2 %
macerozyme R-10	0,1 %	0,2 %	0,4 %

*) cell wall formation;

Results and discussion

Isolation

Besides enzyme concentration and time of digestion, the release of protoplasts from leaf material was influenced by the osmotic substances, mannitol and sucrose, used (Fig. 2).

Mannitol resulted in a typical release of protoplasts depending on time of isolation. An optimum of yield was obtained after 3-4 h of digestion. Longer incubation time resulted in a reduction of yield and viability of protoplasts (Fig. 2.1).

With sucrose as osmoticum (Fig. 2.2), different cellulase concentrations as well as different durations of digestion were tested. Only the highest concentration (3.2 % cellulase, 0.4 % Macerozyme R-10) in combination with the longest duration of digestion (9 h) resulted in a reduced yield and viability of protoplasts. Furthermore protoplast yield was higher with sucrose than with mannitol as osmoticum after digestion using the same enzyme concentrations and durations of digestion.

This phenomenon may be the result of procedural differences since one centrifugation step was omitted when using sucrose. Another explanation could be a direct influence of sucrose on stability of protoplast membranes or on harmful substances in the digestion solution.

A measurement of the protoplast viability is their development in culture. Protoplasts, isolated with different cellulase concentrations and incubation times using sucrose as osmoticum (Fig. 2.2) were cultured and viability and cell wall formation were investigated after 14 d of cultivation. As shown in Table 1, viability as well as cell wall formation were distinctly influenced by incubation time, whereas the effect of enzyme concentration was not clear.

The influence of the intensity of isolation on protoplast development was demonstrated with these experiments. Moreover it is shown, that a short incubation time with an intermediate enzyme concentration is preferable to a longer duration of digestion with lower enzyme concentrations.

Culture

Different factors influencing protoplast culture were tested but few improved the protoplast development (Table 2).

To prolong viability of protoplasts, the addition of BSA (directly from the beginning of cultivation) and PVP-40 (added after 1-2 weeks of cultivation) was important to eliminate harmful phenolics.

Damaging effects could be observed with sucrose as osmoticum in the culture medium when compared to glucose, mannitol and sorbitol containing media.

Table 2: Effect of different factors on the development of leaf protoplasts

	reaction
Growth Conditions of the Donor Plants	
reduced Ammonium concentration	0
growth on hormonefree medium	0
reduced growth conditions	-
Preconditioning of the Leaves	
for 3 days	0
for 7 days	0
Culture Conditions	
reduced temperature (18°C)	0
light intensity (50 $\mu E \cdot m^{-2} \cdot s^{-1}$)	-
Protoplasts Density	
$> 1 \cdot 10^6$ or $< 1 \cdot 10^5$	-
$2\text{-}5 \cdot 10^5$	+
Culture Medium	
Bovine Serum Albumin (2,5 g/l)	+
Polyvinylpyrrolidon (5 g/l)	+
Glucose, Sorbitol, Mannitol as Osmoticum	0
Sucrose as Osmoticum	-
Amino Acids (Alanin, Glutamine Acide, Cystein)	0

+: improved viability and cell wall formation;
0: no clear reaction;
-: accelerated death of the cultures;

Neither different growth conditions of donor plants and the preconditioning of leaves (Haberlach *et al.* 1985) nor the tested culture conditions and media could induce cell division.

Optimal conditions for leaf protoplast cultivation resulted in cell wall formation starting approximately at the 3rd d of culture and in few cases initial cell division occurred. Divisions and further development could not be observed. Cultivation of leaf protoplasts was not possible for longer than 4-5 weeks.

Donor material

As shown in different plant species, division capacity of protoplasts depends on the kind of donor material used for isolation (Potrykus *et al.* 1977; Vasil and Vasil 1979; Binding *et al.* 1981; Lenee and Chupeau 1986).

To examine the regeneration capacity of protoplasts from different tissues and organs of grapevine (leaves, shoot tips, petioles, stems, roots and callus), isolations under under suitable conditions were performed (Table 3).

Table 3: Enzymatic treatments for protoplast isolation from different tissues and organs of grapevine

Donor Material					
Leaves	Shoot tips	Petioles	Stems	Roots	Callus
Enzymatic Treatment					
conc.1 3h	conc.2 5h	conc.1 7h	conc.2 16h	conc.2 8h	conc.2 8h

	conc.1	conc.2
Cellulase *Aspergillus niger*	0,4 %	0,5 %
Cellulase *Penicillium funiculosum*	0,4 %	0,5 %
Cellulase *Trichoderma viride*	0,8 %	1,0 %
Macerozyme R-10	0,2 %	0,5 %

Differences in development between protoplasts from young and older leaves could not be found. Most of the protoplasts died within the first few days of culture followed by a phase of

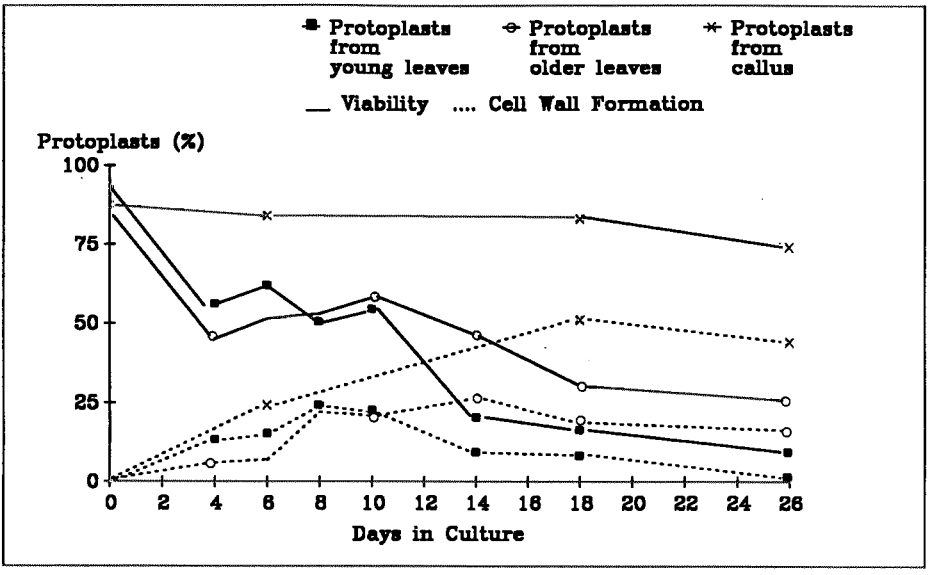


Fig. 3: Process of viability and cell wall formation of protoplasts from different starting material.

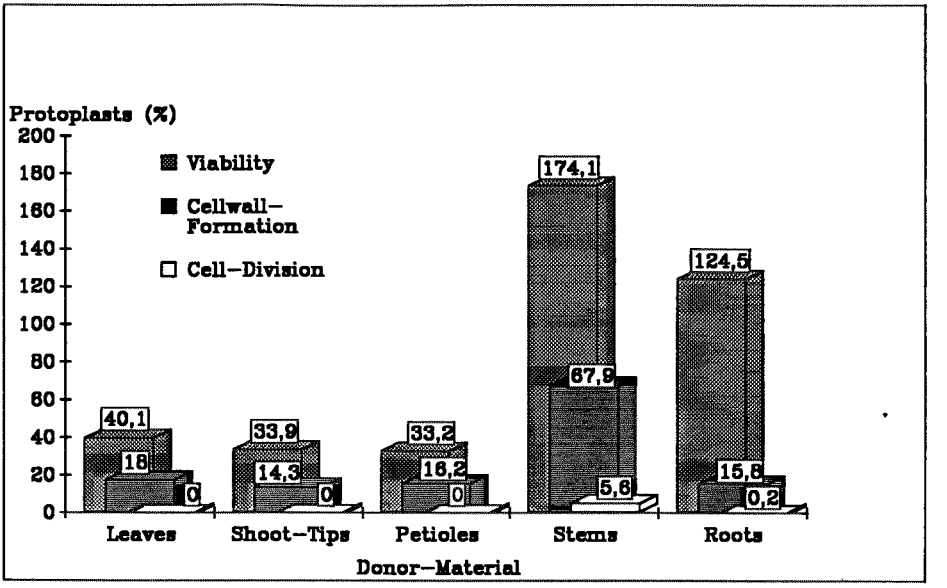


Fig. 4: Development of protoplasts derived from different organs of grapevine after 14 d of culture.

stabilization of the rate of viable protoplasts. After 10-12 d, the rate of viability decreased drastically and could not be stopped by changing or supplementing the medium. In contrast, protoplasts derived from callus suspensions showed high viability and intensive cell wall formation for a duration of 3 weeks (Fig. 3), but no cell division occurred.

The development of protoplasts from petioles and shoot tips was similar to the development of those isolated from leaves. After 14 d of culture, only 33-40 % of the cells were viable and 14-18 % had formed a new cell wall (Fig. 4). In contrast, protoplasts derived from stems and roots showed an intensive development. Cell wall formation started during the first few days and after 3-5 d first cell divisions could be observed.

In case of stem and root protoplasts due to evaporation of medium (10 % in 14 d) and proliferation of cells by cell division, density of viable cells increased. In comparison, the division activity of root protoplasts (0.2 %) was much lower than that of stem protoplasts (5.6 %).

Protoplasts with high division activity are supposed to be of cambial origin. Isolation experiments with stems showed that a reduced incubation time led to protoplasts with reduced division activity (results not shown). The short time of digestion (8 h, Table 3) in which root protoplasts were obtained could be an explanation for the reduced division activity of these protoplasts.

The higher regeneration capacity of stem protoplasts was confirmed in further observations. Only cultures of stem protoplasts showed further divisions and formed microcalli and calli, whereas the cultures of root protoplasts did not show further development and turned brown in the 3rd week of cultivation. The low division activity of root protoplasts was probably responsible for this behaviour.

Different cultivation techniques – liquid culture, solid culture, cultivation in special Biomembrane containers – as well as suitable methods of subcultivation were tested to establish a successful and easy regeneration system for grapevine protoplasts. Changing or supplementing the media to reduce harmful phenolics in the cultures was not effective. Moreover, the cultivation of protoplasts in solid medium could not stimulate protoplast development. The only successful method was cultivation in liquid medium on a solid reservoir-medium. Using this technique, stem protoplasts formed microcalli and within 8-10 weeks after isolation, visible calli developed.

Finally, regeneration capacity of protoplasts from tissue of different cultivars was tested (Table 4). Stem protoplasts from 4 of a total of 8 varieties tested formed callus. In the other cases, either the division activity was too low or the production of phenolics was too high (Rupestris du Lot) and the cultures died. Except of Vidal and Rupestris du Lot, leaf protoplasts did not divide, whereas root protoplasts of all tested varieties showed first division but did not form callus.

Table 4: Regeneration capacity of protoplasts from tissues and organs of different varieties

Varieties	Leaves	Roots	Stems
Optima	CW	D	C
Riesling	CW	D	C
Kerner	CW	/	C
Müller Thurgau	CW	/	D
Orion	CW	/	D
Vidal	D	D	C
Seyval	-	D	D
Rupestris du Lot	D	D	D

/: not tested;
-: no reaction;
CW: protoplasts showed cell wall formation;
D: protoplasts showed first division;
C: protoplasten formed callus;

These results show the importance of the donor material for regeneration experiments of protoplasts. In the case of grapevine, stems seem to be an appropriate material to obtain protoplasts with high division capacity.

Further regeneration of the achieved calli to plants is presently being attempted. Toward this goal, these calli are held on different media and under different culture conditions. In particular, donor material which could yield protoplasts with a high regeneration capacity is used. For improved starting material, suspensions of embryogenic callus could be useful. With 'recalcitrant' grapevine cultivars the production of embryogenic suspensions may be possible. Embryogenic suspensions of several important varieties have been reported by BESSIS and LABROCHE (1985), MULLINS (1987) and STAMP and MEREDITH (1988).

Conclusion

Isolation methods for protoplasts from different organs and tissue of grapevine were established. Cell division could not be induced in protoplasts from leaves, shoot tips, petioles and callus, wehreas root and stem protoplasts showed high division activity. However, stem protoplasts formed microcalli and calli after cultivation in liquid medium on a solid reservoir-medium. Protoplasts from stems of 4 varieties could be regenerated to callus.

References

BARBIER, M.; BESSIS, R.; 1987 a: Isolement et culture de protoplastes de feuilles de vigne (*Vitis vinifera* L., var. Chardonnay). Bull. O.I.V. **60**, 765-775.

-- ; -- ; 1987 b: Régénération de la paroi cellulaire par des protoplastes de feuilles de vigne (*Vitis vinifera* L., var. Chardonnay) et division des cellules. Bull. O.I.V. **60**, 776-788.

BESSIS, R.; LABROCHE, C.; 1985: Callogenèse et régénération chez le Pinot, le Chardonnay et le Gamay. Moët-Hennessy Coll. Amélioration de la Vigne et Culture *in vitro*, 127-134. Moët-Hennessy, Paris.

BINDING, H.; NEHLS, R.; 1977: Regeneration of isolated protoplasts to plants in *Solanum dulcamara* L. Z. Pflanzenphysiol. **85**, 279-280.

-- ; -- ; KOCK, R.; FINGER, J.; MORDHORST, G.; 1981: Comparative studies on protoplast regeneration in herbaceous species of the Dicotyledoneae class. Z. Pflanzenphysiol. **101**, 119-130.

BREZEANU, A.; ROSU, A.; 1984: Isolation and culture of cell protoplasts from the mesophyll callus of *Vitis vinifera* L. Rev. Roum. Biol. - Biol. Veg. **29**, 33-37.

DE FILIPPIS, L. F.; ZIEGLER, H.; 1985: The physiology of grapevine (*Vitis vinifera*) protoplasts isolated from green and senescing leaves. Biochem. Physiol. Pflanzen **180**, 645-653.

HABERLACH, G. T.; COHEN, B. A.; REICHERT, N. A.; BAER, A. M.; TOWILL, L. A.; HELGESON, J. P.; 1985: Isolation, culture and regeneration of protoplasts from potato and several related *Solanum* species. Plant Sci. **39**, 67-74.

KRUL, W. K.; 1989: Recent advances in protoplast culture of horticultural crops: Small fruits. Sci. Hort. **37**, 231-245.

LEE, N.; WETZSTEIN, H. Y.; 1988: Protoplast isolation and callus production from leaves of tissue-cultured *Vitis* spp. Plant Cell Rep. **7**, 531-534.

LENEE, P.; CHUPEAU, Y.; 1986: Isolation and culture of sunflower protoplasts (*Helianthus annuus* L.): Factors influencing the viability of cell colonies derived from protoplasts. Plant Sci. **43**, 69-75.

LINSMAIER, E. M.; SKOOG, F.; 1965: Organic growth factor requirements of tobacco tissue culture. Physiol. Plant. **18**, 120-127.

MULLINS, M. G.; 1987: Propagation and genetic improvement of temperate fruits: The role of tissue culture. In: GREEN, C. E.; SOMERS, D. A.; HACKETT, W. P.; BIESBOER, D. D. (Eds.): Plant Biology, Vol. 3: Plant Tissue and Cell Culture. Alan R. Liss, Inc., New York.

POTRYKUS, I.; HARMS, C. T.; LÖRZ, H.; THOMAS, E.; 1977: Callus formation from stem protoplasts of corn (*Zea mays* L.). Molec. Gen. Genet. **156**, 347-350.

PUITE, K. J.; DONS, J. J. M.; HUIZING, H. J.; KOOL, A. J.; KOORNEEF, M.; KRENS, F. A.; 1988: Progress in plant protoplast research. Proc. 7th Intern. Protoplast Symp., Wageningen, Netherlands. Kluwer Acad. Publ.

SHIMIZU, J.; 1985: Cell regeneration and division of grape mesophyll protoplasts. J. Plant Physiol. **119**, 419-424.

SKENE, K. G. M.; 1975: Production of callus from protoplasts of cultured grape pericarp. Vitis **14**, 177-180.

STAMP, J. A.; MEREDITH, C. P.; 1988: Somatic embryogenesis from leaves and anthers of grapevine. Sci. Hort. **35**, 235-251.

VASIL, V.; VASIL, I. K.; 1979: Isolation and culture of cereal protoplasts. I.: Callus formation from pearl millet *(Pennisetum americanum)* protoplasts. Z. Pflanzenphysiol. **92**, 379-383.

WRIGHT, D. C.; 1985: Factors affecting isolation of protoplasts from leaves of grape *(Vitis vinifera)*. Plant Cell Tissue Organ Cult. **4**, 95-100.

YAMAKAWA, T.; ONOMICHI, K.; KODAMA, T.; MINODA, Y.; 1985: Application of feeder layer method for improved colony formation of grape cells and protoplasts at low cell density. Agricult. Biol. Chem. **49**, 3583-3585.

Seedless x seedless grape progeny: Technique, results and perspectives

P. SPIEGEL-ROY, N. SAHAR and I. BARON

Department of Fruit Breeding and Genetics, Volcani Center, ARO,P.O.B. 6, Bet-Dagan, Israel

S u m m a r y : Recent refinements in the *in vitro* embryo rescue technique employed to raise progenies from abortive ovules of seedless x seedless cultivars include addition of 0.2 ppm NAA (naphtaleneacetic acid) to the medium, inducing highly improved root systems and faster plant development. Selfing seedless *Vitis vinifera* yielded seedless progeny only. Open pollinated Perlette and Flame Seedless gave rise to a high percentage (75 and 87 %, respectively) of seedless progeny. Progeny from various crosses between seedless cultivars segregated into 65 normal seeded and 204 seedless. From the totality of 204 seedless progeny 192 bore fruit with very slight seed traces. Progeny from crosses between seeded and seedless segregated only 7.5-8 % individuals with comparably slight seed traces, amounting to $^1/_3$ of the progeny rated as seedless. Fresh weight determinations of aborted seeds per berry showed a pronouncedly lower weight in progenies from seedless x seedless crosses. Reduction in average berry size in the seedless fraction of seedless x seedless progenies compared to midparent values was of a similar order of magnitude as that obtained in seedless progeny derived from seeded x seedless crosses.

K e y w o r d s : table grape, berry, stenospermocarpy, seed trace, selfing, crossing, genetics, technique, tissue culture.

Introduction

Breeding new seedless cultivars has been performed in the past by selecting from progenies of crosses between seeded (female parent) and seedless (male parent) genotypes. Only about $^1/_4$ of the progeny, on average, proved seedless (SPIEGEL-ROY, unpublished). Moreover, only 6.5-9.5 % of the totality of the hybrids bore fruit without noticeable seed traces (SPIEGEL-ROY *et al.* 1986). Recently, *in vitro* methods for culturing abortive ovules and seeds from seedless cultivars and selections have been described (EMERSHAD and RAMMING 1984, SPIEGEL-ROY *et al.* 1985). These methods enable to raise progeny from crosses between two seedless parents and also from selfed seedless genotypes. The present paper deals mainly with the inheritance of the seedless trait in selfed progeny and crosses between seedless genotypes. New developments in technique and breeding potential of seedless x seedless crosses are also discussed.

Materials and methods

Crosses have been made between seedless parents and their progeny analyzed for seed content, berry size and colour. Progeny from open pollinated Perlette, Flame Seedless and Sultanina was also examined for the same characteristics, as well as progeny obtained from selfing seedless genotypes. A panel consisting of 3 persons classified progeny in 3 categories; normal seeded (N); with noticeable seed traces and texture of the seed deviating from that of the pulp (B); practically seedless, with barely noticeable seed traces (S).

In certain progenies seed trace content has also been evaluated by determining number of aborted seeds per berry and fresh weight of each aborted seed in the berries. On average, 20 berries of each hybrid or genotype have been thus sampled. Berry weight determinations were based on 25 berries. *In vitro* culture was based essentially on the technique developed in our laboratory (SPIEGEL-ROY *et al.* 1985).

Results

Several modifications have been made in the *in vitro* protocol for embryo rescue developed by us (SPIEGEL-ROY *et al.* 1985), successfully adopted since also elsewhere (GRAY *et al.* 1987). At the 4-leaf stage plantlets are transferred from Petri dishes to Magenta vessels (7.6 cm L x 7.6 cm W x 10 cm H). The fresh agar medium contains neither IAA nor GA_3. However, NAA (naphtaleneacetic acid) 0.2 ppm is being added to the solid medium, resulting in a much more profuse root system. As the top of the Magenta vessel is reached, plantlets are transferred to Jiffy pots (No. 7) and these are being placed inside Magenta vessels. Jiffy pots have been moistened with water containing salts of the Nitsch medium ($^1/_2$ strength), but no vitamins or amino acids are added. NAA 0.2 ppm is again included. As soon as roots emerge from the Jiffy pot, plants are transferred to an unsterilized volcanic tuff-peat mixture in the greenhouse for hardening. We have found no advantage by adding BA (benzyladenine) to the solidified medium. A further factor influencing success of culture is size of seed traces; cultivars and selections with at least moderate seed traces (e. g. Flame Seedless, Ruby Seedless) yield a much higher proportion of developing embryos and plants than cultivars with very low ovule size and very small seed traces (e. g. Sultanina).

Table 1: Distribution of normal seeded (N), seedless with seed traces (B), and seedless without noticeable traces (S) in selfed and open pollinated progeny of seedless cultivars and in crosses made between seedless *V. vinifera* parents

female parent	male parent	normal seeded (N)	seedless seed traces (B)	seedless v. slight traces (S)
Perlette	O.P.	3	1	8
Flame Seedless	O.P.	6	1	40
Sultanina	O.P.	17	1	15
L 12	O.P.	1	0	1
Perlette	Perlette	0	0	8
L 12	L 12	0	0	2
Perlette	Beauty Seedless	0	0	3
Perlette	Flame	9	2	6
Perlette	L 12	5	0	12
L 12	Perlette	0	1	17
L 12	Sultanina	0	0	4
L 12	Flame Seedless	6	0	36
L 12	Ruby	11	1	30
L 12	Black Kishmish	1	2	0
Ruby	L 12	6	2	27
Ruby	Sultanina	21	1	35
Flame Seedless	Perlette	3	1	17
Flame Seedless	L 12	0	1	0
Sultanina	Flame Seedless	1	0	2
Sultanina	L 12	0	0	1
Centennial	Flame Seedless	1	0	2
Centennial	Sultanina	1	1	0

So far, 279 plants from seedless x seedless crosses including 10 developed from selfing of seedless parents have fruited. In addition open pollinated progeny of several seedless cultivars have also fruited. Results are summarized in Table 1. All 10 selfed progenies have been seedless without noticeable traces (S). Results from open pollinated progeny show a large difference between Sultanina, in which a close to 1 : 1 distribution between seedless and seeded was noted, and Flame Seedless progeny in which 41 seedless individuals were found compared to 6 normal seeded ones. These results point to a high degree of selfing under natural conditions with Flame Seedless and

Table 2: Distribution of normal (N), seedless with noticeable traces (B), seedless without noticeable traces (S) in progenies a) from crosses between seeded and seedless parents, b) crosses between seedless parents only

Year of observation	Type of cross	Normal seeded (N)	Seedless B	Seedless S
1988	Seeded X seedless	1457	391	140
1988	Seedless X seedless	20	5	71
1989	Seedless X seedless	65	12	192

Table 3: Average fresh weight of single aborted seed and of total of aborted seeds per berry and their average number per berry in Ruby Seedless and L 12, in progenies from their reciprocal cross and in crosses between two seeded cultivars and Ruby Seedless*

Genotype and progenies	Avg. weight of single aborted seed mg	Avg. weight of aborted seed berry mg	Avg. no. aborted seed per berry	Avg. berry weight g	Range berry weight
Ruby Seedless	6.25	16.55	2.65	3.26	
L 12 (seedless)	11.00	37.30	3.40	4.19	
Ruby Seedless X L 12	7.64	19.51	2.52	2.44	1.27-3.94
L12 X Ruby Seedless	8.67	24.94	2.85	3.20	1.92-4.72
M. Alexandria X Ruby Seedless	19.99	56.34	2.70	3.08	1.57-4.54
Italia X Ruby Seedless	24.16	61.30	2.50	2.44	1.40-3.34

* 20 hybrids examined in each progeny. Only progenies rated as seedless have been included.

probably also with Perlette. Of the 269 hybrids derived from various crosses between seedless cultivars, 204 could be rated as seedless, while close to a quarter, 65 were rated as having normal seeds (Table 1). Normal seeded progeny appeared in crosses with all 6 seedless parents used as a female parent, including Perlette which was also shown to segregate only seedless progeny from selfed flowers. Another striking result is the preponderance of seedless progeny with very slight seed traces (S), 192 in number, by far exceeding those with noticeable seed traces (B), 12 only. The significance of this distribution will be even more apparent in comparison with the distribution between seedless (S) and seedless with traces (B) in progenies derived from crosses between seeded cultivars and selections (as a female parent) and seedless cultivars (male parent). Data are given in Table 2. Perusal of the table shows that in progeny from crosses between seeded x seedless only 26.4 % of the individuals rated as seedless were without noticeable traces (S), making out only close to 7.1 % of the total progeny. In contrast, seedless progeny derived from two seedless parents consisted nearly completely of individuals with barely noticeable seed traces (S). These constituted 93.4 % in 1988, 94.1 % in 1989 of the total progeny rated as seedless (B + S) (Table 2). The proportion of individuals devoid of noticeable seed traces in the total progeny (including seeded and seedless) amounted in 1988 to 74.0 %, in 1989 to 71.4 %.

In order to examine nature and size of aborted seed vestiges in seedless progenies by a method other than that of organoleptic determination, fresh weight of each aborted seed was determined in seedless parents and progenies from some crosses between seedless genotypes made in 1989. Similarly, fresh weight of each aborted seed was also determined in seedless hybrids derived from two crosses in which the female parent was a seeded cultivar and the male parent Ruby Seedless. Results are summarized in Table 3. No large differences were noted in the number of aborted seeds per berry, except a larger number with L 12. Average weight of a single aborted seed in the cross between seedless genotypes was between the average weight of the two parents. Average weight of a single aborted seed (column 1, Table 3) was much higher in the progeny classified as seedless derived from crosses of seeded x seedless (19.99 mg and 24.16 mg, respectively). This was reflected also in the much higher proportion of progeny rated as seedless with noticeable seed traces from the M. Alexandria x Ruby Seedless and Italia x Ruby Seedless cross (data not given). The average weight of all aborted seeds, taken together per single berry (column 2, Table 3), showed a similar trend and was nearly 3 times higher in progeny of crosses between seeded x seedless parents compared to the cross between the two seedless parents. Ruby Seedless has figured as a male parent in both types of crosses involved. Only progeny rated as seedless (with and without noticeable traces) has been included in the table. Rather similar berry weights and ranges in the progeny have been obtained in both types of cross. In the reciprocal seedless x seedless cross a rather large difference in berry weight has been obtained, pointing to an effect that could be due to cytoplasmic factors.

Further examination of the inheritance of fruit weight in seedless x seedless progenies has led us to try to compare fruit size in seedless progeny (normal seeded progeny has not been included in the comparison also because of lack of full data) from seedless x seedless crosses with seedless progeny derived from crosses between 4 seeded cultivars and 5 seedless parents. The comparison is somewhat incomplete as no hybrids or only a small number are available from certain crosses. Results are given in Table 4, presented in 4 columns. The 1st column shows progeny mean. The 2nd column represents the midparent value, while the 3rd column gives the percentage decrease of the progeny mean from the midparent value. Number of progeny in which berry weight of the seedless progeny has been determined is given in brackets in the 4th column. No definite conclusions concerning parental contribution can be inferred, though mean decrease in fruit weight with Sultanina seems indicated; however, only 2 crosses are involved. In progeny from selfed Perlette a similar berry weight decrease has been noted as that observed with Sultanina crosses (average of selfed Perlette 1.6 g against 2.56 g in Perlette, a 37.5 % decrease). On the whole, the 7 seedless x seedless populations averaged a similar decrease to the 11 progenies from seeded x

Table 4: Average berry weight in seedless fraction of progenies between seedless parents and between four seeded parents with seedless male parents. 1st column: Average berry weight (g) of progeny (seedless progeny only). 2nd column: midparent berry weight (g). 3rd column: percent berry weight decrease of seedless fraction of progeny compared to midparent. In brackets: number of seedless hybrids in the progeny

Female/male parent	Ruby Seedless	Perlette	L 12	Flame Seedless	Sultanina
Seedless:Ruby Seedless			2.44		1.50
			3.66		2.38
			33.3		37.0
			(20)		(27)
" Perlette			2.70	2.00	
			3.40	2.61	
			20.6	23.4	
			(9)	(16)	
" L 12	3.20	2.20		2.70	
	3.66	3.40		3.33	
	12.6	35.3		18.9	
	(20)	(10)		(30)	
Seeded Early Muscat		2.35	2.89		
		2.87	3.58		
		18.1	19.3		
		(33)	(12)		
" Muscat Hamburg	2.34	1.95		2.44	
	3.50	3.24		3.17	
	33.1	39.8		31.0	
	(62)	(19)		(69)	
" Italia	2.61		3.55	2.60	
	4.10		4.58	3.80	
	36.7		22.5	31.5	
	(25)		(17)	(41)	
" Muscat Alexandria	2.75			2.93	1.84
	3.85			3.52	3.03
	28.6			16.8	39.3
	(40)			(26)	(9)

seedless crosses, also displaying a similar range. While the lack of realization of a partial diallele setup does not allow the drawing of final conclusions, results seem to point to an essentially similar mode of inheritance of berry weight in other types of crosses, and as already noted with a much

better distribution of desirable seedless types (without noticeable seed traces) in progeny derived from seedless x seedless crosses.

Discussion

269 hybrids from crosses between stenospermocarpic *V. vinifera* parents, as well as a small selfed progeny and a sizable open pollinated progeny have fruited so far. In *in vitro* method used by us for the rescue of embryos and aborted seed (SPIEGEL-ROY *et al.* 1985), Ga_3 and IAA proved to be necessary additions to the solid medium during the first phase of culture. While the addition of NAA was highly effective in enhancing at an early stage the development of a large root system of the *in vitro* grown hybrids, contrary to other findings (GRAY *et al.* 1987), no further benefit accrued from the addition of a cytokinin (BA). Though results obtained from selfing the seedless Perlette genotype do confirm the recessive nature of the seedless trait postulated by WEINBERGER and HARMON (1964) and LOOMIS and WEINBERGER (1979) and strongly indicated by results of our seeded x seedless crosses, the results obtained by us from crosses between seedless cultivars seem much more difficult to interpret. Such crosses segregated into 65 normal seeded and 204 seedless individuals. Contamination by airborne pollen during emasculation could not possibly account for such a relatively high rate of normal seeded progeny. Not taking into account the very small progenies from certain crosses between seedless parents (Table 1), only 1 cross, between L 12 and Perlette, did yield 100 % seedless progeny, while of 7 other crosses all gave rise to a certain number of seeded individuals in the progeny. These results contrast with the postulated 3 : 1 seeded x seedless ratio obtained in most seeded x seedless populations examined by us during 5 nearly consecutive years (SPIEGEL-ROY, unpublished results).

Analysis of results obtained from open pollinated progeny of seedless genotypes discloses some data of interest. With Flame Seedless and to a lesser extent with Perlette, the bulk of the open pollinated progeny was found to be seedless and, moreover, belonging to type S, with very slight seed traces. This suggests the possibility of selfing having occurred on a large scale, possibly before anthesis. This might not have been the case with Sultanina as in its progeny from open pollination a close to 1 : 1 ratio between seeded and seedless was found.

A distinct feature of the seedless progeny derived from seeded x seedless crosses is the preponderance of individuals with very slight seed traces. While in progeny of crosses from seeded x seedless genotypes only 6.5-9.5 % bore fruit with barely noticeable seed traces (SPIEGEL-ROY *et al.* 1986), constituting less than $^1/_3$ of the total progeny rated as seedless, analysis of seedless progeny from seedless x seedless crosses yielded over 94 % hybrids rated as seedless with slight seed traces. Thus, while crosses between seeded and seedless yield only less than 8 % progeny with slight seed traces, a total 71 % of the progeny from seedless x seedless crosses have borne seedless fruit without noticeable seed traces. This would amount in selection blocks from seedless x seedless crosses about 9 times more truly seedless progeny (from an identical number of hybrids) compared to that to be obtained from seeded x seedless progeny. Methods for measuring seed traces more objectively have been developed (MERIN *et al.* 1983; PERL *et al.* 1989). We have reported here on another simplified approach, namely determining fresh weight of aborted seed in individual berries. Differences between progenies of seedless x seedless crosses and progenies of seeded x seedless crosses are rather large with much smaller weight of aborted seed in progenies sampled from crosses between two seedless parents. Ruby Seedless has been a common parent in the progenies analyzed, including those with a seeded female parent.

Lower berry weight and perhaps also some inbreeding effect could have been anticipated in at least certain seedless x seedless crosses. It was therefore of interest to follow the inheritance of berry weight in seedless progeny from both seeded x seedless and seedless x seedless crosses. Berry size is known to be quantitatively inherited (SPIEGEL-ROY *et al.* 1981). Seeded parents used in crosses with seedless cultivars often have large berries. As we are interested primarily in seedless progeny

from seeded x seedless and seedless x seedless crosses, the analysis of berry size or weight from that portion of the progeny only will obviously give rise to a biased distribution. This will amount to a pronouncedly lower average size or weight compared to midparent values, as normal seeded fruit is potentially larger (MÜLLER-THURGAU 1898; WINKLER 1932). Analyzing, however, seedless progeny from seedless x seedless crosses and from seeded x seedless crosses, we found wide variation but no substantial difference in fruit size diminution compared to midparent values between the two groups. Moreover, in the latter group a much larger part of the progeny had noticeable seed traces, and still no better average berry weight was manifested in comparison to progenies originating from seedless x seedless crosses. In one case examined by us notable differences especially as to berry weight occurred in a reciprocal cross between two seedless genotypes, pointing to a cytoplasmic factor or factors in addition to that of nuclear genes. The development of the *in vitro* technique for embryo rescue will allow in the future, though more laborious, to test also the effect of reciprocal crosses between seeded and seedless genotypes. First selections with satisfactory berry size and with negligible traces have been already made by us from seedless x seedless progenies.

As transmission of the fruit size trait does not seem to differ essentially in crosses between seedless parents from that found in seeded x seedless crosses, use of two different seedless genotypes with large sized berries as male and female parents in a cross should prove effective in obtaining individuals with good fruit size in the progeny. Interspecific crosses between seedless types (GRAY *et al* 1987) may perhaps enhance plant vigour of hybrids along with some further contribution to fruit size.

Literature cited

EMERSHAD, R. L.; RAMMING, D. W.; 1984: *In ovulo* embryo culture of *Vitis vinifera* L. c.v. Thompson Seedless. Amer. J. Bot. **71**, 873-877.

GRAY, D. L., FISHER, L. C.; MORTENSEN, J. A.; 1987: Comparison of methodologies for *in ovulo* embryo rescue of seedless grapes. HortScience **22**, 1334-1335.

LOOMIS, N. H.; WEINBERGER, J. H.; 1979: Inheritance studies of seedlessness in grapes. J. Amer. Soc. Hort. Sci. **104**, 181-184.

MERIN, U.; ROSENTHAL, I.; LAVI, U.; 1983: A chemical method for the assessment of grapes according to their seed content. Vitis **22**, 306-310.

MÜLLER-THURGAU, H.; 1898: Abhängigkeit der Ausbildung der Traubenbeeren und einiger anderer Früchte von der Entwicklung der Samen. Landw. Jahrb. Schweiz **12**, 135-205.

PERL, M.; LAVI, U.; SPIEGEL-ROY, P.; 1989: Estimation of seed traces in grape berries by inhibition of luciferase activity. HortScience [in press].

SPIEGEL-ROY, P.; ASSAF, R.; BARON, I.; 1981: Inheritance of some characters in progenies of *Vitis vinifera* from crosses with Dabouki and Alphonse Lavallée. Proc. 3rd Intern. Symp. Grape Breeding, Davis, Ca., 210-219.

– – ; SAHAR, N.; BARON, I.; LAVI, U.; 1985: *In vitro* culture and plant formation from grape cultivars with abortive ovules and seeds. J. Amer. Soc. Hort. Sci. **110**, 109-112.

– – ; – – ; – – ; – – ; 1986: Ovule and seed culture from early ripening seedless and seeded cultivars. Vignevini **13** Suppl. al (12), 34-36.

WEINBERGER, J. H.; HARMON, F. N.; 1964: Seedlessness in *vinifera* grapes. Proc. Amer. Soc. Hort. Sci. **85**, 270-274.

WINKLER, A. J.; 1932: Maturity tests for table grapes. Calif. Agricult. Exp. Sta. Bull. 529, 1-35.

Hybridization of seedless grapes

D. W. Ramming, C. A. Ledbetter and R. Tarailo

Horticultural Crops Research Laboratory, USDA/ARS, 2021 South Peach Avenue, Fresno, CA, USA

Summary:

1. Complete seedlessness was not achieved in any seedless x seedless families produced. This clearly shows the seedless trait is not controlled by a single recessive gene.

2. The percentage of seedlessness obtained was not the same for all families. This indicates the importance of parental combinations and the need for progeny testing to increase the efficiency of producing seedless offspring.

3. All seedless genotypes used except Thompson Seedless have a seeded female parent, indicating heterogenity for seeds/aborted seeds. It will be interesting now to use seedlings from the seedless x seedless families to see if 100 % seedless offspring can be obtained.

4. Three seedless x seedless families compared to their complementary families from seeded x seedless were significantly different for percent seedless. *In ovulo* embryo rescue of seedless x seedless hybrids is also advantageous as it allows direct hybridization without seeded genotypes. This eliminates the introduction of genes from seeded genotypes and one generation – a savings of 5 years. The ability to achieve complimentary crosses between seedless genotypes directly can be achieved.

Key words: table grape, berry, seedlessness, stenospermocarpy, crossing, genetics, tissue culture.

Introduction

Consumer demands for stenospermocarpic seedless grapes have increased as they become familiar with this type of grape in the market. Grape breeders have tried to meet this demand by hybridizing seeded by seedless genotypes to create new seedless cultivars. The production of seedless genotypes by this method is quite low, ranging from 10 to 30 % (Loomis and Weinberger 1975). Table grape breeders have dreamed of hybridizing seedless genotypes directly to increase the percentage of seedless offspring obtained and increase the efficiency of the breeding program.

The development of *in ovulo* embryo rescue techniques for grapes (Emershad and Ramming 1984; Spiegel-Roy *et al.* 1985) has now made direct hybridization of seedless genotypes possible. The improvement of culture procedures (Emershad *et al.* 1989) and production of plants (Ramming 1989) has been reported elsewhere. The results obtained from 9 families produced in 1983 or 1984 are reported.

Materials and methods

Seedless genotypes with aborted seeds ranging from 0 to 25 mg fresh weight (FW) were hybridized by emasculation and controlled pollination (Table 1). P79-101 has only female flowers and was not emasculated but covered with paper bags before blooming and after pollination. All families had 24 or more fruiting seedlings and the percentage of seedlings bearing fruit ranged from 64 to 93 %. The average FW of the seed/aborted seed was determined by taking the largest seed from each berry of the 10 largest berries in a cluster. This allows us to determine the maximum expression of seed size for each seedling. Statistical differences between populations for the seedless character were determined with Chi square analysis.

Results and discussion

The distribution of seedlings with varying size of seeds/aborted seeds is shown in the figure. The histograms indicate that there are two groups of seedlings based on the bimodal distribution of seed FW. After comparing the seedling distribution in the histograms and observing the morphological traits of the seeds/aborted seeds, 25 mg FW was selected as the best division

Table 1: The seedless x seedless crosses made in 1983-84, the average weight of the seeds for each parent and the percent seedless offspring

Female	seed[1] (mg FW)	Male	seed[1] (mg FW)	Mid-parent Mean	% Sdlss[2] (<25mg)	% Sdlss[2] (<10mg)
C85-82	10.6	C20-149	6.6	8.6	78	58
P60-58	13.8	Thompson Seedless	3.2	8.5	70	60
B46-112	5.7	C18-36	5.9	5.8	67	67
A71-185	19.9	C32-68	14.2	17.1	56	33
P79-101	25.0	C32-68	14.2	19.6	83	67
P79-101	25.0	C33-199	0.0	12.5	76	56
P79-101	25.0	Flame Seedless	5.9	15.5	73	52
P79-101	25.0	B31-164	13.8	14.4	54	29
P79-101	25.0	C35-33	14.0	14.5	44	24

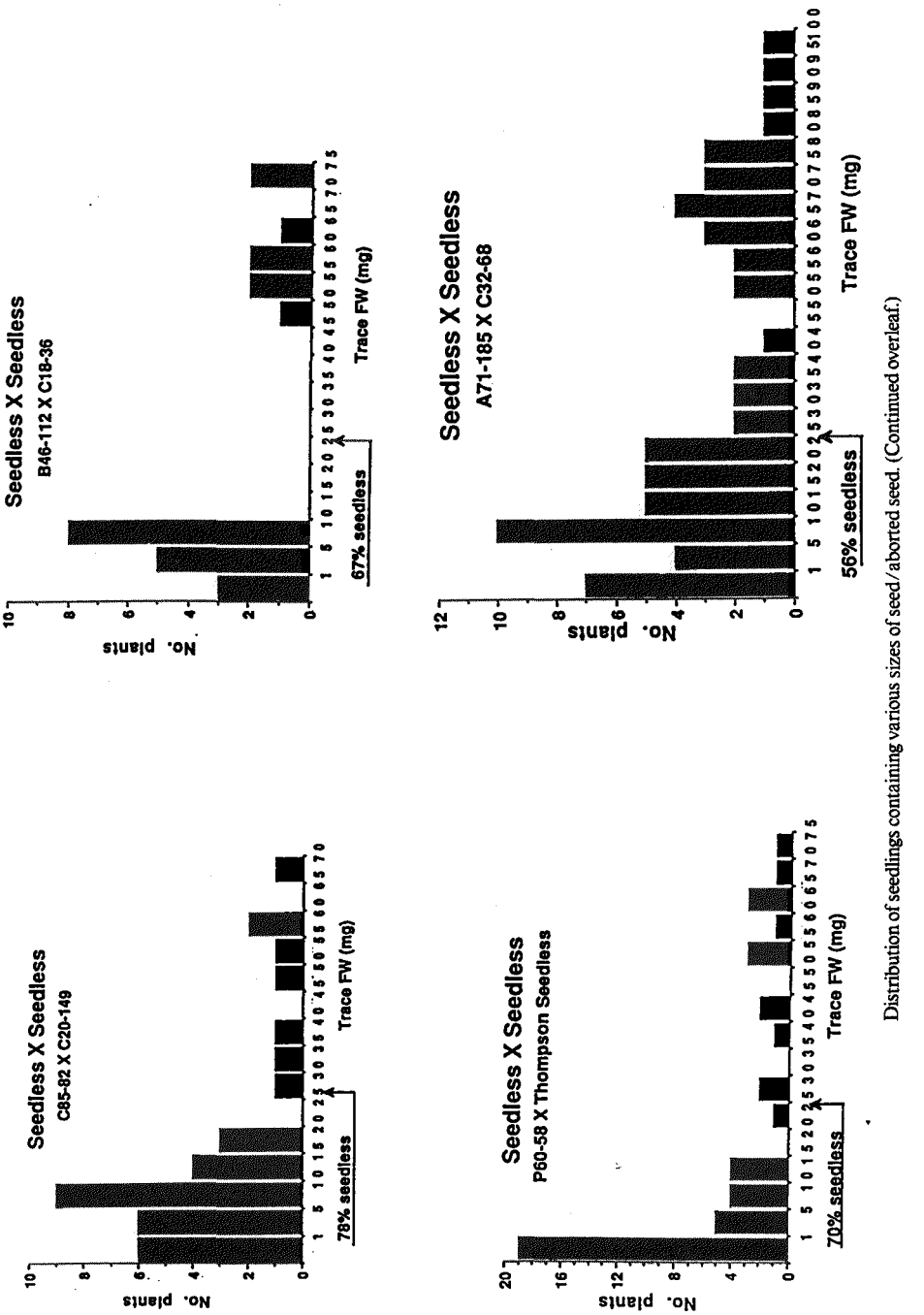
1)Seed weight determined by weighting the largest seed from the 10 largest berries per cluster.
2)Division between seeded and seedless = 25 or 10mg FW as indicated.

Table 2: The percent seedless offspring from seedless x seedless families with the same maternal parent, P79-101

Seedless Pollen Parent	% sdlss[1]	n
C32-68	83	24
C33-199	76	34
Flame Seedless	73	111
B31-164	54	24
C35-33	44	78
Significance[2]	***	

1)Seedless = aborted seeds less than 25 mg.
2)*** significant at the 0.5% level.

between seeded and seedless genotypes from an anatomical point of view. The maximum seed size for consumer acceptance as seedless is probably nearer 10 mg FW, but is influenced by flesh texture.



Distribution of seedlings containing various sizes of seed/aborted seed. (Continued overleaf.)

Every seedless x seedless family examined contained seedlings with seeds. This shows that seedlessness is not controlled by a single recessive gene as postulated in some reports (CONSTANTINESCU *et al.* 1975; DUDNIK and MOLIVER 1976).

The percentage of seedless offspring for the families ranged from 83 to 44 % (Table 1). The correlation coefficient between percent seedless offspring and mid-parent values for the 9 families

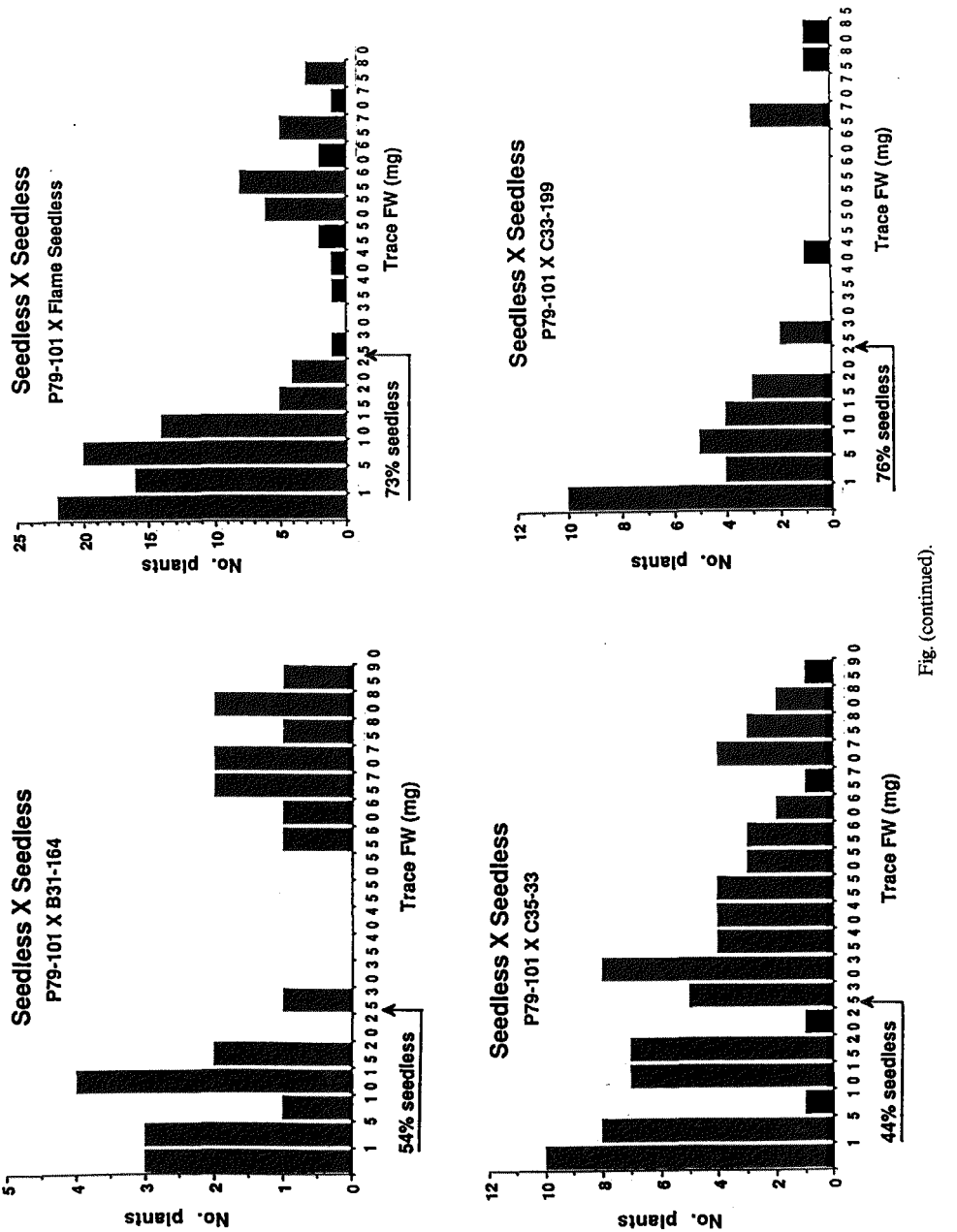


Fig. (continued).

Table 3: Comparison of families from seeded x seedless with seedless x seedless for percent seedless offspring

	Seedless pollen parents					
	Flame Seedless		C32-68		C35-33	
Females	% sdlss[1]	n	% sdlss[1]	n	% sdlss[1]	n
Seeded[2]						
P45-98	36	(281)	44	(249)	31	(35)
C15-133	32	(208)	39	(64)	25	(83)
Kishmiski	14	(20)	26	(99)	23	(103)
Seedless[3]						
P79-101	73	(111)	83	(24)	44	(78)
Significance[4]	***		***		NS	

[1] Seedless = aborted seeds less than 25mg FW.

[2] Seed/aborted seed weight based on average FW of all seeds in 10 average berries.

[3] Seed/aborted seed weight based on largest seed in 10 largest berries.

[4] NS,*** = Nonsignificant or significant at the 0.5% level respectively.

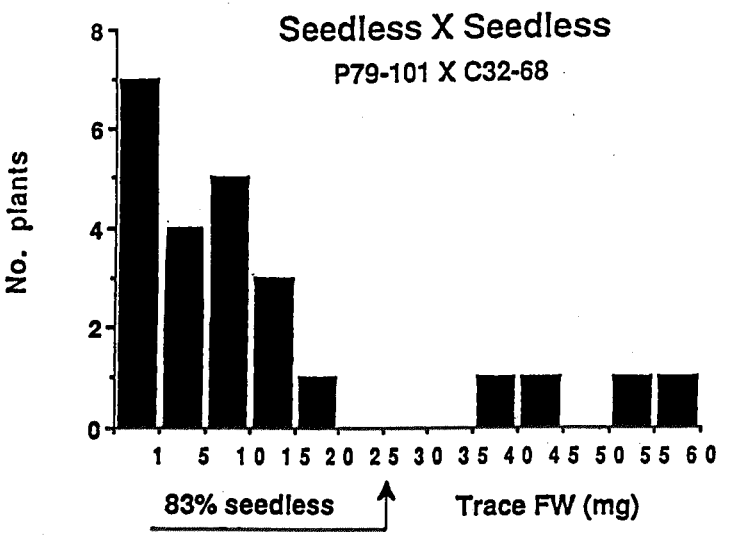


Fig. (continued).

was -0.4. This shows a low negative correlation between the aborted seed size of the parents and the percent seedless offspring, suggesting a low relation between phenotype and genotype for seedlessness. However, when individual families are compared it is seen that phenotype of the parents does not always correlate to the percent seedlessness in the family. For example, A71-185 x C32-68 and P79-101 x C32-68 representing parents with large aborted seeds had low (56 %) and high (83 %) seedless offspring, respectively, showing parental combination is important. The 5 families with P79-101 as the common female were compared (Table 2) and significant differences between percent seedlessness in the families were found. Even though C32-68 has a large aborted seed, in this case the family had the highest percent seedless offspring. This again points out the importance of parental combination and the need for progeny testing to achieve maximum efficiency in breeding for seedless genotypes.

The families from 3 seedless selections used as pollen parents and hybridized with the seedless selection P79-101 were compared to families from the same 3 seedless pollen parents hybridized with 3 seeded selections (Table 3). The families from the seeded females were studied earlier and their seed/aborted seed FW is based on the a v e r a g e seed weight from 10 largest berries. This measurement actually increases the percent seedless found compared to the method used for determining the FW of the seedless x seedless crosses. The seedless x seedless families were significantly different from the seeded families in 2 of the 3 cases. If the average FW for all traces instead of the largest trace is used for the seedless x seedless family, P79-101 x C35-33, 3 more seedlings are now classified as seedless instead of seeded. This increases the percent seedless to 47 % which is significantly different from the seeded x seedless families.

Literature

CONSTANTINESCU, G.; PENA, A.; INDREAS, A.; 1975: Inheritance of some qualitative and quantitative characters in the progeny of crosses between functionally female (gynodynamic) and apyrene (androdynamic) varieties. Probl. Genet. Teor. Apl. 7, 213-241.

DUDNIK, N. A.; MOLIVER, M. G.; 1976: Inheritance of seedlessness in grape in the south of the Ukrainian SSR. Ref. Zh. **5.55.118**, 105-113.

EMERSHAD, R. L.; RAMMING, D. W.; 1984: *In ovulo* embryo culture of *Vitis vinifera* L. c. v. 'Thompson Seedless'. Amer. J. Bot. **71**, 873-877.

---;---; SERPE, M. D.; 1989: *In ovulo* embryo development and plant formation from stenospermic genotypes of *Vitis vinifera*. Amer. J. Bot. **76**, 397-402.

LOOMIS, N. H.; WEINBERGER, J. H.; 1979: Inheritance studies of seedlessness in grapes. J. Amer. Soc. Hort. Sci. **104**, 181-184.

RAMMING, D. W.; 1989: The use of embryo culture in fruit breeding. HortScience [in publication].

SPIEGEL-ROY, P.; SAHAR, N.; BARON, J.; LEVI, V.; 1985: *In vitro* culture and plant formation from grape cultivars with abortive ovules and seeds. J. Amer. Soc. Hort. Sci. **110**, 109-112.

In vitro selection for tolerance to magnesium deficiency in grapevine rootstocks

A. Bouquet, A.-M. Chabbert, Y. Danglot

Station de Recherches Viticoles, Centre INRA de Montpellier, Domaine du Chapitre, F-34750 Villeneuve les Maguelonne, France

Summary: Some grapevine rootstocks (44-53 Malègue, Fercal) are known as very susceptible to magnesium deficiency, whether it is induced by low level of magnesium in the soil, particularly in acidic soils or, more generally, by high levels of potassium fertilization in soils normally provided with magnesium. So, their cultivation is strongly hindered in many vineyards, despite their other interesting cultural characteristics: very high chlorosis resistance for Fercal, drought tolerance, low vigor and beneficial effects on wine quality for 44-53 Malègue. *In vitro* tissue culture offers new prospects to solve this problem. In order to select somaclonal variants of these rootstocks exhibiting a lower level of susceptibility to magnesium deficiency, we compared the results of *in vitro* tests made on different varieties known as tolerant or susceptible to magnesium deficiency with the behavior of these varieties in the vineyard.

The varieties chosen were 44-53 Malègue and Fercal (susceptible), 41 B and 140 Ruggeri (tolerant). Plants were grown for 2 months on culture media with different concentrations of magnesium and potassium. Tissues of samples constituted of the foliar system (leaf blades, petioles and stems) of 6-15 plants (100-300 mg of dry matter) were extracted by HCl 0.1 N (10 ml for 100 mg of dry matter) and ions K^+, Mg^{++} and Ca^{++} were assayed by flame spectrophotometry.

An initial experiment was performed using one-node microcuttings and micrografting (scion: *Vitis vinifera* cv. Syrah) grown on a half-strength Murashige and Skoog medium with three levels of potassium and magnesium fertilization. It shows that the uptake of magnesium by vitroplants grown on this medium is not suitable to characterize the differences in susceptibility of rootstock varieties to magnesium deficiency. Fercal was more susceptible than 41 B (difference slightly significant, $P = 0.05$), 44-53 Malègue appeared curiously more tolerant than 140 Ruggeri, but the difference was not significant. The effect of grafting was not significant and there was no interaction between variety and grafting.

A second experiment was performed using one-node microcuttings grown on a Galzy medium (modified Knoop 1/2), with a low content in magnesium and increasing levels of potassium fertilization (0, 500, 750 and 1000 mg/l of KNO_3). It showed that the uptake of magnesium was significantly ($P < 0.01$) higher in 140 Ruggeri and 41 B than in Fercal and 44-53 Malègue. The inhibitory effect of increasing levels of K^+ on the absorption of Mg^{++} was clearly demonstrated.

Somatic embryos of 44-53 Malègue were obtained by anther culture and selected in conditions of magnesium deficiency: After four sub-cultures in liquid medium, 37 embryos were retained from which 4 germinated and developed into plants. Attempts to obtain somatic embryos by anther culture were unsuccessful with the variety Fercal.

3 selected somaplants of 44-53 Malègue (S_1, S_2 and S_4) were compared to an unselected somaplant (T_0) and the original clone (Cl. 120). After micropropagation of the somaclones, one-node microcuttings and micrografting (scion: *Vitis vinifera* cv. Cot) were grown on Galzy medium with reduced level of magnesium and two levels of potassium fertilization (0 and 1000 mg/l KNO_3). There were no significant differences in the uptake of potassium and calcium between clone and somaclones. With low potassium fertilization, the uptake of magnesium by somaclone S_2 was significantly lower ($P < 0.01$). With high potassium fertilization, somaclone S_2 and clone 120 were significantly lower than the other somaclones S_1, S_4 and T_0. So, there is evidence of somaclonal variation, but it is possible that the occurrence of this variation is not linked to the selection pressure applied *in vitro*. The amplitude of this variation is small and its stability and expression need to be confirmed in the vineyard. So far, the somaplants have been acclimated in the greenhouse and will be planted next year in the field for further investigations.

Key words: magnesium, potassium, calcium, deficiency, tolerance, rootstock, tissue culture, somaclonal variation, test, selection.

The detailed results will be published elsewhere.

Conservation of the genetic resources of *Vitis*

M. HARST-LANGENBUCHER and G. ALLEWELDT

Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, F. R. Germany

Summary: As an alternative or supplement to field collections, a repository of 35 grapevine genotypes and 15 clones of cv. Optima was set up under minimal growth conditions. To prolong the storage period between subculturing to at least 12-18 months, some factors of long-term storage were optimized and the causes responsible for early senescence of sensitive genotypes were investigated.

Besides indirect effects due to excision time, origin of the starting material and preculture, there is a direct influence of the light conditions on the survival rate of cultures. CCC application showed, in some cases, positive effects only after long-term storage.

An increase of carbon dioxide concentration during storage is considered to be responsible for the early death of some cultures.

Key words: gene resources, tissue culture, long-term storage, preculture, light condition, growth regulator, carbon dioxide.

Introduction

For grapevines, as with most heterozygotic plants, maintainance of homologous material is normally carried out vegetatively in living collections. This form of preservation, however, is highly vulnerable by biotic and abiotic factors e. g. pests, pathogens, climatic stress or air pollution.

An ideal alternative or supplement to field collections is preservation *in vitro*. Under normal culture conditions (NC), maintainance *in vitro* is laborious, time and space consuming. For storage purposes plants are maintained under reduced culture conditions (RC) to prolong the period between subculturing.

In 1986 a repository of 35 grapevine genotypes and 15 clones of cv. Optima was set up under minimal growth conditions. A wide range of genotypical reactions was observed during a storage period of 12 months. To optimize the storage conditions and to determine the causes of early senescence, various examinations were carried out.

Material and methods

Plant material

Rooted plants deriving from nodal cuttings were used as storage material. The plantlets were cultivated under NC up to a shoot length of 5-7 cm and then transferred to RC.

Culture conditions

- NC: 25 °C, 16 h photoperiod, 50 $\mu E \cdot m^{-2} \cdot s^{-1}$
- RC: 8 °C, 10 h photoperiod, 10 $\mu E \cdot m^{-2} \cdot s^{-1}$

The medium of LINSMAIER and SKOOG (1965) supplemented with hormones (0.01 mg/l NAA and 0.03 mg/l BA), vitamins (0.40 mg/l thiamine), 30 g/l sucrose and 8 g/l agar, was used under NC and RC.

CCC was applied to the nutrient medium before cultivation of the cuttings according to ALLEWELDT and HARST-LANGENBUCHER (1987).

Carbon dioxide determination

The determination of CO_2 was carried out with a special CO_2 detector Ultramat 22p Siemens modified for use with *in vitro* cultures.

Abbreviations used

NAA, 1-naphtaleneacetic acid; BA, 6-benzyladenine; CCC, chlorocholinechloride (2-chlorethyl-trimethyl-ammonium-chloride); NC, normal culture conditions; RC, reduced culture conditions.

Results and discussion

Optimization of the long-term storage conditions

For a successful *in vitro* cultivation and long-term storage of nodal cuttings the starting material is important.

Best results were obtained when the explants were taken in the months of June and July. Cuttings from greenhouse-grown plants performed better than cuttings from the field (Table 1).

Prior to long-term storage the plants have to be subcultured twice after excision and establishment *in vitro*. Plants which were stored directly after sprouting of just excised cuttings were highly infected or died within a few weeks of storage (Table 2).

Table 1: Survival rate (%) of *in vitro* cultures after 12 months of long-term storage in dependence on excision time and origin of the plant material (n = 20)

excision time / origin	JUNE	JULY	AUGUST
FIELD	70	80	0
GREENHOUSE	90	100	50

Table 2: Survival rate (%) of *in vitro* cultures after 3, 6 and 12 months of long-term storage in dependence on the number of subcultures (n = 20)

number of subcultures after excision	months of storage		
	3	6	10
0	100	100	55
1	100	95	37
2	100	90	80

Table 3: Survival rate (%) of *in vitro* cultures from cv. Riesling and the wild species *V. rupestris* after 9 months of long-term storage in dependence on light intensity (n = 16)

light intensity	10 $\mu E \cdot m^{-2} \cdot s^{-1}$	1 $\mu E \cdot m^{-2} \cdot s^{-1}$
genotype		
RIESLING	50	3
V.RUPESTRIS	88	81

Light conditions also seem to play an important role on survival rate. Within a few months of storage, the cultures stored under continuous light or in darkness died. Under short-day conditions and low light intensity ($10 \mu E \cdot m^{-2} \cdot s^{-1}$), most of the cultures survived a storage period of 15 months. A further reduction of the light intensity down to only $1 \mu E \cdot m^{-2} \cdot s^{-1}$ caused rapid losses of sensitive cultivars within a few weeks under RC (Table 3).

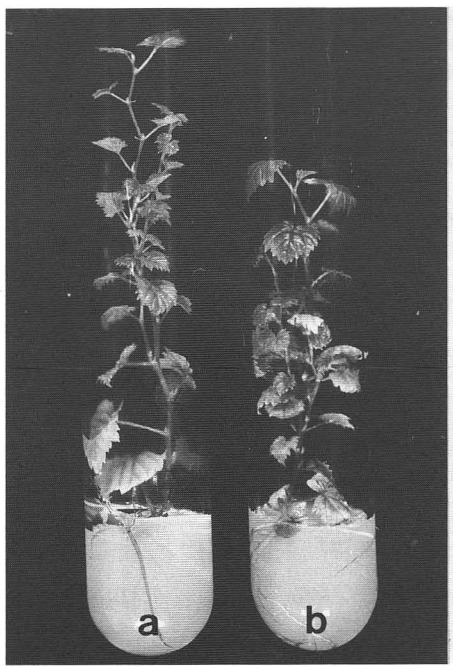


Fig. 1 (left): Improvement of sprouting and shoot development after subculturing onto fresh medium in dependence on CCC treatment during long-term storage. a) Subculture of a CCC-treated plant, b) subculture of an untreated plant.

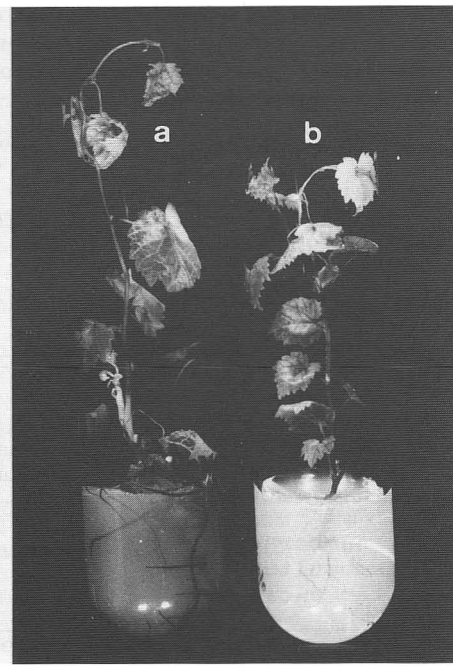


Fig. 2 (right): Induction of senescence symptoms on *in vitro* plantlets by long-term storage under RC (a) and by increasing the CO_2 concentration under NC (b).

Comparable results had been achieved by BARLASS and SKENE (1983) and FAUSTINI (1984).

In a further attempt to improve the survival rate, CCC was applied to *in vitro* cultures. Under field conditions an increase in cold tolerance had been induced by treating grapevines with this growth inhibitor (BOURQUIN and ALLEWELDT 1970).

Table 4: Survival rate (%) of *in vitro* cultures of *V. riparia* in dependence on CCC treatment during different storage periods (n = 16)

treatment	untreated	CCC
storage period (months)		
3	100	100
6	71	100
12	36	64

Table 5: Comparison of the survival rates (%) from different genotypes of *Vitis* after a long-term storage of 12 months. Storage periods 1986/87 and 1987/88

genotype	storage period I 1986-1987	storage period II 1987-1988
V.RUPESTRIS	31	88
V.RIPARIA	0	63
SO 4	56	53
RIESLING	63	81
KERNER	81	100
FABERREBE	56	100
OPTIMA	56	100
ORION	6	100
VIDAL	50	81
mean	44	84

In vitro cultures of only a few cultivars showed a better survival rate in RC when treated with CCC (Table 4). Some cultivars could not be stored longer than when untreated. However, after long-term storage these CCC-treated plants exhibited earlier sprouting and faster shoot development after subculturing onto fresh medium (Fig. 1). This result has to be confirmed in subsequent examinations.

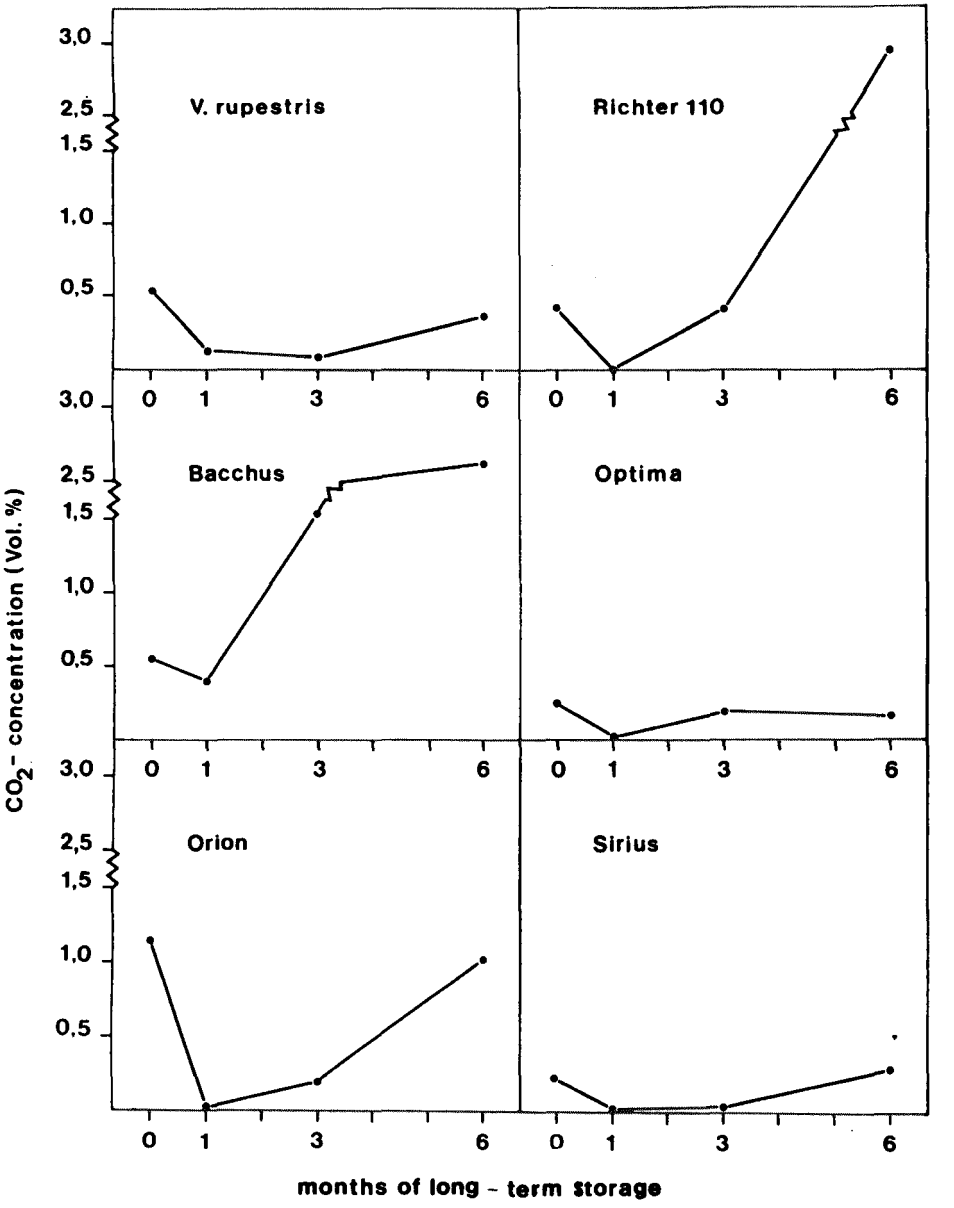


Fig. 3: CO_2 development during a storage period of 6 months in dependence on the genotype.

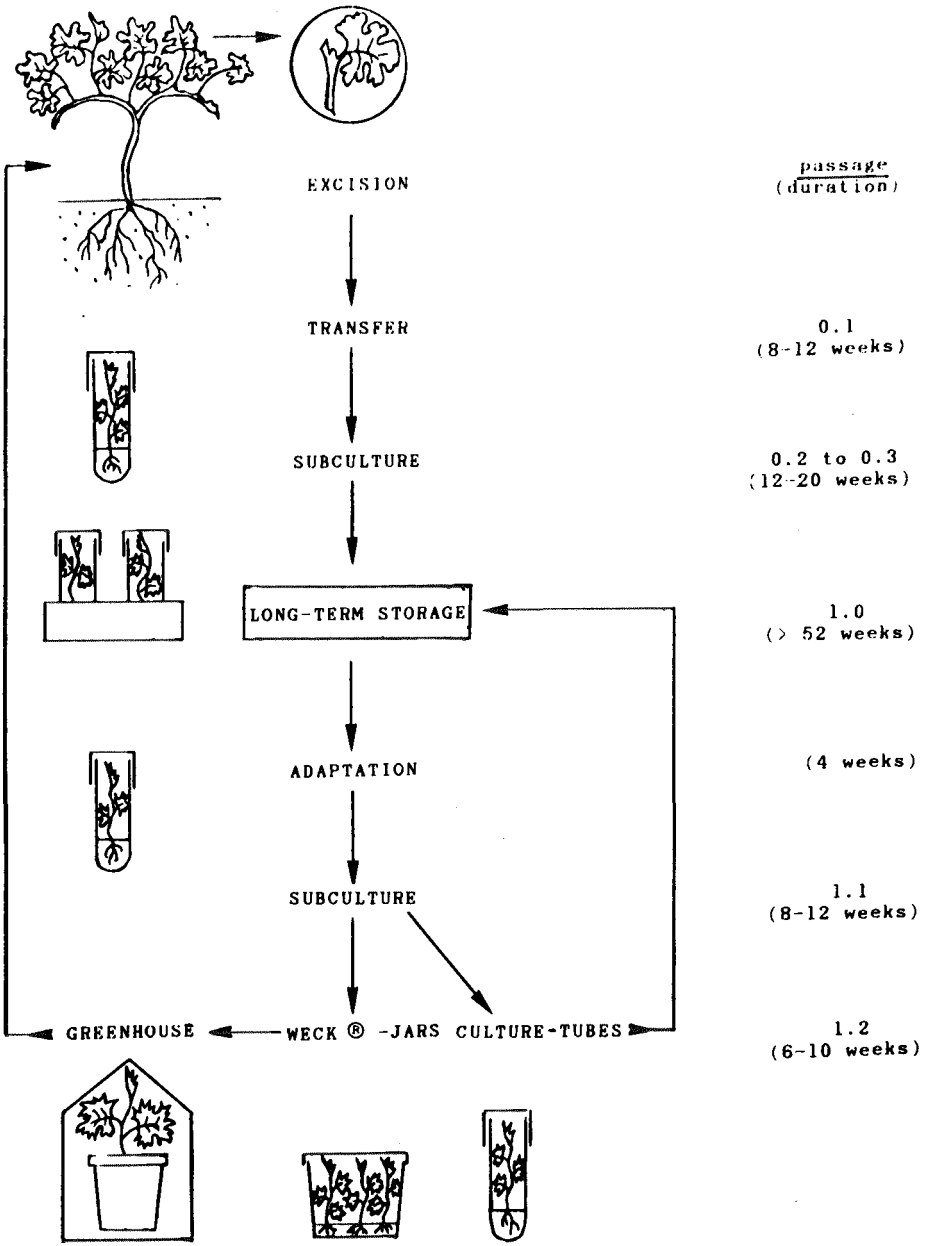


Fig. 4: Long-term storage under reduced culture conditions during maintainance and propagation of *Vitis* material by tissue culture techniques.

Causes of senescence

Besides these tests for optimizing the long-term storage conditions, the causes of early senescence exhibited by some genotypes were of great interest. Many authors have supposed that CO_2 becomes concentrated under *in vitro* conditions in the culture tubes (Donovan and Murashige 1979; De Proft *et al.* 1985; Fournioux and Bessis 1986; Zobel 1987).

In a special examination, senescence symptoms similar to those observed during long-term storage could be induced by artificial rising the CO_2 concentration up to 3 % (v/v) under NC (Fig. 2).

The development of CO_2 concentrations during a storage period of 6 months is demonstrated in Fig. 3. During the period under NC the CO_2 concentration increased to more than 0.5 % (v/v) of the environmental CO_2 content of the laboratory (start of storage). Within the first 4 weeks after transfer of the plantlets to RC, the CO_2 concentration declined rapidly (1 month of storage). During the following 5 months under RC the CO_2 concentration showed a characteristical rise dependent on genotype (6 months of storage).

Fig. 5: Resumption of growth vigour after 20 months of long-term storage when subcultured onto fresh medium. a) Plantlet after 20 months of long-term storage, b) subcultivation onto fresh medium, c) sprouting 7 d after subcultivation, d) shoot development 21 d after subcultivation.

In the culture tubes of cvs Richter 110, Bacchus and Orion, which showed a very marked response to RC, a high CO_2 concentration of almost 3 % (v/v) was determined.

To avoid high CO_2 concentration, the N_2 content in the atmosphere of the culture tubes could be risen to keep the CO_2 content on a non-toxic level. The long-term storage of ornamentals is carried out in this way (PREIL 1989, personal communication).

Due to these tests for optimizing the long-term storage conditions and the examination of the development of CO_2 concentration during the storage period, some general recommendations for carrying-out conservation of *in vitro* cultures of grapevine can be given (Fig. 4):

After excising the starting material from field- or greenhouse-grown grapes, the cultures have to be propagated twice under NC before they can be transferred to RC. If more than 50 % of each cultivar have died, the surviving cultures should be re-transferred into NC and adapted for about 4 weeks before subculturing onto fresh medium. After being subcultured twice, the plants can be stored again or, if necessary, adapted to soil.

By following this procedure the survival rate was almost doubled in a further storage period, as demonstrated on some cultivars (Table 5).

The stored material did not loose vitality even when the plants were subcultured onto fresh medium after a storage period of more than 20 months under RC (Fig. 5). After adaptation to soil and acclimatization to field, the long-term stored plant material is true to type (Fig. 6).

So far, some important factors influencing the survival rate under RC have been described. Other factors, like an adaptation to short-day conditions before and an adaptation to long photoperiod after the storage, have to be examined. A further perspective for optimization of the long-term storage can be the examination of the composition of the nutrient medium (full- or half-strength salt concentration, NH_4NO_3 concentration, reduction of sucrose concentration, activated charcoal, etc.).

For almost all of the stored genotypes a minimum storage period of 12 months was realized. There are no problems in subculturing and in the acclimatization to field conditions after long-term storage. By using differenciated explants like shoot-tips or nodal cultures, somaclonal variation can be excluded (D'AMATO 1978).

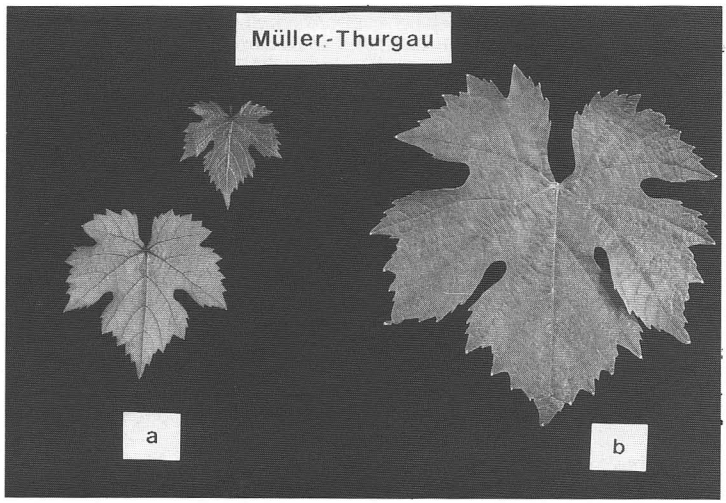


Fig. 6: Plants are true to type when adapted to field conditions. a) Leaves from an *in vitro* long-term stored plant after adaptation to field conditions, b) leaves from the same cultivar derived from the field collection.

At the present situation of plant cell and tissue culture research of the grapevine this conservation method of *in vitro* plantlets seems to be well-suited for a safe maintainance of healthy plant material. Thus, the establishment of a gene repository and the long-term storage of clonal material for breeding and/or propagation has been initiated.

Conclusion

A simple and safe maintainance procedure for rooted plantlets of *in vitro* cultures of grapevine has been established by improving the storage conditions (starting material, preculture, light conditions, CO_2 development). The period between two subcultivations could therefore be prolonged to 12-18 months. For those genotypes which show yet an unsatisfying survival rate, optimization of the long-term storage has to be continued.

References

ALLEWELDT, G.; HARST-LANGENBÜCHER, M.; 1987: Der Einfluß von Wachstumsinhibitoren auf die Langzeitlagerung von *in-vitro*-Kulturen der Rebe. Vitis **26**, 57-64.

BARLASS, M.; SKENE, K. G. M.; 1983: Long-term storage of grape *in vitro*. FAO/IBPGR Plant Genetic Resources Newsletter **53**, 19-21.

BOURQUIN, H. D.; ALLEWELDT, G.; 1970: Der Einfluß von CCC und B-995 auf das Triebwachstum der Reben. Vitis **9**, 105-120.

D'AMATO, F.; 1978: Chromosome number variation in cultured cells and regenerated plants. In: THORPE, T. A. (Ed.): Frontiers of Plant Tissue Culture, 287-295. International Association of Plant Tissue Culture, Calgary.

DONOVAN, S. T.; MURASHIGE, T.; 1979: Volatile emissions of plant tissue cultures. I. Identification of the major components. In Vitro **15**, 654-658.

FAUSTINI, R.; 1982: Metodi alternativi di conservazione del materiale genetico della vite: "colture *in vitro*". Vignevini **9** (9), 11-13.

FOURNIOUX, J. C.; BESSIS, R.; 1986: Influence de la teneur en gaz carbonique sur la morphogenèse de la vigne en culture *in vitro*. Can. J. Bot. **64**, 2608-2616.

LINSMAIER, E. M.; SKOOG, F.; 1965: Organic growth factor requirement of tobacco tissue culture. Physiol. Plant. **18**, 100-127.

PROFT, M. P. DE; MAENE, L. J.; DEBERGH, P. C.; 1985: Carbon dioxide and ethylene evolution in the culture atmosphere of *Magnolia* cultured *in vitro*. Physiol. Plant. **65**, 375-379.

ZOBEL, R. W.; 1987: Gaseous compounds of soybean tissue cultures: Carbon dioxide and ethylene evolution. Environ. Exp. Bot. **27**, 223-226.

Meristem culture for clonal micropropagation of grapevines

H. ÇELIK and M. BATUR

Department of Horticulture, Faculty of Agriculture, University of Ankara, 06110 Ankara, Turkey

Summary: Experiments were carried out to determine the effects of MS medium concentrations (4/4 MS, 3/4 MS, 2/4 MS) combined with GA_3 at 0.0, 0.1 and 0.5 mg/l, and vitamin formulations (MS, Morel, thiamine-inositol) on success of meristem culture and of 15 auxin (IBA) x cytokinin (BAP) combinations on shoot and root formation for clonal micropropagation of the wine grape cv. Kalecik Karasi and the rootstock cv. 41 B M.G.

Best results were obtained from standard MS mineral composition and vitamin formulation combined with 2.5 mg/l BAP and 0.5 mg/l GA_3 for primary meristem cultures of both genotypes; 0.0 mg/l IBA x 1.0 mg/l BAP for shoot subculture and 1.0 mg/l IBA x 0.0 mg/l BAP for root subculture of Kalecik Karasi; 0.0 mg/l IBA x 1.0 mg/l BAP for shoot subculture and 5.0 mg/l IBA x 0.0 mg/l BAP for root subculture of 41 B, considering shoot, explant, root, callus and particularly entire plant formation in the cultures of both stages.

Key words: tissue culture, micro-propagation, growth, meristem, root, shoot, culture medium, vitamin, growth regulator.

Introduction

Meristem-tip culture has been widely used in clonal micro-propagation of higher plants during the last three decades. The most important application of meristem culture is in the production of virus-free plants, rapid multiplication and also in the long-term storage of such virus-free clonal germplasm through cryopreservation techniques (QUAK 1977; KARTHA 1981).

Although the theoretical aspects of virus eradication from plants by meristem-tip culture is not clear yet, ever since the pioneering work of LIMASSET and CORNUET (1949) this technique has been the most efficient method of eliminating viral pathogens from crop plants.

Several workers reported that grapevines have been fully freed of certain wide-spread viral pathogens such as fan leaf, leaf roll and yellow speckle, either by meristem culture alone or meristem culture in combination with thermotherapy (BARLASS *et al.* 1982; GRENAN 1984; BLAICH 1985).

In the past decade, numerous studies were carried out to explore the reactions of *Vitis* species to meristem culture for the above-mentioned purposes (FAVRE 1978; BARLASS and SKENE 1980, 1981, 1982; CHÉE and POOL 1983, 1985; GRAY and FISHER 1987; FANIZZA 1987; ALLEWELDT and HARST-LANGENBUCHER 1987).

Materials and methods

As the basic plant material, apical meristems that are 0.5 mm in length with two or three leaf primordia, isolated from growing tips of current shoots of Kalecik Karasi (*V. vinifera* L.) which is a superior red wine variety, and 41 B M.G. (Chasselas x *V. berlandieri*) which is a difficult-to-root and lime resistant rootstock variety, were used in the experiments which were carried out in three consecutive steps:

Experiment I: Effects of various concentrations (4/4 MS, 3/4 MS, 2/4 MS) of agar-solidified (0.8 %) standard MURASHIGE and SKOOG (1962) medium supplemented with sucrose (3.0 %) and BAP (2.5 mg/l) (CHÉE and POOL 1985) with three combinations of Ga_3 at 0.0, 0.1 and 0.5 mg/l on success of the meristem cultures in establishment medium.

Table 1: Effects of MS concentrations, GA_3 and vitamin formulations on success of the cultures in establishment medium

MS	GA_3 (mg/l)	Survival rate (%)		Growth (%)	
		K.Karasi	41 B	K.Karasi	41 B
4/4	0.0	57.1	71.4	21.4	3.6
	0.1	53.6	54.3	17.9	37.1
	0.5	75.0	67.9	64.3	53.6
3/4	0.0	67.9	51.4	42.9	17.1
	0.1	71.4	64.3	25.0	7.1
	0.5	60.7	28.6	32.1	17.9
2/4	0.0	67.9	64.3	32.1	3.6
	0.1	53.6	39.3	39.3	-
	0.5	57.1	20.0	50.0	-
	Vit. Formul.				
4/4	MS Vit.	100.0	92.8	100.0	83.3
	Morel Vit.	100.0	72.5	100.0	60.7
	Thia.-Mes.In.	100.0	93.8	100.0	79.6

Experiment II: Effects of three vitamin formulations (MS vitamin, Morel vitamin and thiamine + meso-inositol) supplemented in standard MS medium on success of the meristem cultures in establishment stage.

Experiment III: Effects of 15 auxin (IBA) x cytokinin (BAP) combinations in the above-mentioned MS medium supplemented with 0.5 mg/l GA_3, designed with a predominance of BAP in shoot proliferation medium and IBA in rooting medium, on shoot and root formation and development of the cultures.

Cultures were incubated at constant temperature of 25 °C, 16 h photoperiod (4000 lux) and 70 % relative humidity during establishment (3 weeks), shoot proliferation and rooting subcultures (5 weeks in each stage) (Wetherell 1982; Harris and Stevenson 1982).

Results and discussion

Experiment I

Highest survival rates of the meristem cultures for both Kalecik Karasi (75.0 %) and 41 B M.G. (67.9 %) were obtained from 4/4 MS x 0.5 mg/l GA_3. Similarly, the same combination also gave better results in the development of survival meristems for both genotypes (Table 1, Fig. 1).

Experiment II

As shown in Table 1, all meristems of Kalecik Karasi which were cultured in standard MS medium with the combination of the three vitamin formulations were still alive. However, survival rates of 41 B meristems were above 90.0 % in MS vitamin and thiamine + meso-inositol, but 72.5 % in Morel vitamin. The rates of growing meristems are also similar to that survival data.

Experiment III

For both genotypes, 0.0 mg/l IBA x 1.0 mg/l BAP and 0.5 mg/l IBA x 1.0 mg/l BAP combinations gave better shoot proliferation and development in shoot medium (Table 2, Fig. 2). On the other hand, satisfactory rooting rates (above 50.0 %) were obtained with those combinations in Kalecik Karasi. The combination containing only BAP at 1.0 mg/l also produces entire plants at the rate of 42.9 % in the same genotype. These data are in agreement with the reports of SKENE and BARLASS (1980), HARRIS and STEVENSON (1982), MASAHIKO *et al.* (1982). Although the data concerning shoot development in 41 B were generally similar to those of Kalecik Karasi, as a result of inefficient rooting, an entire plant production rate of 18.8 % was only achieved in the combination containing 0.5 mg/l IBA and 0.5 mg/l BAP.

Results on rooting and entire plant formation in rooting medium were found to be rather different according to the genotypes. Highest root formation was obtained in the combinations of 1.0 mg/l IBA x 0.0 mg/l BAP (86.7 %) and 1.0 mg/l IBA x 0.5 mg/l BAP (85.7 %) for Kalecik Karasi; 5.0 mg/l IBA x 0.0 mg/l BAP (100.0 %), followed by 2.5 mg/l IBA x 0.0 mg/l BAP (90.0 %) and 5.0 mg/l IBA x 0.5 mg/l BAP (90.0 %) for 41 B. In entire plant formation, similar combinations as above gave the best results: 1.0 mg/l IBA x 0.5 mg/l BAP (71.4 %) and 1.0 mg/l IBA x 0.0 mg/l BAP (66.7 %) for Kalecik Karasi; 5.0 mg/l IBA x 0.0 mg/l BAP (80.0 %) and 5.0 mg/l IBA x 0.5 mg/l BAP (70.0 %) for 41 B (Table 3, Fig. 3).

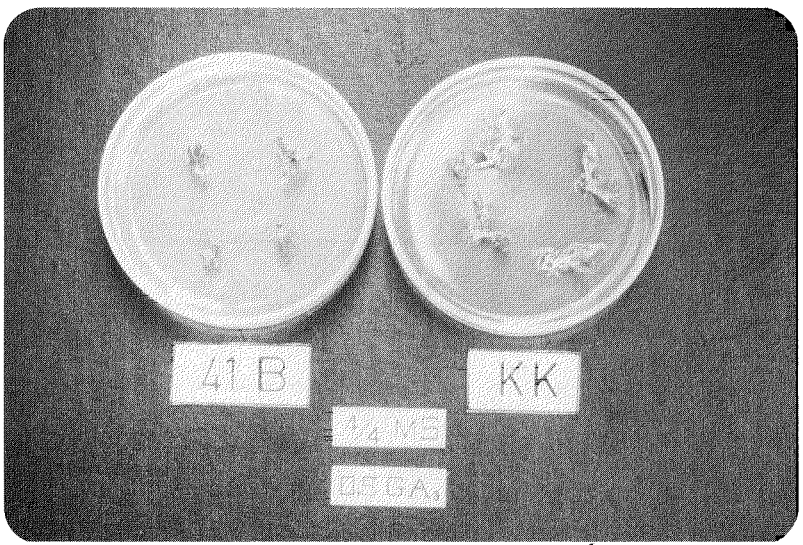


Fig. 1: Development of apical meristems in the establishment medium of MS supplemented with 0.5 mg/l GA_3.

Table 2: Effects of IBA x BAP combinations on growth of the apical meristems of wine grape cv. Kalecik Karasi and rootstock cv. 41 B M.G. in shoot medium

IBA (mg/l)	BAP (mg/l)	Shoot Formation(%)		No.of Shoots/ Culture		No.of explants/ Culture		Total wt.of culture(mg)		Entire plant(%)	
		K.K.	41 B	K.K.	41 B	K.K.	41 B	K.K.	41 B	K.K.	41 B
0.0	0.0	-	-	-	-	1.66	2.00	537.7	239.3	-	-
0.0	0.5	20.0	25.0	0.20	0.25	2.53	2.56	911.3	528.8	20.0	-
0.5	0.5	13.3	18.8	0.13	0.18	2.93	2.31	930.5	1174.3	13.0	18.8
0.0	1.0	42.9	43.8	0.57	0.43	3.64	3.12	2439.0	909.4	42.9	-
0.5	1.0	42.9	40.0	0.78	0.60	4.07	3.00	1039.7	1731.9	28.6	-
1.0	1.0	33.3	18.8	0.33	0.18	3.16	2.43	1793.5	1857.6	33.3	-
0.0	2.5	-	18.8	-	0.25	4.18	3.68	2643.2	1207.8	-	-
0.5	2.5	6.7	31.3	0.06	0.31	3.80	2.62	1702.4	1407.6	6.7	-
1.0	2.5	13.3	31.3	0.13	0.31	3.06	2.68	2180.3	1968.5	6.7	-
2.5	2.5	18.7	25.0	0.18	0.25	3.75	1.68	1718.6	1984.5	-	-
0.0	5.0	-	-	-	-	2.50	2.56	1071.6	778.0	-	-
0.5	5.0	13.3	-	0.13	-	4.06	2.62	1785.3	1173.0	-	-
1.0	5.0	-	-	-	-	3.53	2.25	2265.7	1301.5	-	-
2.5	5.0	-	-	-	-	3.56	2.43	2318.9	1752.1	-	-
5.0	5.0	7.2	-	0.07	-	3.28	1.86	2516.2	514.5	-	-

Table 3: Effects of IBA x BAP combinations on root and shoot growth of the apical meristems of wine grape cv. Kalecik Karasi and rootstock cv. 41 B M.G. in rooting medium

IBA (mg/l)	BAP (mg/l)	Root formation(%) K.K.	41 B	No.of roots/ culture K.K.	41 B	Total wt.of culture(mg) K.K.	41 B	Entire plant(%) K.K.	41 B
0.0	0.0	66.7	33.3	1.58	0.75	1012.5	321.4	13.3	25.0
0.5	0.0	76.9	53.8	2.23	1.92	1040.6	617.9	53.8	38.5
0.5	0.5	69.2	54.5	0.07	1.72	1786.6	1468.0	53.8	45.5
1.0	0.0	86.7	63.6	2.86	2.81	1957.2	722.0	66.7	36.4
1.0	0.5	85.7	70.0	1.92	1.70	1206.1	1515.0	71.4	40.0
1.0	1.0	50.0	25.0	0.90	0.33	1624.9	1336.8	30.0	16.7
2.5	0.0	63.6	90.0	2.00	5.30	1367.6	916.2	54.5	60.0
2.5	0.5	78.6	75.0	3.14	2.83	1796.5	2008.9	57.1	66.7
2.5	1.0	66.7	41.7	1.73	1.33	1767.2	1897.2	46.7	41.7
2.5	2.5	41.7	33.3	1.07	0.58	2671.0	2175.4	33.3	16.7
5.0	0.0	64.3	100.0	1.42	4.60	1612.1	1002.9	50.0	80.0
5.0	0.5	84.6	90.0	2.53	3.90	1926.0	2107.8	61.5	70.0
5.0	1.0	83.3	30.0	2.50	1.10	2536.1	1669.9	58.3	30.0
5.0	2.5	72.7	10.0	2.09	0.50	2253.3	1525.1	27.3	10.0
5.0	5.0	30.8	-	0.50	-	3296.9	509.6	-	-

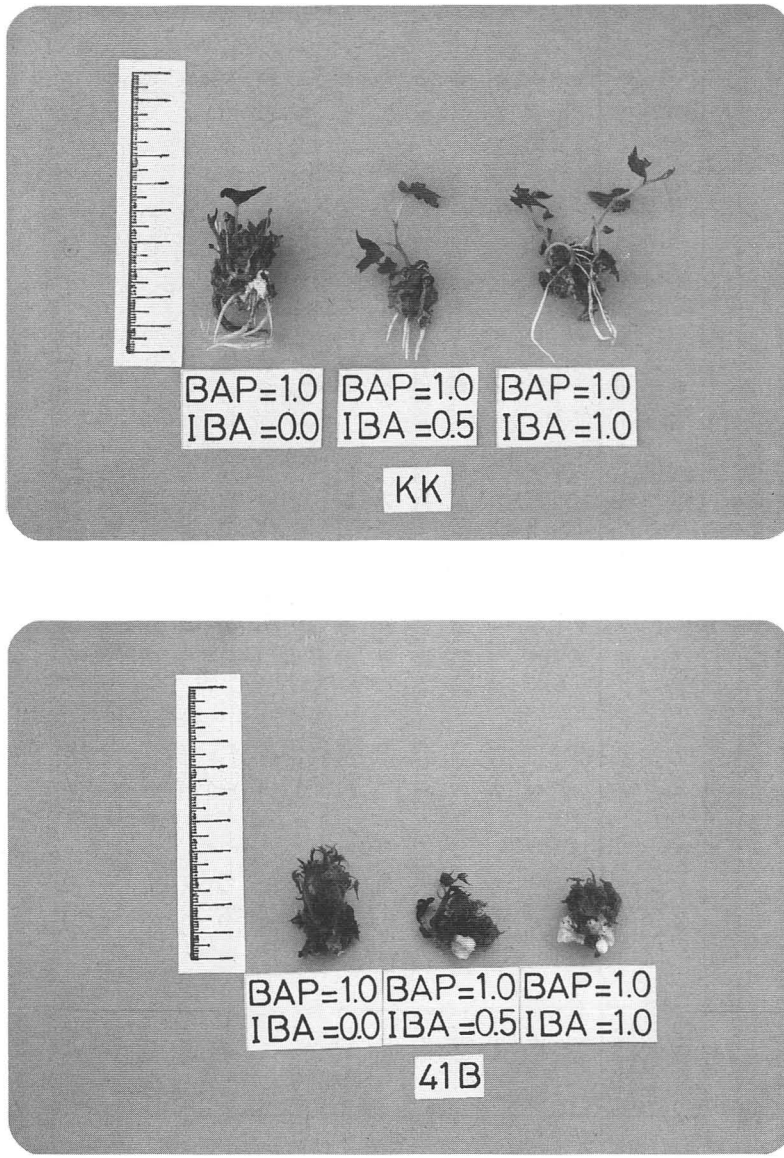


Fig. 2: Growth patterns of Kalecik Karasi and 41 B M.G. apical meristems in shoot subculture.

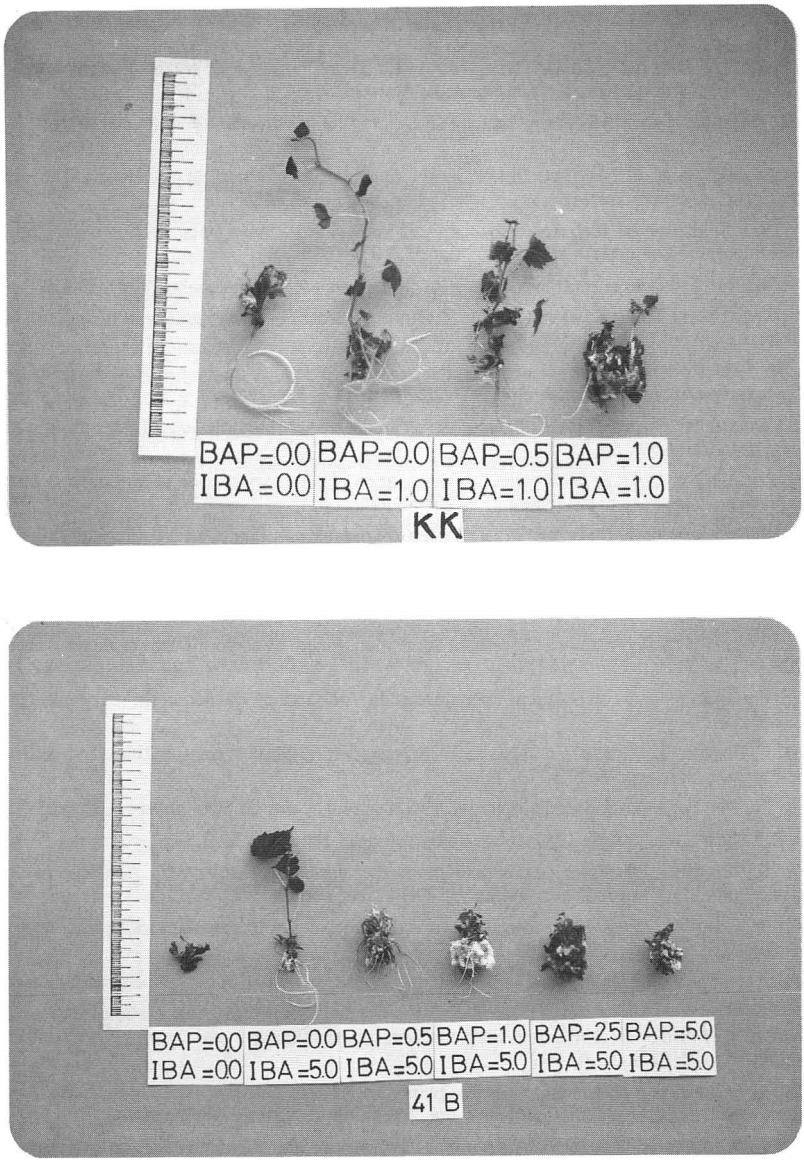


Fig. 3: Growth patterns of Kalecik Karasi and 41 B M.G. apical meristems in root subculture.

Literature

ALLEWELDT, G.; HARST-LANGENBUCHER, M.; 1987: Der Einfluß von Wachstumsinhibitoren auf die Langzeitlagerung von *in-vitro*-Kulturen der Rebe. Vitis **26**, 57-64.

-- -- ; 1987: The effect of growth inhibitors on long-term storage of *in vitro* cultures of grapevine. Hort. Abstr. **57**, 9309.

BARLASS, M.; SKENE, K. G. M.; 1980: Studies on the fragmented shoot apex of grapevine. I. The regenerative capacity of leaf primordial fragments *in vitro*. J. Exp. Bot. **31**, 483-488.

-- -- ; -- -- ; 1981: Studies on the fragmented shoot apex of grapevine. III. A scanning electron microscope study of adventitious bud formation *in vitro*. J. Exp. Bot. **32**, 1079-1083.

-- -- ; -- -- ; 1982: Virus-free vines from tissue culture. Austral. Grapegrower Winemaker (224), 40-41.

-- -- ; -- --.; WOODHAM, R. C.; KRAKE, L. R.; 1982: Regeneration of virus-free grapevines using *in vitro* apical culture. Ann. Appl. Biol. **101**, 291-295.

BLAICH, R.; 1985: Recherches sur les cultures de méristèmes et d'organes de vigne *in vitro* en vue de la sélection et de la conservation de génotypes. Bull. O.I.V. **58** (650/651), 391-395.

-- -- ; 1985: Studies on *in vitro* cultures of grape organs and meristems with the aim of selecting and preserving genotypes. Hort. Abstr. **55**, 8502.

CHÉE, R.; POOL, r. M.; 1983: *In vitro* vegetative propagation of *Vitis*: Application of previously defined culture conditions to a selection of genotypes. Vitis **22**, 363-374.

-- -- ; -- -- ; 1985: *In vitro* propagation of *Vitis*: The effects of organic substances on shoot multiplication. Vitis **24**, 106-118.

FANIZZA, G.; 1986: BAP induces rooting *in vitro* shoot tip culture in *Vitis*: genotypic variation. Genet. Agrar. **40**, 448-449.

FAVRE, J. M.; 1977: Premiers résultats concernant l'obtention *in vitro* de néoformations caulinaires chez la vigne. Ann. Amélior. Plantes **27**, 151-169.

-- -- ; 1978: Preliminary results on obtaining spontaneous *in vitro* shoot production in the grapevine. Hort. Abstr. **48**, 240.

GRAY, D. J.; FISHER, L. C.; 1987: *In vitro* shoot propagation of grape species, hybrids and cultivars. Proc. Florida State Hort. Soc. **98**, 172-174.

GRENAN, S.; 1984: Polymorphisme foliaire consécutif à la culture *in vitro* de *Vitis vinifera* L. Vitis **23**, 159-174.

HARRIS, R. E.; STEVENSON, J. H.; 1982: *In vitro* propagation of *Vitis*. Vitis **21**, 22-32.

KARTHA, K. K.; 1981: Meristem culture and cryopreservation. In: THORPE, T. A. (Ed.): Methods and Applications. Plant Tissue Culture. Academic Press, New York.

LIMASSET, P.; CORNUET, P.; 1949: Recherche du virus de la mosaïque du tabac dans les méristèmes des plantes infectées. C. R. Acad. Sci., Paris, **288**, 1971-1972.

MASAHIKO, I.; TOMIO, S.; HIDEO, T.; KATSUYASU, U.; 1982: Elimination of grapevine viruses by meristem tip culture. Proc. 5th Intern. Congr. Plant Tissue and Cell Culture, 409-413.

MURASHIGE, T.; SKOOG, F.; 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 473-497.

QUAK, F.; 1977: Meristem culture and virus-free plants. In: REINERT, J.; BAJAS, Y. P. S. (Eds.): Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. Springer-Verlag, Berlin.

SKENE, K. G. M.; BARLASS, M.; 1980: Micropropagation of grapevine. Proc. Intern. Plant Propagators Soc. **30**, 564-570.

WETHERELL, D. F.; 1982: Introduction to *in vitro* propagation, 1-85, University of Connecticut. Avery's Plant Tissue Culture Series, New Jersey.

The use of *in vitro* apical culture of grapevines to eliminate pathogens (different viruses, *Agrobacterium tumefaciens*)

B. ALTMAYER

Landes- Lehr- und Forschungsanstalt für Landwirtschaft, Wein- und Gartenbau, Breitenweg 71, D-6730 Neustadt, F. R. Germany

A b s t r a c t : Grapevines infected with different nepo-viruses (ArMV, RRV, GFV, TmBRV, SLRV) and grapevine leafroll (GLR), respectively, were propagated using *in vitro* apical culture. Shoot tip explants including the meristem and 1-3 leaf primordia (max. size 0.5 mm) were cultivated on a Linsmayer-Skoog medium, containing 1 mg/l IAA and 2 mg/l BAP at 28 °C. After regeneration of shoots and rooting in a modified White's medium, the completed plants could be transferred to the glasshouse and finally planted in the field.

None of these grapevines, regenerated from virus infected mother plants in 1984 and 1985 without heat treatment, shows symptoms of virus infection or reaction in the serological test whereas all control plants derived from cuttings of the same mother plants are virus-infected. Using the same method, *in vitro* apical culture can be used to eliminate *Agrobacterium tumefaciens* from infected grapevines.

To investigate the occurrence of modifications as well as to produce healthy plants many different grape varieties were propagated by this method. Ampelographic characteristics (size and form of leaves and grapes) were modified in at least two cases (clones of Silvaner and Riesling).

In vitro micropropagation of grape varieties

A. DALLOUL, S. MISIK and L. OLAH

Ltd. Company for Micropropagation of Grape, P.O.Box 25, H-6001 Kecskemét, Hungary, and P.O.Box 83, H-3301 Eger, Hungary

A b s t r a c t : Micropropagation – by shortening the time of production of propagation material – has an important role in the breeding of grape. Since one of the main tasks at the Eger-Mátra wine region is the breeding and clonal selection, we have started to examine the possibility of *in vitro* propagation of varieties and clones which are promising for this wine region.

Till now 9 varieties and clones have been examined by the method of HAYDU (1984).

We have found that the adaptability of varieties to micropropagation depends on the genotype. This is proved by similar behaviour among related clones.

In our experiments, propagation rates (= number of new buds/number of initial buds) have varied from 0.00 to 5.08 according to the genotype. The higher expenses of micropropagated plants can be recovered in the higher biological value of the virus-free propagation material. The expenses of micropropagation can be reduced by developing variety-specific technology.

Production of somatic embryos and plantlets in seedless grapevine varieties (*Vitis vinifera* L.) by anther culture

A. Bouquet, G. Benchetrit and Y. Danglot

Station de Recherches Viticoles, I.N.R.A., Domaine du Chapitre, F-34750 Villeneuve lès Maguelonne, France

A b s t r a c t : Breeding for seedless table grapes is now currently done by hybridization of seedless varieties using the technique of *in ovulo* rescue. The qualities required for the new varieties are mainly improved bud fertility, earlier ripening and increased berry size. Somaclonal variation induced by *in vitro* tissue culture can be an interesting alternative to hybridization for improving seedless varieties of major importance, like Sultanina, or seedless varieties having technological characteristics difficult to recover after cross-breeding, like aptitude for canning. With this objective, the embryogenic ability of the anther somatic tissues was measured on 8 seedless varieties: Sultanina, Perlette, Delight, Beauty Seedless, Canner Seedless, Sultana Moscata (75 Pirovano), 45 Bruni and 116 Bruni. Callus induction, somatic embryo production and somatic embryo conversion into plantlets depend considerably on the variety used. From this point of view, the most interesting ones are Sultanina, Canner Seedless, 75 Pirovano and 116 Bruni. On the whole, 952 plantlets were obtained from 3213 somatic embryos. The influence of developmental stage of the anther on the different parameters of embryo somatic production was studied on the variety 116 Bruni, and results show that some of these parameters are antagonistic processes: Promotion of one generally results in depression of the other. Somaclonal variation among the somatic embryos of 116 Bruni was observed with the occurrence of sub-lethal plantlets showing a delayed chlorophyll deficiency.

Vegetative multiplication of five Dalmatian autochthonous grape cultivars *in vitro*

D. Hartl, P. Males and S. Jelaska

Institute for Adriatic Agriculture, University of Split, Put Duilova 11, YU-58000 Split, Yugoslavia

A b s t r a c t : Possibilities of micropropagation of Debit, Plavac mali, Plavina, Posip and Vugava cultivars were investigated.

Explants for culture were prepared from the sterilized shoot apices. All tested genotypes were successfully established in culture when placed on the medium containing the salts of Murashige and Skoog, 0.5 mg l^{-1} IAA, 0.1 mg l^{-1} BA and 3 % sucrose. Vigorous plantlets were consistently produced by transferring to media with BA (0.02-2.2 mg l^{-1}) and 0.1-0.5 mg l^{-1} IAA. The growth habit of the *in vitro* grown shoots exhibited three main characteristics of grape seedling morphology: lack of tendrils, spiral phyllotaxy and leaves lacking the lateral sinuses.

The goal of this work was to test the feasibility of *in vitro* propagation of grapevine. Furthermore, this method may be of significance for virus elimination of infected plants.

Elimination of virus diseases by *in vitro* culture

G. Staudt and H.-H. Kassemeyer

Staatliches Weinbauinstitut Freiburg, Merzhauser Str. 119, D-7800 Freiburg, F. R. Germany

Abstract: Arabis mosaic virus (ArMV) and raspberry ringspot virus (RRV) are causal agents of grapevine fanleaf disease, one of the most damaging virus diseases of grapevines occurring in Germany. A simple method has been developed to eliminate the viruses using *in vitro* propagation of single nodes.

Nodes from ArMV or RRV-infected vines were micropropagated to complete plants and cultivated in a modified Murashige-Skoog medium. The position of individual nodes on their mother plant was documented. For any new subculture, the plants were dissected to single nodes when they reached a certain length. These were cultivated separately. After three *in vitro* subculturings, the plants were transferred to pots with a substrate containing a disinfected loess/sand mixture and cultivated under greenhouse conditions.

After each *in vitro* subculture and 18 months after the beginning of the *in vitro* culture, the resulting plants were tested using ELISA for the presence of ArMV and RRV, respectively. After the first and second subculture, a certain number of plants already showed no detectable viruses. After 18 months, no correlation could be detected between the origin of the plant from any particular node and virus elimination.

The results suggest that this *in vitro* culture method makes elimination of ArMV and RRV possible within 18 months without any thermotherapy.

Agrobacterium-mediated transformation of grapevine (*Vitis vinifera* L.)

V. Guellec [1]), C. David, A. Petit, J. Tempe and M. Branchard

[1]) L.A.P., UA 115, Bt 360, Université de Paris-Sud, F-91405 Orsay Cedex, France

Abstract: Genetically transformed grapevine (*Vitis vinifera* L.) roots were obtained after inoculation of *in vitro* grown whole plants (cv. Grenache) with *Agrobacterium*. The strain used contains two independant plasmids: the wild type Ri-plasmid pRi 15834 (agropine type) and a Ti-derived plasmid which carries the neomycin phosphotransferase II gene (NPTII) and the nopaline synthase gene. Expression of the NPTII gene can confer kanamycin resistance to transformed plant cells. Axenic root cultures derived from single root tips were obtained. Opine analysis indicated the presence of agropine and/or nopaline in established root cultures. For one of the cultures the presence of Ri and Ti derived DNA was confirmed by Dot-blot hybridizations of genomic root DNA with pRi 15834 TL-DNA and the NPT II gene. Callogenesis was induced by subculturing root fragments on Murashige and Skoog (1962) medium supplemented with benzylamino purine (0.2 mg/l) and indoleacetic acid (0.5 mg/l).

Vitis sp.: Somatic embryos obtained on medium inducing calcareous chlorosis

M.H. NETZER and M. BRANCHARD

L.A.P., UA 115, Bt 360, Université de Paris-Sud, F-91405 Orsay Cedex, France

A b s t r a c t : Somatic embryogenesis is one of the techniques used to screen for plants resistant to calcareous chlorosis. In the Plant Breeding Laboratory in Orsay, LEBRUN and BRANCHARD (1987) have obtained somatic embryos for different genotypes. Also, CHIADMI and BRANCHARD (1985) defined ferric and bicarbonic ions which are able to induce a resistance to calcareous chlorosis.

Anthers of diverse grapevine rootstocks, susceptible (Riparia Gloire, Rupestris du Lot, 3309 Couderc) and resistant (Fercal, 41 B) to calcareous chlorosis were cultivated in chlorosis inducing medium with increasing concentration of ferric sulfat and of potassium bicarbonate (from 50 to 1000 mg/l). For each replicate, 30 sterilized flowers were dissected and stamens were cultivated in an Erlenmeyer flask and maintained at 28-30 °C ± 1 °C on a rotary shaker in the dark for one month.

At present, the results observed in chlorosis medium indicate an important stamen necrose at high concentration. However, callus appearance is delayed when low concentrations are used. The first somatic embryos have appeared and have begun regenerating.

Grapevine shoot formation *in vitro*

J. KAROGLAN, N. MIROSEVIC and S. JELASKA

Faculty of Agriculture, Institute of Fruit Trees, Grapes, Oenology and Horticulture, Kaciceva 9, YU-41000 Zagreb, Yugoslavia

A b s t r a c t : In studies on *in vitro* propagation of *Vitis vinifera* L. (cvs Chardonnay, Pinot white, Sultanina, Plavac mali and Plavac mali sivi, a spontaneous variant), shoot explants, 1-2 mm long, were grown on media with different types and concentrations of growth regulators, macronutrients and carbohydrates.

Excised buds from canes, collected in March and held in water and in a controlled laboratory environment, were the source of explants. Very successful surface sterilization of buds was achieved with 1.5 % Izosan (chlorine product, Pliva, Zagreb) and 0.01 % Tween 20 for 15 min, and rinsed in sterile distilled water (3 x 5 min).

Two basal media with macronutrients according to MURASHIGE and SKOOG (MS), full and half strength, and LLOYD and MCCOWN (WPM) supplemented with 2 % sucrose, 0.8 % Difco Bacto agar and (mgl^{-1}): 1 thiamine : HCl, 0.5 pyridoxine : HCl, 2 glycine, 100 myo-inositol were tested. Cytokinin BA and auxins IAA and NAA in different concentrations and combinations were studied. The full strength MS medium with 1 mgl^{-1} BA and 0.3 mgl^{-1} IAA gave the best results in establishing multiple shoot cultures.

Effects of cultivar and time length in culture on multiplication rate, leaf shape deviation and vitrification was analysed.

Embryogenic cell lines from somatic embryos of grape (*Vitis vinifera* L.)

N. MATSUTA and T. HIRABAYASHI

Fruit Tree Research Station, Tsukuba, Ibaraki 305, Japan

Abstract: Somatic embryo formation occurred on leaf callus of grape (*Vitis vinifera* cv. Koshusanjaku). An embryogenic callus was induced from somatic embryo clusters cultured on vitamine-, inositol- and glycine-free NITSCH and NITSCH (1969) medium supplemented with 1.0 μM 2,4-D. This callus has retained a high embryogenic activity after repeated subculture on the same medium for over 2 years and has produced numerous embryos after transfer to a hormone-free medium.

The effect of cytokinin treatment on somatic embryogenesis from leaf callus was also examined. N-(2-chloro-4-pyridyl)-N'-phenylurea (KT-30) and N-(1,2,3-thiadiazol-5-yl)-N'-phenylurea (TAG), both synthetic cytokinins, were found to be effective for the induction of somatic embryogenesis. When leaf callus was induced by these cytokinins combined with 2,4-D at either 5.0 or 10.0 μM, somatic embryos were produced.

The regeneration of plantlets from culture anthers picked from anther culture derived plants in grape *in vitro*

ZI YI CAO, WEI LI, CAI YUE GUO and YU SHU QI

Gansu Agricultural University, Wuwei, Gansu, China

Abstract: The induction of plantlets by grape anther culture is difficult because only a few genotypes can induce plantlets from anthers and the frequency of plant regeneration is very low. The goal of the present study is to determine if the frequency of plantlet regeneration can be increased by the culture of anthers picked from the anther culture derived plants as it does in cereal crops. The grape inflorescences of V70 line, a line derived from the anther culture, which were in uninucleate pollen stage and the flower buds just separated, were inoculated on the B5 medium supplemented with 0.5 ppm 2,4-D, 2 ppm BA, 3 % sucrose, and cultured under 28 °C ± 2 °C for 1-1.5 months, and then transferred on regeneration medium (B5 supplemented with 4 ppm BAP and 0.2 ppm NAA). After 4 months, the embryoids regenerated from the calli of anthers, and plantlets formed when the embryoids were transferred onto shoot regeneration medium. The results showed that the frequency of plantlet regeneration from anthers picked from anther culture derived plants is higher than that from anthers picked from the stock plants. Thus, crosses between genotypes which can be induced to form plants from anther culture and others which cannot will possibly increase the inducible materials.

Section 6: Clonal selection

Effects of *in vitro* gamma irradiation on two grapevine cultivars (*Vitis vinifera* L.) [1])

P. Rosati, O. Silvestroni, C. Intrieri and G. Murri

Istituto Coltivazioni Arboree, Università degli Studi, Via Filippo Re 6, I-40126 Bologna, Italy

Summary: The acute γ-irradiation (0, 20, 30, 40 Gy) of two white wine grape varieties, Trebbiano Romagnolo and Albana, during *in vitro* proliferation was tested. Trebbiano R. had a higher *in vitro* proliferation rate, but also showed a higher number of cultures with vitrification and morphological abnormalities. Albana withstood a maximum dose of 30 Gy, while Trebbiano R. withstood the highest dose used.

Among the field planted vines, variants with shorter internodes were found in the first 2 years after planting.

Key words: tissue culture, irradiation, mutation, breeding, variety of vine, shoot, malformation, internode length.

Introduction

Mutation breeding has been applied to grapevine seeds and somatic tissues, as to other fruit species, using both physical and chemical mutagens. After irradiation, two or more vegetative multiplications are required for the induced mutations to become evident (Lapins 1983).

By combining irradiation of proliferating *in vitro* cultures with micropropagation, the time required for vegetative multiplication can be reduced from 2-3 years to a few months. For this reason, the two techniques have been applied to two Italian white wine grape varieties, Trebbiano Romagnolo and Albana (*Vitis vinifera* L.). Both of these cultivars are important and well established in North Italy Po Valley, where their grapes are mainly used to produce appellation wines (VQPRD). These varieties normally require cane pruning since their basal bud fruitfulness is low. An improvement in their basal bud fertility would enable growers to adopt spur-pruned training systems. In addition, both cultivars have very long internodes, while short internode variants, with more erect growth habit, would also be desirable. Finally, Trebbiano R. has tight bunches and looser bunches would decrease the incidence of the bunch rot in years of adverse weather conditions.

Material and methods

Culture establishment

In vitro cultures of the two wine grape cultivars Trebbiano R. and Albana were initiated in the early spring (1985). Individual buds were aseptically excised from uninodal cuttings that were collected in the field while still dormant and forced in greenhouse.

The standard proliferation medium (SPM) was composed as following: Murashige and Skoog (1962) mineral salts and vitamins (MS) with the addition of 2 mg/l benzylaminopurine (BA), 30 g/l sucrose, 7 g/l agar (Bacto-Difco). The pH was adjusted with 0.1 N KOH to 5.7 before autoclaving for 20 min at 120 °C.

Initially, the subculture interval was 1 month for both cultivars. Cultures were maintained in a growth room at a temperature of 23 ± 1 °C, 16/8 h (light/dark) photoperiod with a PPFD of 30 μmol $m^{-2}s^{-1}$. The explants were at first individually grown in test tubes (20 x 150 mm) and later

[1]) Research funded by the Italian Ministry of Education.

transferred into 500 mm glass jars with metallic screw caps which were filled with 70 ml of medium and 10 cultures each.

To improve the unsatisfactory proliferation results of Trebbiano R., the subculture interval was shortened from 4 to 2 weeks and the SPM modified by using 1) different hormone combinations and 2) a double layer culture system with the MS salts of the liquid medium reduced to half and BA 0.5 mg/l applied over solid medium.

Irradiation

Irradiation was performed in cooperation with the FARE Department of ENEA (Casaccia, Rome) by applying γ-rays from a ^{60}Co source. The following acute treatments (varied length of jar exposition, 1.53 Gy/min) were applied to proliferating and still actively growing cultures in 500 ml glass jars: control (no irradiation), 20, 30, and 40 Gy. Cultures were irradiated 10 d (Trebbiano) and 20 d (Albana) after transplant on SPM.

Vegetative multiplication after irradiation

The cultures were transferred to fresh SPM 2 d after irradiation. A different protocol was then followed for the two cultivars.

Albana: When irradiated tufts were first subcultured (MVO-MV1; Lacey 1984), the longer shoots (> 15 mm) were individually placed in test tubes on SPM (to induce basal buds proliferation) and the shorter ones on a medium having the same composition but without hormones (to obtain shoot elongation). In the subsequent two subcultures only SPM was used.

Before transfer on the rooting medium, the shorter shoots were placed for one subculture on an elongation medium having the same composition as the SPM but with BAP reduced to 0.2 mg/l.

Trebbiano R.: In the first subculture after irradiation (MV1) all shoots, irrespective of their length, were placed in 500 ml jars on SPM medium. Unlike Albana, a 5 mm layer of liquid medium (composition cited above) was added on the top of the gelified medium to ensure proper nutrition. No elongation treatment was applied to short shoots since we had observed in previous trials that a high BA concentration was essential for satisfactory growth.

In proliferation, at the end of each subculture, the following data were recorded for both cultivars:

1) Number of living and dead cultures.
2) Proliferation rate (final/initial number of shoots), total length (mm) and node number of each culture.
3) Number of green, yellow, brown and vitreous cultures.
4) Number of normal and morphologically abnormal (leaf margin and/or blade) cultures.

After counting, brown and vitreous shoots were discarded as well as many shoots of the control to prevent an excessive number of control vines.

The MV3 shoots (fourth division after irradiation) were transferred on rooting medium having the following composition: MS mineral salts (reduced to half-strength and vitamins, naphthalene acetic acid (NAA) 0.5 mg/l, sucrose (commercial) 20 g/l, agar (Japan, unlabelled) 6.5 g/l.

The rooting phase *in vitro* occurred in the same growth room as the previous proliferation phase. After 20 d, the rooted cuttings were counted and transferred to soil (weaning), the dead cultures counted and discarded, and the unrooted cuttings placed on a fresh rooting medium after a renewal of the basal cut. After a further 20-d rooting period, the counting was repeated and the results expressed as the sum of the two rooting cultures.

Soil establishment and field evaluation

Rooted cuttings were transferred to a peat, sand and perlite (1 : 1 : 1) mixture in plastic containers in a growth room at 23 ± 1 °C and PFD 100 μmol $m^{-2}s^{-1}$ where the relative humidity was

kept as high as possible for the 1st week. 40 d after transfer, culture survival was expressed as percentage of the initial number of plantlets. At the end of one full growing season in a nursery bench (1987), the developed plants were transferred to the field and cut back to two buds. The next winter (1988-89), the vines were again cut back to two buds. At the end of August 1988 and 1989, total shoot length (m) and number of nodes were recorded for each field planted vine.

Data concerning rooting and soil establishment were statistically analyzed with chi-square test, and linear regression calculated for the association shoot length and internode number of field-grown vines.

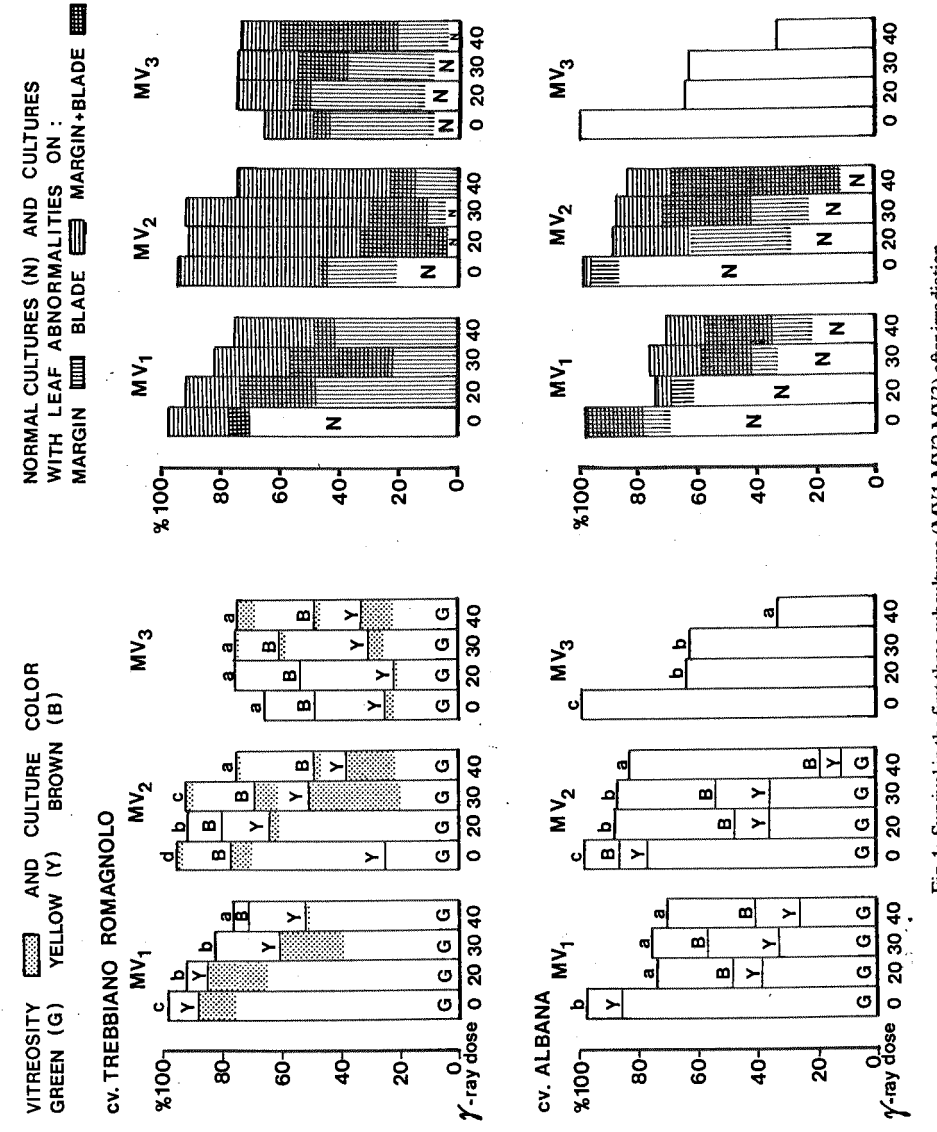


Fig. 1: Survival in the first three subcultures (MV1-MV2-MV3) after irradiation.

Results

The *in vitro* cultures of Trebbiano R. had more problems than those of Albana. 15 months from initial explant were needed to obtain a sufficient number of uniform green Trebbiano R. shoots for irradiation and only 6 months for Albana. SPM induced the best proliferation and survival for both cultivars, however, the subculture interval for Trebbiano R. had to be reduced from 4 to 2 weeks.

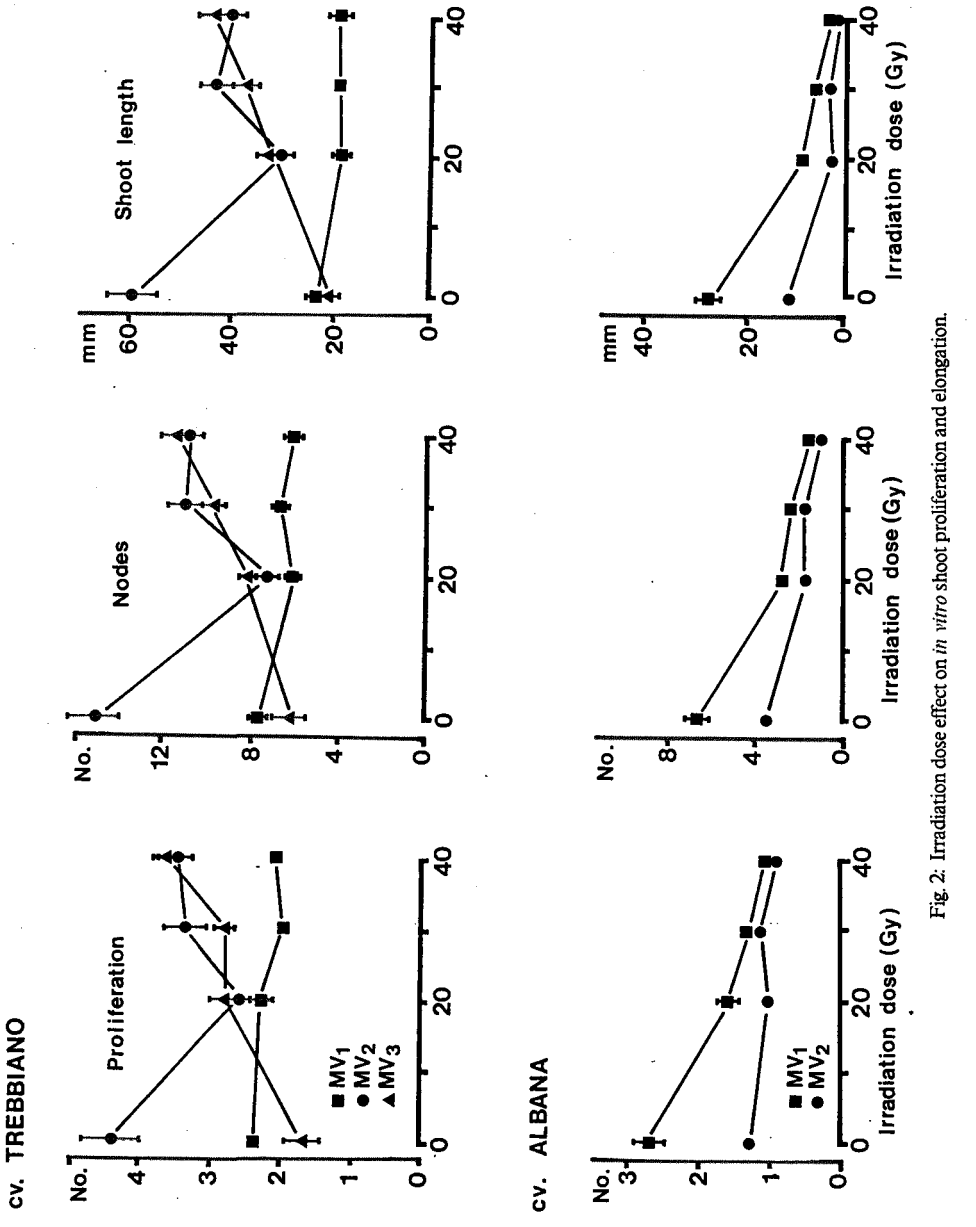


Fig. 2. Irradiation dose effect on *in vitro* shoot proliferation and elongation.

Irradiation dose effect on *in vitro* shoot proliferation and rooting. Vines transferred to weaning, nursery and field

γ-ray dose Gy	In-vitro shoots MVO No.	MV3 No.	Rooted %	Vines transferred to: Weaning No.	Nursery No.	Field 1988 No.(z)
cv.TREBBIANO ROMAGNOLO						
0	154	45[y]	100 c	45	36	19
20	93	234	86 a	202	87	21
30	106	235	92 a	215	48	0
40	72	177	98 b	173	107	23
cv. ALBANA						
0	50	85[y]	100 b	85	68	16
20	252	114	72 a	82	54	54
30	194	132	67 a	88	52	52
40	166	13	61 a	8	7	7

Mean separation within cultivars, chi-square test, P=0.05

(y) Only a few of the control shoots were maintained

(z) Several Trebbiano R. vines were field planted only in 1989

Proliferation and survival

Fig. 1 shows the effects of irradiation. Culture survival decreased as the doses increased except in Trebbiano MV3; the phenomenon was particularly evident in Albana MV3.

Vitrification occurred in all Trebbiano R. treatments and subcultures, but was not present in Albana. Leaf browning appeared in MV1 in the latter cultivar and its percentage increased in MV2 particularly at 40 Gy. In contrast, leaf browning was found in Trebbiano R. starting from MV2 with no differences between irradiation doses.

Abnormalities in leaf margin and/or blade were higher in Trebbiano R. (reaching 100 % in irradiated MV1 material). In Albana, abnormalities increased along with doses and from MV1 to MV2 (Fig. 1).

In light of the proliferation and shoot elongation data, the different response to irradiation and tissue culture of the two cultivars was evident (Fig. 2). Proliferation in Trebbiano R. was higher than in Albana, which later showed a constant decrease of values from control to Gy 40. Irradiation had a depressive effect in Trebbiano R. MV1 and MV2, while in MV3 it stimulated proliferation.

Mortality, shoots discarded for browning and low proliferation rate, lowered the final number of transferred Albana shoots below the initial one, particularly at Gy 40. In contrast, the final number of Trebbiano R. shoots was higher than the initial number, mainly because of the high proliferation rate, irrespective of the irradiation dose (Table and Fig. 2).

Irradiation, as expected, decreased rooting in both cultivars, but its effect was less marked in Trebbiano R. Albana had the best weaning results as shown by the higher percentage of vines transferred to nursery (Table).

Field growth

Some variants (4 of Trebbiano R. and 4 of Albana) for vegetative characters were found in vine populations in 1988, 5 of them with longer and 3 with shorter internodes. In 1989, only variants with shorter internodes were observed (1 in Trebbiano R. and 6 in Albana); 1 of them, in Albana, performed exactly as in the 1st year. In 1989, the vines showed longer shoots because of longer internodes as compared to 1988 (Fig. 3).

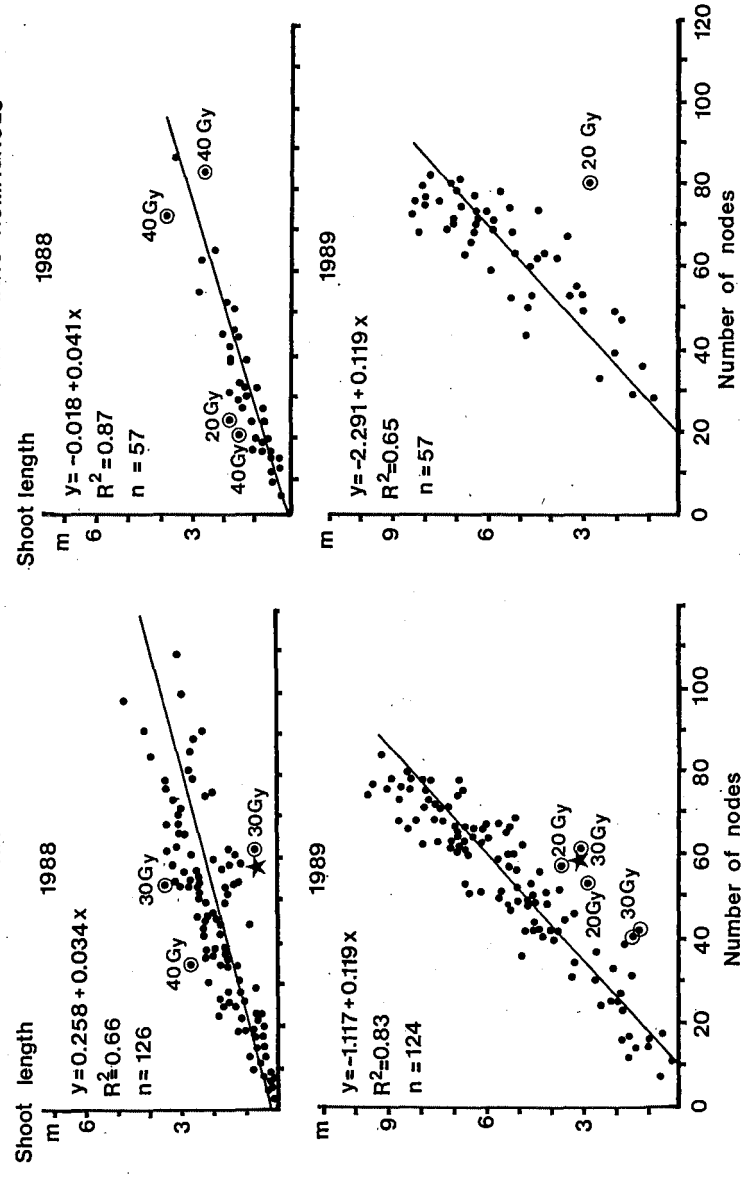


Fig. 3: Correlation between number of nodes and shoot length for field-grown Albana and Trebbiano R. grapevines in 1988 and 1989.

Discussion and conclusions

The response of the two grapevine cultivars to tissue culture on the same medium was different and not completely satisfactory. Trebbiano R. had a good proliferation rate which should have meant a successful *in vitro* propagation. But the vitreousity, leaf yellowing or browning and morphological abnormalities clearly indicated that the adopted medium can be improved. For example, vitrification has been linked to metabolic perturbations induced by chemical or physical factors related to medium composition (DEBERGH *et al.* 1981; PAQUES and BOXUS 1987; PASQUALETTO *et al.* 1988). In addition, Albana evidenced low proliferation rate which may be enhanced by increasing the cytokinin concentration as is done in other grape cultivars (HARRIS and STEVENSON 1982).

The two tested cultivars also proved to have a different sensitivity to irradiation: Albana withstood a maximum dose of 30 Gy while Trebbiano R. withstood the highest dose used (40 Gy). These results concur with the findings on irradiation *in situ* of dormant grapevine material in which doses ranging from 50 Gy (REICHARDT 1955) to 30 Gy (DONINI 1975) were suggested. *In vitro* irradiation of grapevine required a lower or similar dose than that applied *in situ*, as suggested for other top fruit species (LACEY and LENNARD 1977; PREDIERI *et al.* 1986).

Despite the difficulties encountered, the techniques employed yielded in a short time weaned and field-transferred grapevines (January 1988). In these plants, the only vegetative trait that could be considered during 1988 and 1989 was the internode length. The first season after transplant to the field, variants with longer and shorter internodes were found. The former disappeared the following season.

A variant of Albana, isolated from 30 Gy irradiated material, exhibited shorter internodes in both seasons, differing significantly from the mean of the population observed. Further observations of this and of the other variants will be needed over the next few years to confirm these preliminary results. Fruiting of the irradiated vines in 1990 will also allow the evaluation of productive traits.

References

DEBERGH, P.; HARBAOUI, Y.; LEMEUR, R.; 1981: Mass propagation of globe artichoke (*Cynara scolymus*): Evaluation of different hypotheses to overcome vitrification with special reference to water potential. Physiol. Plant. **53**, 181-187.

DONINI, B.; 1975: Improvement of vegetatively propagated plants through induced mutations. Proc. Res. Coordin. Met. Tokai, 1974, IAEA, 35-51.

HARRIS, R. E.; STEVENSON, J. H.; 1982: *In vitro* propagation of *Vitis*. Vitis **21**, 22-32.

LACEY, C. N. D.; LENNARD, J.; 1977: Apple meristem culture. Long Ashton Res. Stat. Rep. 1976, 21.

LAPINS, K. O.; 1983: Mutation breeding. In: MOORE, J. N.; JANICK, J. (Eds.): Methods in Fruit Breeding. Purdue University Press, West Lafayette, Indiana.

MURASHIGE, T.; SKOOG, F.; 1962: A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. **15**, 473-497.

PAQUES, M.; BOXUS, P.; 1987: 'Vitrification': review of literature. Symp. *In Vitro* Problems Related to Mass Propagation of Horticultural Plants. Acta Hort. **212**, 155-166.

PASQUALETTO, P. L.; ZIMMERMANN, R. H.; FORDHAM, I.; 1988: The influence of cation and gelling agent concentrations on vitrification of apple cultivars *in vitro*. Plant Cell Tissue Organ Cult. **14**, 31-40.

PREDIERI, S.; ROSATI, P.; FORNASINI, B.; 1986: Mutagenesi del pero in micropropagazione: messa a punto del metodo. Riv. Ortoflorofrutticolt. Ital. **70**, 369-379.

REICHARDT, A.; 1955: Experimentelle Untersuchungen über den Effekt von Röntgenstrahlen in der vegetativen Vermehrung einer alten Rebensorte. Gartenbauwiss. **20**, 355-413.

Selection advance and environmental variance in clonal selection of the wine grape variety Kövidinka

EDIT HAJDU

Research Institute for Viticulture and Oenology, H-6000 Kecskemét, Hungary

Summary: The wine grape variety Kövidinka has been cultivated in Hungary for centuries. Clones were obtained using the 4-step method of NÉMETH and some clones showed considerable selection advance. The performance of 2nd vegetative progenies of the selected clones were evaluated over 7 years (1981-1988) based on the following characters:

growth vigour of the stock	tendril atrophy
fertility of buds	millerandage
grape yield	berry rot
mean grape weight	sugar and acid content of the must

Mathematical evaluations of the characters can give useful information as to the performance of the clone stocks. In the years of the study, large environmental variation was observed in the tested characters. At the same time, the slight environmental variation found in some individuals indicated their genetic stability. They will be selected and propagated as sub-clones in the future.

Key words: variety of vine, Hungary, clone, selection, performance, modificability, mutation, genetics.

Introduction

In the selection of wine grape clones, breeding work is greatly encumbered by environmental effects. In natural surroundings, grapevine stocks cultivated during centuries in the same area tend to change in morphology and inner characters due to selection pressure. The stand turns heterogeneous with one stock becoming weak and susceptible to diseases and with the other maintaining its original character (BALINT 1977; VIDA 1981). But some stocks can also be found which exhibit positive changes. Changes are expressed in the phenotype and, if fixed in the genotype and manifested through generations of clones, they could be very valuable.

Whether the positive properties, expressed as morphological or internal characters of a clone, are caused by modifications or changes fixed in the genotype (mutation), can be proved by clone selection of several steps.

In our Institute, clonal selection has been performed with good success in table and wine grape varieties for several decades (HAJDU 1980, 1988; FÜRI and NÉMETH 1972).

Of the cloned varieties, I want to present the effect of some environmental factors on the wine grape variety Kövidinka on the mean of years and clones, first within the population and then at stock level.

Material and method

The cloned variety Kövidinka is usually cultivated in the largest wine district of Hungary, on the Great Plains, corresponding to 45 % of the vine area of the country. The variety is well adapted to extreme conditions. It can be grown on sandy soils under arid conditions. It is productive, sensitive to frosts but regenerates easily. Both primary and secondary buds are fertile. It yields an ordinary white wine.

Clonal selection began in 1963 (KWAYSSER and KISHONTI 1965); following the 4-step clonal selection method of NÉMETH was used (NÉMETH 1967; HAJDU 1980,). In the past 25 years, 3 valuable clones (8/59, 10/60, and 11/59) were selected and propagated from the mother stocks (HAJDU 1988). Stock performance of the selected clones was studied in the 2nd vegetative

generation during 7 years from 1981 to 1988 in a row design. In the year 1987 frost damage thwarted evaluation. High cordon training and Sylvoz pruning method was used with 12-14 buds/m^2. The trial site was level with a sandy soil.

In the clone and control stocks the following parameters were studied:

growth vigour and health of stocks	tendril atrophy
bud fertility	millerandage
cluster yield	rot of berries
mean cluster size	sugar content and acidity of the must

The data obtained give useful information as to the performance of stocks and correlation of the characters studied.

Results

High environmental variations were observed in the characters studied between the different years. Stocks were symptomatically healthy. Several stocks were found which varied little when exposed to different environmental effects, that is, they were genetically stable.

Evaluation means and deviations are represented in Figs. 1-7 to simplify their analyses.

Fig. 1 shows the performance of the selected Kövidinka clones with 60 stocks/clone related to the control in correlation of 9 characters and averaged over 7 years. Besides qualitative and quantitative mean values, their deviations were also taken into account. In most characters the clone 8/59 was superior. Here deviations showed variations in stock performance on the mean of years.

Fig. 2 represents the yearly variation of the studied characters with means calculated from all the stocks in the clone trial. Stock means and deviations are given for every year. In most characters, high variations were observed between years. The absolute bud fertility coefficient was relatively stable, independent of years. The growth vigour of stocks was uniform except in 1988 when winter frosts intervened. Millerandage, rot and tendril atrophy varied highly in the years. Mean cluster weight did not follow that high variation. Fig. 2 reveals clearly the year when high values were found for a studied character. That year is also most efficient for selection. For

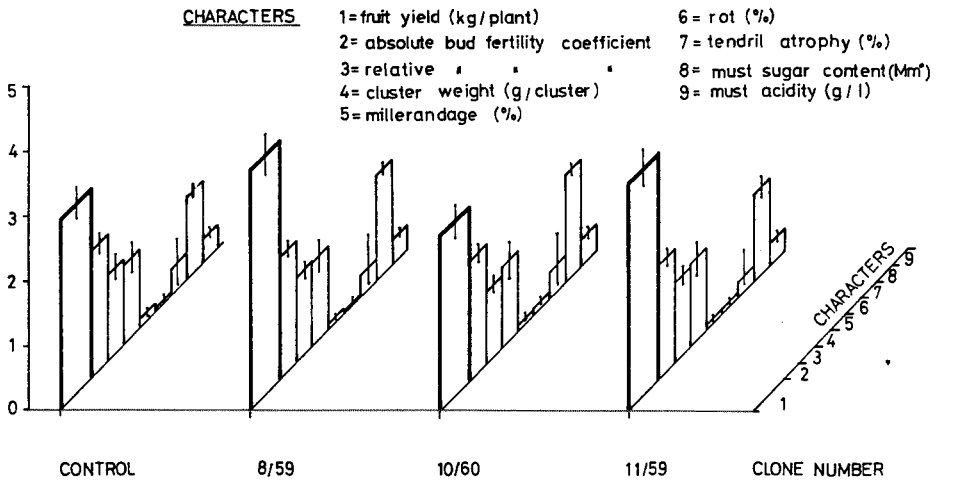


Fig. 1: 7-year means and distribution of qualitative and quantitative characters in Kövidinka clones and control. Kecskemét-Miklóstelep.

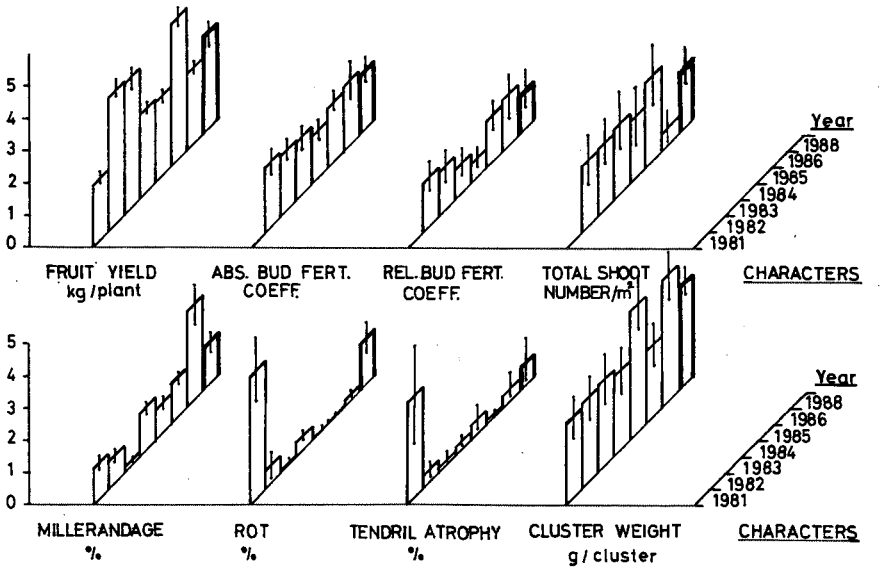


Fig. 2: Changes and distribution of quantitative characters in the mean of Kövidinka clones and control during 7 years. Kecskemét-Miklóstelep.

instance: for cluster weight, bud fertility, cluster size and stock vigour, 1986 and 1988 proved to be very advantageous. For selection for millerandage and tendril atrophy, the years 1981 and 1986 were suitable. That is, it was in those years that highest mean and deviation values were observed in the studied characters.

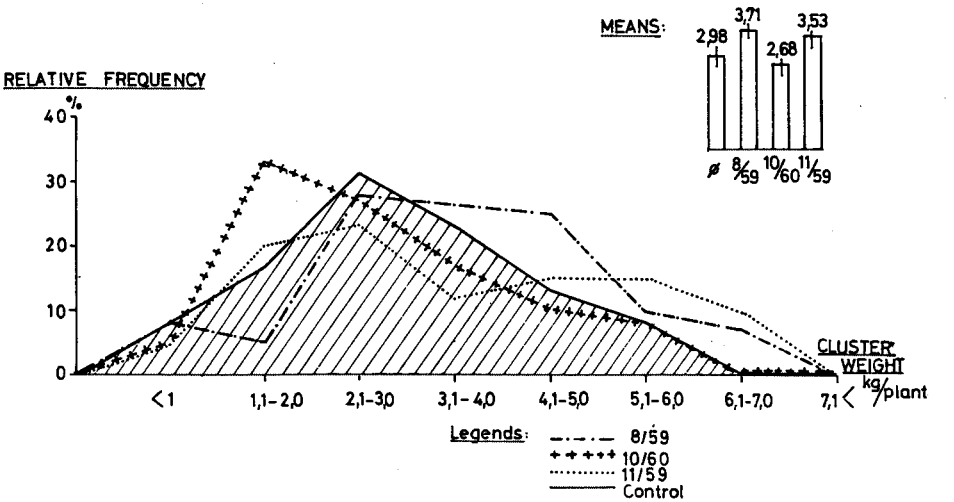


Fig. 3: Plant distribution of Kövidinka clones and control based on 7-year yield data. Kecskemét-Miklóstelep.

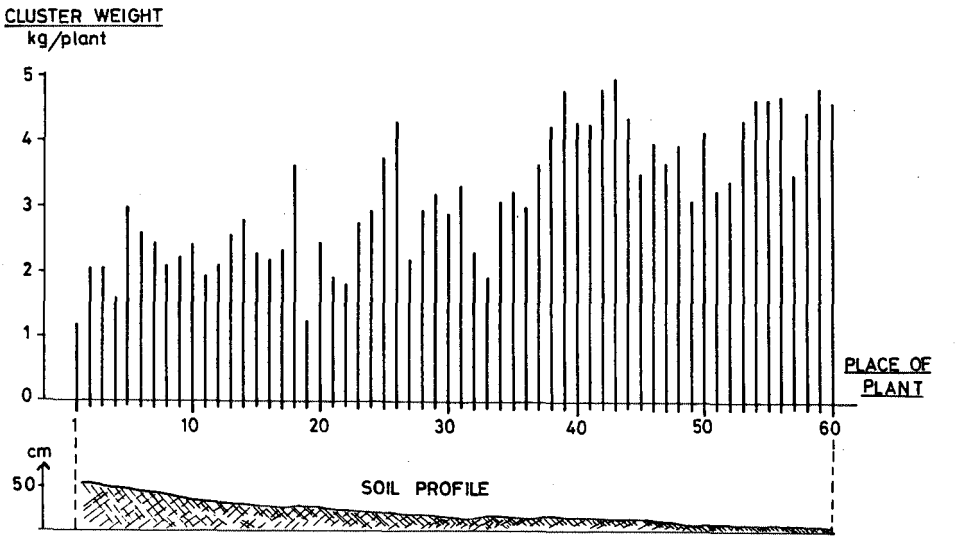


Fig. 4: Cluster weight of Kövidinka plants in the mean of 7 years and clones depending on their place in the trial. Kecskemét-Miklóstelep.

Fig. 3 shows the distribution of stocks according to cluster yield within clones and control populations, respectively. Related to control data, the highest selection advance was obtained in the clone 8/59 and 11/59, respectively. In both clone populations, more stocks of high yields were found than in the control or in the clone 10/60.

Evaluating the 7-year means of cluster yield/stock according to the place of stocks within the rows of the trial area, correlation was found between soil surface condition and cluster yield on a

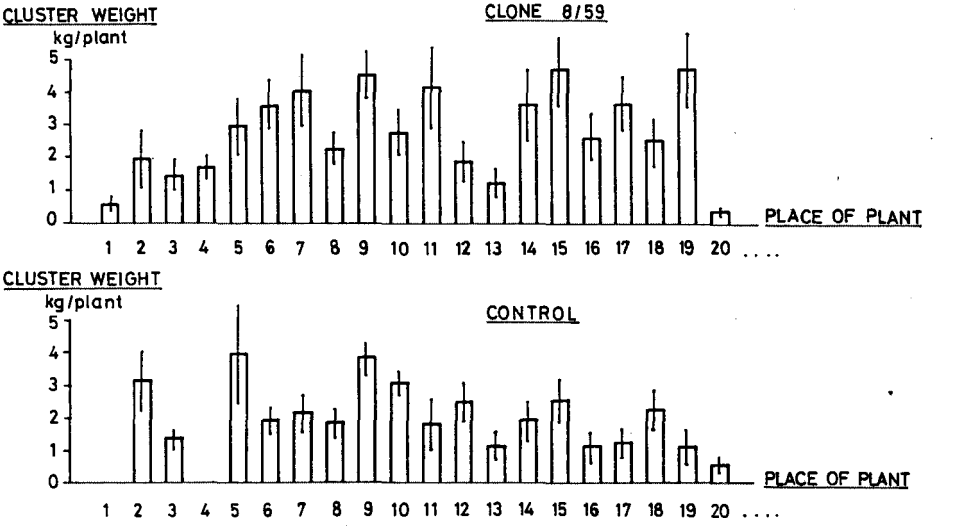


Fig. 5: Cluster weight mean and distribution of Kövidinka 8/59 clone and control depending on the place of the plants. Kecskemét-Miklóstelep.

homogeneous soil with slight slope (0.3 % slope). The lines in the upper part of Fig. 4 represent cluster yield and those at the bottom the soil slope. The effect of soil surface level was evaluated for each character, correlation is, however, most convincingly manifested in cluster yield. Stocks situated deeper in a different microclimate may have a better access to water table and accordingly superior nutrient utilization.

The cluster yield of stocks was analysed as to their place in the row. In Fig. 5 we can see the mean yield and deviation values of the clone 8/59 and that of the control from the 1st to the 20th stock, one after the other, in the row. Of course, the best stock is the one with the highest mean values and lowest deviation, that is, with a consistent yield. In this clone, more highly productive stocks can be found than in the control.

The relative frequencies of cluster weight for the entire stand in the clone trial are indicated in Fig. 6. Yield means in the 7 years are grouped according to their frequency. Below the principal means, the cluster yield of 5 stocks in the clone 8/59 and that of 5 control stocks are given for each year. Data help to choose stocks which produce a stable number of clusters – low or high – independent of environment.

Besides climatic conditions, nutrients play an important part in yield. In the clone trial, uniform nutrient supply was provided. We wanted to establish whether there was a difference between nutrient uptake and utilization in these stocks. Leaf samples were taken 3 times from each stock. Unfortunately, data of only 1 year are available.

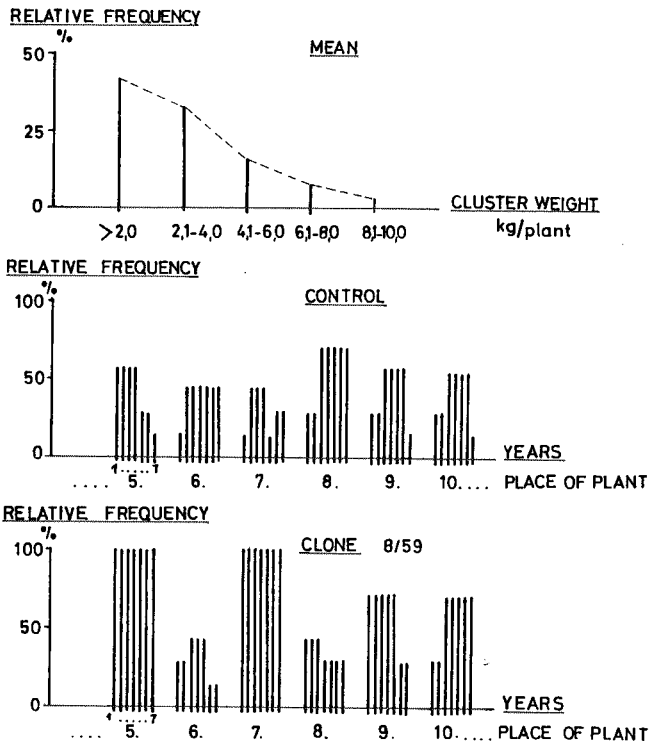


Fig. 6: Distribution of Kövidinka clone 8/59 and control based on their cluster weight depending on the place of plants over 7 years. Kecskemét-Miklóstelep.

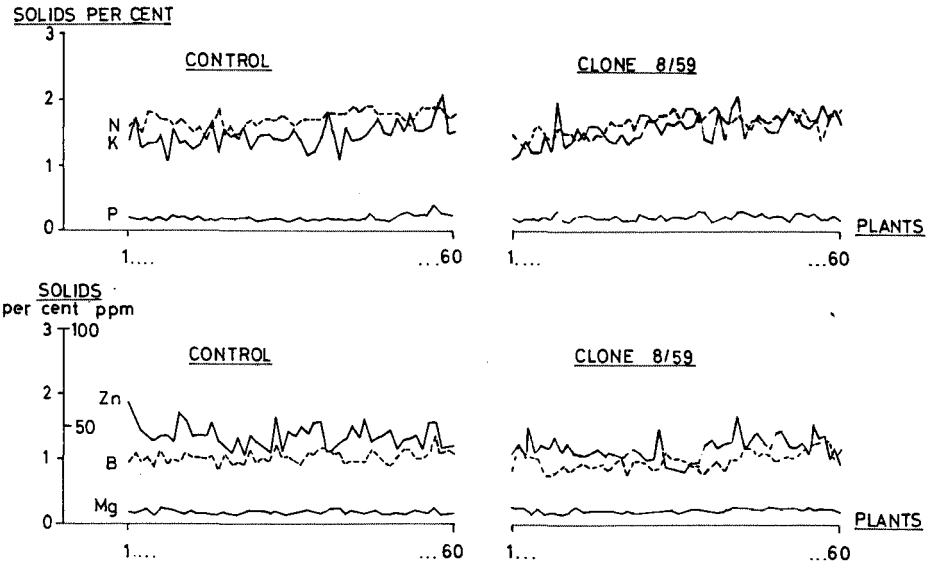


Fig. 7: Leaf analysis results of Kövidinka clone 8/59 and control plants in 1988. Kecskemét-Miklóstelep.

Values of the most important nutrients, N, P, K, Mg, B and Zn, are shown in Fig. 7. Zn and Mg uptake of stocks was almost identical while that of N, P and, particularly, K varied. The phenomenon can be explained by differences in the root system and assimilation. It is very interesting over the life of a stock or a clone. There are individuals of good nutrient uptake and utilization and individuals of poor nutrient uptake and utilization.

Conclusion

The statements are based on observations of the wine grape variety Kövidinka in the 3rd step of clone selection where the 2nd clone generation of selected mother stocks was studied for 10 characters. In the entire plantation, clones were superior to the control in every character. When studying single stocks, high environmental variance was found for several characters in the mean of 7 years, but only the evolution of cluster yield is presented. Depending on environment (year, soil conditions) typical phenotypic variations were observed.

Under identical environmental conditions, the stability of phenotypic variance in the stocks assists in selecting individuals of superior genotype. A thorough knowledge of the stocks and the elimination of environmental variances will allow the selection and propagation of the best clones. Phenotypic variances due to environmental changes and genotypes can also be evaluated in other wine grapes.

Literature

BALINT, A.; 1977: Az öröklés- és származástan alapjai. Mezögazdasági Kiadó, Budapest. 310-311.

FÜRI, J.; NÉMETH, M.; 1972: Stand der Leistungsselektion bei der Rebe in Ungarn. Wein-Wiss. 27, 79-96.

HAJDU, E.; 1980: Genetikai és növényegészségügyi változások a szölö klónszelekciójánánál. Doktori értekezés. Gödöllö. 45-48.

---; 1988: A Kövidinka borszölöfajta klónozásának eddigi eredményei. Kertgazdaság. Budapest. 20, 47-57.

KWAYSSER, I.; KISHONTI, A.; 1965: Borvidákeink legfontosabb szölöfajtáinak klónszelekciója. Témabeszámoló. SZBKI. Kecskeméti Kutató Allomásának évi kutatási jelentése. Kecskemét, 95-99.

NÉMETH, M.; 1967: A klónszelekciós szölönemesités módszere Pécsett és az elért eredmények. Szölö- és Gyümölcstermesztés, Budapest. Vol. III, 41-83.

VIDA, G.; 1981: Az evolueiós genetika alapjai. Evolúció I. Natura Kiadó. Budapest. 317, 61-70.

Progress in mass and clonal selection of grapevine varieties in Portugal

A. MARTINS [1]), L. C. CARNEIRO [2]) and R. CASTRO [1])

[1]) Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, P-1399 Lisboa Codex, Portugal

[2]) Estacao Agronómica Nacional, Quinta do Marquez, P-2780 Oiras, Portugal

S u m m a r y : Since 1978 we have developed methods for mass and clonal selection of grapevine well adapted to the conditions of Portuguese viticulture. The most remarkable innovation of this methodology is the establishment of large experimental populations of clones in which good estimates of yield heritability and genetic gain can be obtained.

The aim of the present paper is to clarify some methodological aspects such as (1) the ideal composition and structure of the experimental populations of clones and (2) the application of this methodology to a large number of Portuguese varieties, in order to maximize the rate of yield improvement. At present we can point out the following results:

1. According to experimental data and computer simulations, we may conclude that the optimal number of clones to be included in each population is about 200-300, each one represented by 4 vines in 5 replications. By this way we can usually obtain heritability estimates between 30 and 80 % and genetic gain values higher than 15 %.

2. Until now, we have succeeded in applying this methodology to 30 of the most important Portuguese varieties, which represent more than 80 % of all varieties normally propagated in the country.

K e y w o r d s : clone, selection, variety of vine, Portugal, yield, analysis, computer simulation, genetics.

Introduction

The methodology of selection followed in different countries usually comprises several cycles of selection from an initial cycle, with a high number of genotypes up to a final cycle in which a unique genotype is selected. Clonal selection may take from 20 to 25 years (SCHÖFFLING and BENDER-BERLAND 1983).

However, the genotypes selected during the first cycle may already be used by vinegrowers as mass vegetative material, supplying high yield gains in a very short time. In countries that have recently started systematic selection, such as Portugal, there is urgent need to obtain better propagation materials, making this procedure particularly attractive.

In order to develop this mass selection methodology we are introducing statistical and genetical instruments of analysis – heritability (h^2) and genetic gain (R) – which have not yet been used in clonal grapevine selection of other countries. These techniques are applied to data analysis of clone populations yield, which corresponds to the second cycle of the integrated selection methodology that has been used since 1978 (MARTINS *et al.* 1987).

The possibility of obtaining useful results with this data analysis depends on the factors included in the expression for calculation of the genetic gain $R = i \cdot s \cdot h^2$. Owing to the lack of bibliographic information on this subject, the main purpose of this paper is to obtain, using computer simulations, the most favourable values of these factors. In the second part of this paper, the application of the described methods to the most important Portuguese grapevine varieties selection and the obtained data will be discussed.

Material and methods

The initial data consisted of the yield of individual plants out of a population of 189 clones of cv. Periquita, set up in two blocks and two replications of seven plants with the following statistical

indicators: mean of first block – 1.972; mean of second block – 1.472; general mean – 1.722; standard deviation – 0.556; heritability – 0.378. Using as standard values the yield of this population, BASIC programs (VAX BASIC V3.2) have been written to generate populations of random numbers (corresponding to total fruit weight per vine) with estimated variation values – medium per block, variance of media and heritabiity (h^2) – similar to those of the Periquita population.

The subroutine that generates the individual weights is, according to the classical model, $Y_{ij} = m + g_i + b_j + e_{ij}$ and it has the following values:

$$\text{vine} = 1.722 + r(\text{rep}) + f(c) + \text{rnd} \cdot m(\text{rep}) - m(\text{rep})/2$$

(vine = individual vine fruit weight; r(rep) = between block variation; f(c) = genotypic variation; m(rep) = modified factor of individual variance according to the block).

The intensity of selection (i) values in the calculation of the genetic gain (R), has been obtained from the SMITH's (1969) formula:

$$i = 0.8 + 0.4 \cdot \log(1/P-1).$$

Results and discussion

The selected number of clones in an experimental population has a high influence on the estimated gain (through i). However, this number has to be chosen regarding other aims, such as: a guarantee of a good stability for the observed gains, a high level of genetic variability and a maintenance of the initial enological quality level of the grapevine variety.

All the statements mentioned above suggest that a high number of clones is needed, while the value of gain and management of the propagation procedure recommends low numbers. In an empirical approach, we have adopted numbers around 40 clones.

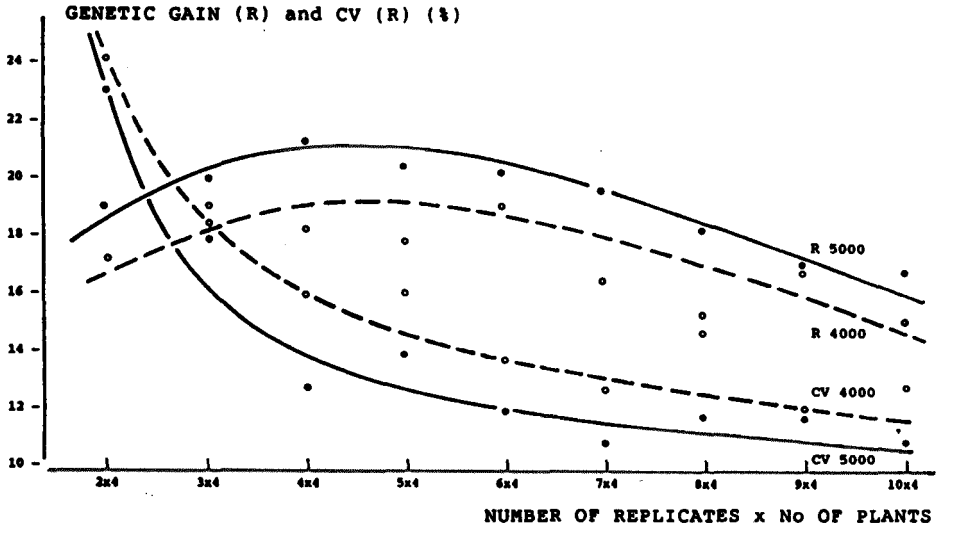


Fig. 1: Genetic gain (R) and coefficient of variation of the gain (CV(R)) when the number of replications varies from 2 to 10, the total numbers of plants in the experiment are 4000 or 5000 and the number of plants per replication is constant.

As far as the remaining gain factors are concerned, they depend on the number and the nature of the selected clones in the population and on the experimental design adopted. Moreover, some aspects of the experimental design adopted are due to some practical considerations that are independent from the methodology: (1) the size of plots (number of vines in a minor experimental unit) can hardly be less than 4, because this is a fairly small number and easy to limit in the field (half of the numbers of vines between two posts in our vineyard); (2) the total number of plants in an experiment which can hardly go beyond 4000-5000 (= 1.3-1.6 ha) otherwise it would drastically increase the environment variability and would make the completion of many experimental operations difficult.

According to what has been said, populations with areas of 1.3-1.6 ha (4000-5000 plants) having 4 vines per replicate, have been used in several computer simulations to better clarify the balance between the number of replications and the number of clones and to obtain the maximum rate of gain. We used the estimated gain (R) and the respective coefficient of variation among several experiments with the same structure as leading indicators of goodness of several types of experiments. The data corresponding to 1000 simulations are shown in Fig. 1.

The results show that the gain is maximum when experiments with 4-5 replications are considered, and the coefficient of variation decreases even more beyond this limit, although slowly. We may conclude that experiments with 5 replications of 4 vines each will give the highest and most accurate gain estimate (low coefficient of variation).

It may be verified that the genetic gains are higher and the coefficient of variation lower when the number of vines increases in the experiment. However, these data have to be carefully considered, as the environment variability increases when the area of the experiment increases, and this factor is not included in the simulated model which generated these data.

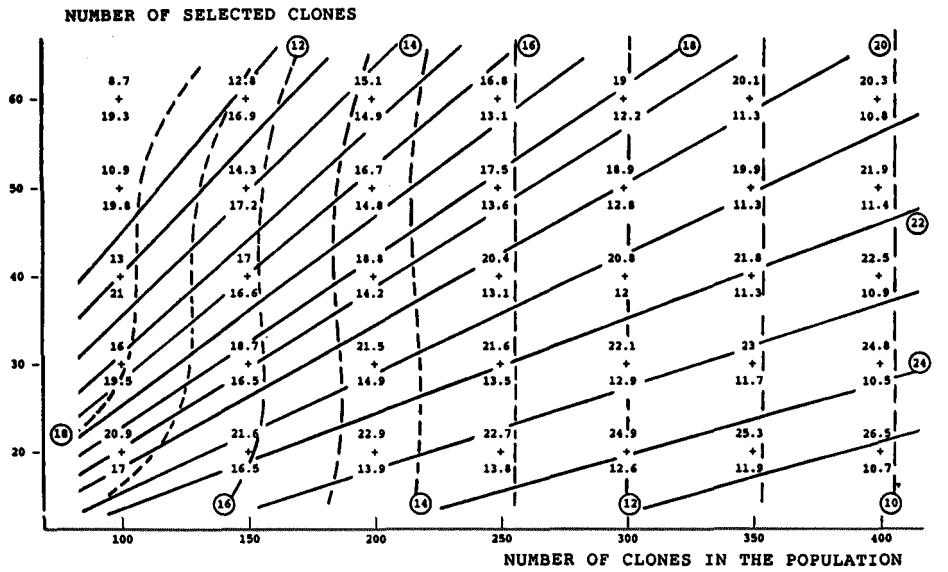


Fig. 2: Isolines of the gain (sloped lines, numbers above the chart) and of coefficient of variation of gain (vertical lines, numbers below the chart) as a function of the clones number in the experimental population and of the number of selected clones. Average data of 600 computer simulations regarding plots of 5 replications of 4 vines each (all values in the chart are in percent).

Estimate of heritability (h^2) and genetic gain of yield (R) in several experimental populations of clones

POPULATION-SITE, YEAR	No. CLONES POPULATION/SELECTED	MEAN	STAND. DEV.	HERIT. (h^2)	GENETIC GAIN (R)
Touriga-Douro,84	69 / 20	0.675	0.269	0.656	30.3 %
Periquita-Ribatejo,84	189 / 40	1.722	0.556	0.378	16.2 %
Touriga- Dão,85	49 / 20	1.777	0.712	0.780	29.6 %
Alvarelhão-Dão,86	32 / 20	2.429	0.658	0.738	11.9 %
Loureiro-V. Verdes,87	132 / 30	2.248	0.591	0.356	12.1 %
Touriga-Dão,87	52 / 20	3.354	1.047	0.805	24.8 %
Trajadura-V. Verdes,88	191 / 40	0.751	0.447	0.201	15.9 %
Vinhão-V. Verdes,88	211 / 40	0.697	0.351	0.386	26.8 %
Avesso-V. Verdes,88	164 / 40	1.553	0.427	0.228	8.8 %
Siria-Pinhel,88	239 / 40	2.282	0.799	0.777	39.2 %
Trincadeira-Ribatejo,88	272 / 40	0.413	0.136	0.587	29.1 %
Arinto-Oeste,88	266 / 40	0.522	0.268	0.650	49.8 %
Antão Vaz-Alentejo,88	210 / 40	1.465	0.322	0.423	12.8 %
Aragonêz-Alentejo,88	231 / 40	2.134	0.599	0.474	19.0 %

In a second set of computer simulations, the model structure was set to 5 replications of 4 plants each, and the number of population clones was varied as well as the number of selected clones.

The results are plotted in Fig. 2. We can observe that to the right hand of the chart the gain and the coefficient of variation of the gain isolines are progressively separating and the isolines of gain are also becoming flatter. In the population band of 250-300 clones we can visualize an area which divides the chart in two. On the left side the isolines are spaced closely with steep slopes, and on the right side they are separated by greater distances with shallow slopes. In reference to the numbers of clones, below 250-300 clones small increases of this number result in great increases of gain (R) and great decreases of coefficient of variation, above this limit the tendencies are reduced.

The data plotted in Figs. 1 and 2 suggest that populations of 250 clones and 5 replications of 4 plants each should be used. This design results in a suitable mass selection procedure when the 40 best clones are selected. Under these conditions and if the variation of genetic gain and environmental indicators are similar to those of the Periquita populations values (which have been taken as a standard value), the gains of estimated yield will be approximately 20 % and the coefficient of variation about 14 %.

According to these conclusions, we have been applying this methodology of mass selection (in the first cycle of clonal selection) to the most important Portuguese grapevine varieties and some of these results are shown in the table.

The field data, when compared with the results of computer simulations, revealed a satisfactory fit. Meanwhile, we are actually carrying out the first experiments to determine the gains of selected material and then we will be able to verify the consistency with the estimated gains.

Conclusions

The computer simulations allowed us to establish an adequate structure for experimental populations of grapevine clones – 250 clones, 5 replications and 4 plants each – which enable a 20 % gain in yield per cycle of mass selection, with each cycle lasting approximately 5 years.

The application of this methodology to Portuguese grapevine varieties supports the results foreseen in computer simulations and proves to be a valuable contribution to the improvement of Portuguese viticulture.

References

MARTINS, A.; CARNEIRO, L.; CASTRO, R.; MAGALHAES, N.; 1987: Analyse de la variabilité génétique de populations de clones de vigne (*Vitis vinifera* L.). Rech. Agron. Suisse **26**, 261-264.

SCHÖFFLING, H.; BENDER-BERLAND, G.; 1983: La clonalisation des cépages de raisins de cuve. Progr. Agric. Vitic. **100** (15): 396-400, (16): 415-420.

SMITH, C.; 1969: Optimum selection procedures in animal breeding. Anim. Prod. **11**, 433-442.

Wine test results from clones of the varieties Kerner, Müller-Thurgau, Gewürztraminer and Riesling during the development and redevelopment phases

H. Schöffling and K. H. Faas

Zentralstelle für Klonenselektion, LLVA Trier, Egbertstr. 18/19, D-5500 Trier, F. R. Germany

Summary: In the field of clone development in the Mosel-Saar-Ruwer region, in 5 selection phases microvinifications were carried out. Included were the varieties Kerner, Gewürztraminer, Riesling and Müller-Thurgau. According to the harvest and wine data, the findings in the sensorial assessment of the wine, as well as the results of the correlation and regression analysis, the following was established:

1. There were differences in the harvest data.
2. There were differences in the sensory assessment.
3. The must weight alone as a selection criterion for wine quality was insignificant.

It is strongly recommended that a microvinification with at least 2 replicates should be carried out in each selection phase.

Key words: variety of vine, clone, selection, analysis, yield, must quality, wine quality, sensory rating, microvinification, Moselle.

Introduction

Vine selection in Germany began more than 200 years ago in 1787 (Schöffling and Faas, in print). Clonal selection started in 1876. The first clone vineyard was planned in 1900. Vine clones result from mutation and selection. Their development requires approximately 20 years (Schöffling 1989). It includes 4 test phases, as shown in Fig. 1. These are followed by a subsequent selection with a redevelopment, which undergoes 2 test phases (Fig. 2).

Today over 465 grapevine clones are available. They originated from 34 grapevine varieties. Their cultivation is managed by 54 clone breeders, of which 70 % are private and 30 % publicly funded.

Nearly all clones are virus-tested (compare Stellmach 1987). Their performance at harvest is examined (Faas and Schöffling 1986). While their harvest data are adequate, there is still a need for quality rating. A great deal of research work has been carried out on clones already released

Table 1: Varieties, test phases and locations of grapevines and clones

Variety	Vine (V) Clone (C)	Test phase	Location
Kerner	24 V	First -Selection (FS)	Wintrich
Gewürztraminer	5 C	Pre -Selection (PS)	Wormeldange
W. Riesling	5 C	Interim -Selection (IS)	Kanzem
Müller-Thurgau	5 C	Main -Selection (MS)	Köwerich
W. Riesling	5 C	Subsequent-Selection (SS)	Kanzem

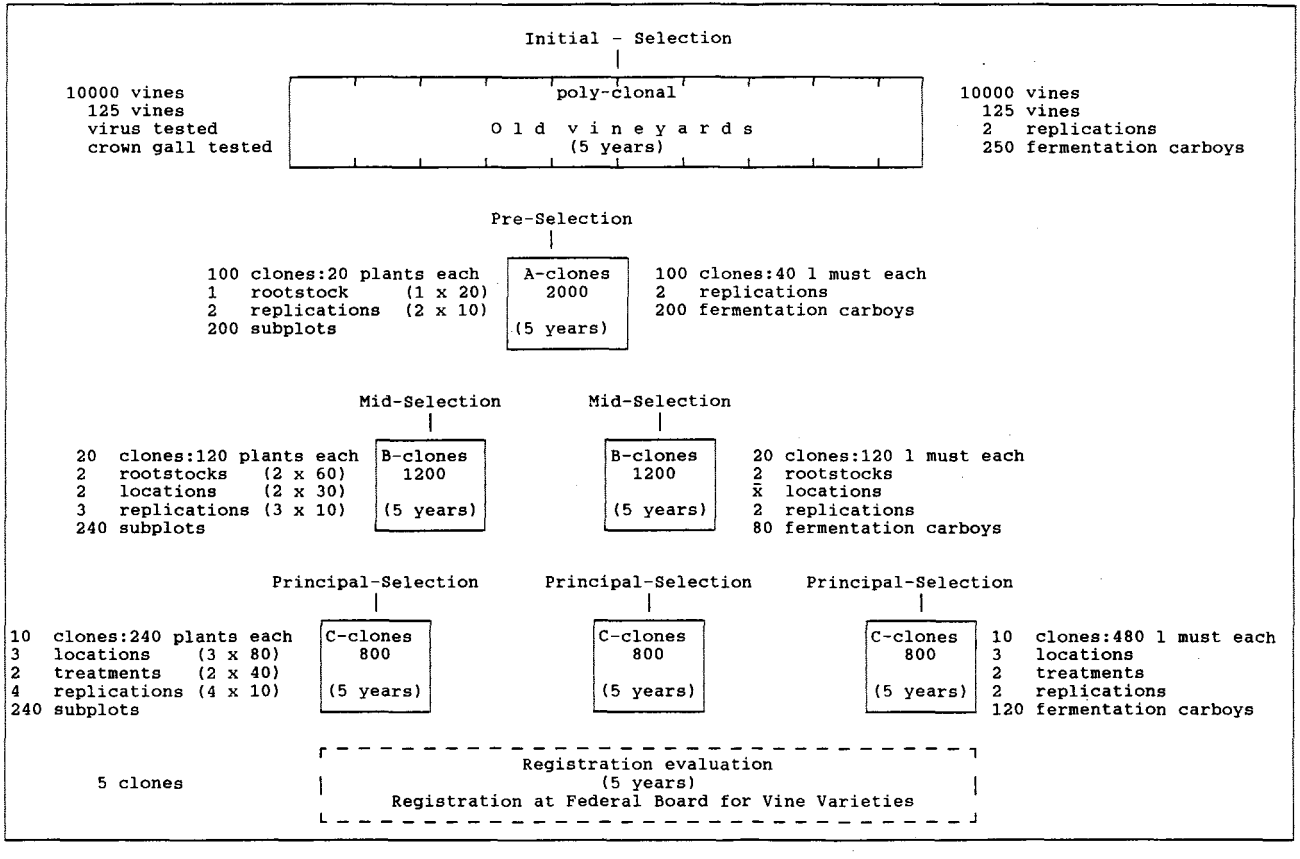


Fig. 1: Development within initial, pre-, interim and principal clonal selections. Duration: 20 years.

(Schöffling and Faber 1987; Rapp 1989). It relates to determination of wine quality sensorially and by gas chromatography. The results are encouraging.

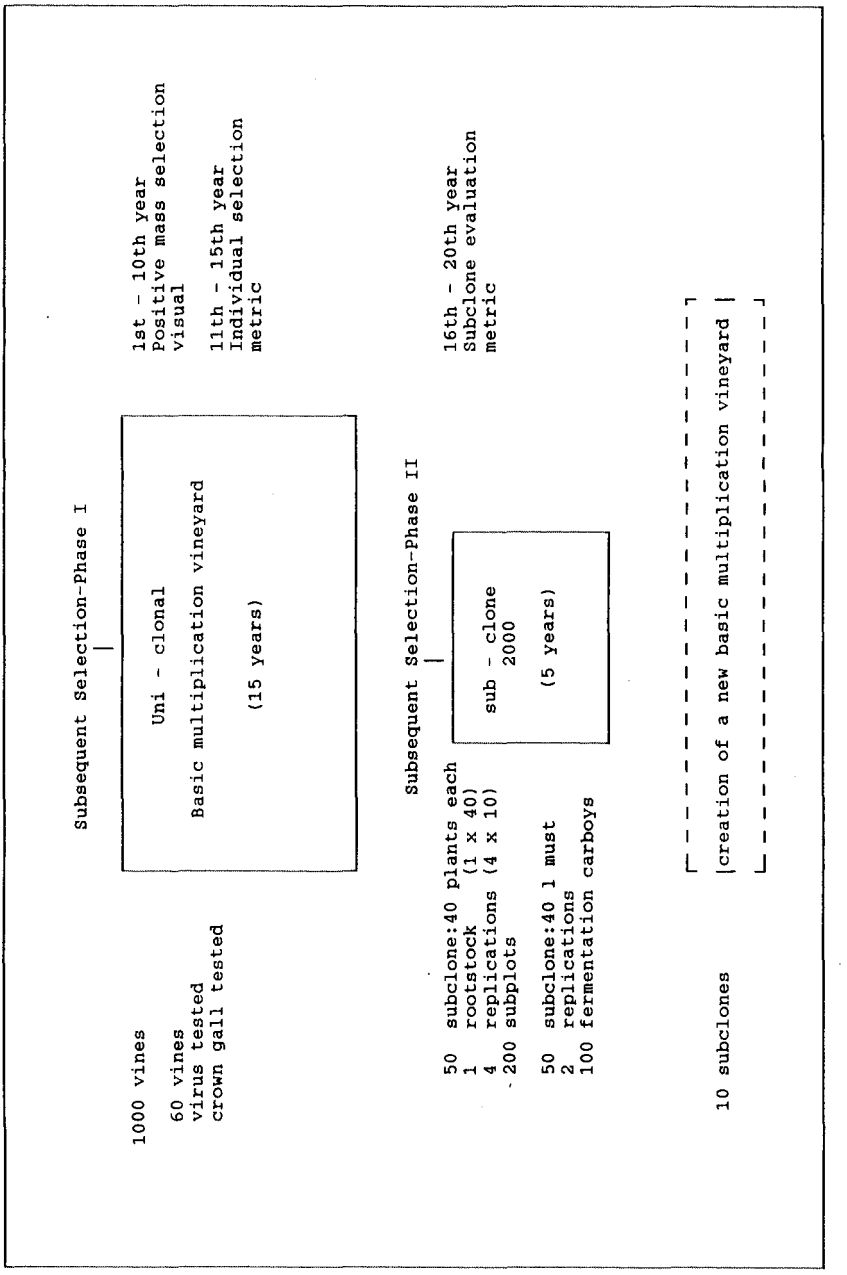


Fig. 2: Redevelopment within subsequent clonal selections, phase I and II. Duration: 20 years.

The experiments in question were carried out on clones which had not yet been released. We studied, by sensory evaluation, which differences arose in blind testings as a result of a certain number of chemicals present in very low concentrations in the clonal wines of the species *Vitis vinifera*. The varieties included are shown in Table 1.

Results

1. Harvest data

Table 2 shows a survey of the harvest data. Where possible, the values obtained were taken with replications. Naturally they showed great differences. In must weight they varied, according to the selection phase, between 4, 7, 9, 13 and 43 %.

2. Treatment of must

The musts were fermented with selected yeast, with 2 replicates. The experimental parameters are shown in Table 3.

Table 2: Harvest data ($\bar{x}$, maximum difference as %) of grapevines and clones in different test phases

Variety	Test	Year	Grape yield kg/vine bzw. kg/are		Must weight °Oe		Total acidity g/l		pH Value pH		Grape-rot %	
			$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%
Kerner	FS	1988	4.1	400	64.9	43	8.5	23	3.06	3	1.9	0
Gewürz-traminer	PS	1988	83.9	24	72.8	7	9.2	24	3.11	3	0.0	0
W. Riesling	IS	1985	142.4	46	60.2	9	15.0	17	2.83	7	0.0	0
Müller-Thurgau	MS	1988	197.4	28	67.6	13	6.5	6	3.27	2	18.4	64
W. Riesling	SS	1987	96.4	63	56.2	4	16.3	9	2.94	3	5.0	0

Table 3: Must treatment of grapevines and clones in different test phases

Variety	Test phase	Chaptalization	De-acidification	Fermentation
Kerner	FS	no	no	fermented out
Gewürztraminer	PS	increased by 16 g/l	no	fermented out
W. Riesling	IS	increased by 28 g/l	reduced to 9.5 g/l	fermented out
Müller-Thurgau	MS	increased by 20 g/l	no	fermented out
W. Riesling	SS	increased by 20 g/l	reduced to 9.0 g/l	fermented out

Table 4: Sensory data ($\bar{x}$, maximum difference as %) of grapevine and clone wines in different test phases

Variety	Judge-ment	Test	Year	Aroma 0-5		Taste 0-5		Harmony 0-5		Quality No. 0-5	
				$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%
Kerner	22	FS	1988	2.11	280	2.06	473	2.01	415	2.49	372
Gewürz-traminer	30	PS	1988	2.56	12	2.47	10	2.44	9	2.50	9
W. Riesling	536	IS	1985	5.78	5	5.86	6	5.80	5	6.02	5
Müller-Thurgau	30	MS	1988	2.61	15	2.59	13	2.60	8	2.60	11
W. Riesling	102	SS	1987	2.82	18	2.72	14	2.63	17	2.72	15

Within the individual research groups, the same treatments were carried out. The residual sugar contents and the total acids varied around 1 g/l. Before bottling both replicates were combined together. In case of the variety Riesling, the replications were bottled separately. So we can conclude that the prerequisites are sufficient to allow a sensory evaluation of the wines.

3. Sensory data

Experts were brought in to make observations on the wine. We used the DLG 5-point scheme and in one case a 10-point scheme. The results are shown in Table 4.

The observations within the research groups have, to some extent, revealed great variations, especially in aroma.

Differences were ascertained by means of the F-test and the Duncan test. The analysis showed significant differences as presented in Table 5.

Out of 208 possibilities, 82 could be identified. That is about 40 %.

Table 5: Significant differences in grapevine and clone wines in different test phases

Variety	Test	Aroma n	Taste n	Harmony n	Quality No. n
Kerner	FS	13	10	12	12
Gewürztraminer	PS	-	-	-	-
W. Riesling	IS	5	4	4	6
Müller-Thurgau	MS	-	-	-	-
W. Riesling	SS	5	3	4	4

Table 6: Significant relationships between harvest and wine data, respectively, and sensory data in different test phases

x Must data Wine data	y Aroma F P I M S S S S S S	y Taste F P I M S S S S S S	y Harmony F P I M S S S S S S	y Quality No. F P I M S S S S S S	To- tal
Must					
Grape yield	* *	* *	* *	* * *	9
Must weight	*	*	*	*	4
Total acidity			* *		2
pH-Value		*	*	*	3
Grape rot					0
Wine					
Alcohol	* *	*	*	*	5
Residual extr.			*		1
Residual sugar	*	* *	*	*	5
Total acidity	*	*	* * *	* *	7
pH-Value	*	*		*	3
Total	8	9	12	10	39

4. Correlations

The sensory results were examined to determine a relationship between the harvest data and the wine data. The findings are shown in Table 6.

At first, in all selection phases correlations to the sensory evaluation were indicated. From 200 possible cases, however, only 39 were significant. That is around 20 %. These were apportioned half from the must data and half from the wine data. The highest number of correlations were found with the grape yield.

5. Regressions

The regression coefficients for the 39 relationships with selected parameters are shown in Table 7.

The first thing to recognize about the regression coefficients is that the wine was judged better when, for example, the grape yield and the acidity decreased. In about 50 % of possible cases this relationship applied. So far the results suggest a limitation of yield, which is carried out worldwide.

To which extent can must weights be valued as a quality factor ? – In German winemaking this is considered as an important criterion of quality. For example, the quality classification of the wine law is based on the must weight at harvest. The analogy of this is that the must weight has always played a leading role in the area of quality analysis in clonal selection. Thus, when so great a significance is attributed to the must weight in wine production, an equally close correlation had to be given to the sensory assessment. But only in 4 cases, that is 20 %, was the correlation found.

Table 7: Significant regression coefficients of the relationships between harvest and wine data, respectively, and sensory data (n = grapevines, clones or clones x replications) in different test phases

Relationship		Test			Regressionanalysis			
y	x		n	$\bar{y}$	$\bar{x}$	r^2 = B	a	byx
Aroma-Grape yield		MS	10	2.63	197.4	0.423*	3.69	-0.0054
Aroma-Grape yield		SS	10	2.82	96.4	0.679**	3.64	-0.0080
Aroma-Must weight		SS	10	2.82	56.2	0.686**	-7.80	0.1900
Aroma-Alcohol		FS	23	2.11	68.6	0.124*	0.07	0.0302
Aroma-Alcohol		SS	10	2.82	75.1	0.428*	-8.77	0.1540
Aroma-Residual sugar		IS	5	5.78	11.7	0.773*	2.48	0.2808
Aroma-Acidity (wine)		IS	5	5.78	7.9	0.929**	22.06	-2.0509
Aroma-pH-Value (wine)		FS	23	2.11	2.85	-0.130*	10.55	-2.9516
Taste-Grape yield		MS	10	2.60	197.4	0.527**	3.87	-0.0064
Taste-Grape yield		SS	10	2.72	96.4	0.806**	3.44	-0.0070
Taste-Must weight		SS	10	2.72	56.2	0.543**	-4.84	0.1300
Taste-pH-Value (must)		PS	5	2.47	3.11	0.736*	-4.89	2.3655
Taste-Alcohol		SS	10	2.72	75.1	0.298*	-5.02	0.1030
Taste-Residual sugar		IS	5	5.84	11.7	0.870**	2.15	0.3139
Taste-Residual sugar		PS	5	2.47	0.9	0.790*	1.74	0.7871
Taste-Acidity (wine)		IS	5	5.84	7.9	0.671*	20.40	-1.8341
Taste-pH-Value (wine)		IS	5	5.84	3.32	0.748*	9.24	-1.0232
Harmony-Grape yield		MS	10	2.61	197.4	0.494*	3.66	-0.0053
Harmony-Grape yield		SS	10	2.63	96.4	0.659**	3.29	-0.0070
Harmony-Must weight		SS	10	2.63	56.2	0.527**	-5.06	0.1400
Harmony-Acidity (must)		PS	5	2.44	9.2	0.834*	3.23	-0.0854
Harmony-Acidity (must)		MS	10	2.61	6.5	0.348*	6.33	-0.5725
Harmony-pH-Value (must)		PS	5	2.44	3.11	0.785*	-2.81	1.6885
Harmony-Alcohol		PS	5	2.44	95.3	0.743*	0.02	0.0254
Harmony-Residual extract		PS	5	2.44	6.5	0.920**	0.55	0.2931
Harmony-Residual sugar		IS	5	5.80	11.7	0.799*	2.13	0.3128
Hamrony-Acidity (wine)		PS	5	2.44	7.4	0.823*	3.25	-0.1085
Harmony-Acidity (wine)		IS	5	5.80	7.9	0.774*	22.08	-2.0507
Harmony-Acidity (wine)		MS	10	2.61	5.0	0.313*	5.33	-0.5430
Qual. No.-Grape yield		IS	5	6.01	171.0	0.686*	6.70	-0.0049
Qual. No.-Grape yield		MS	10	2.61	197.4	0.564**	3.74	-0.0057
Qual. No.-Grape yield		SS	10	2.72	96.4	0.778**	3.45	-0.0080
Qual. No.-Must weight		SS	10	2.72	56.2	0.645**	-5.86	0.1500
Qual. No.-pH-Value(must)		PS	5	2.49	3.11	0.714*	-3.34	1.8741
Qual. No.-Alcohol		SS	10	2.72	75.1	0.328*	-5.74	0.1130
Qual. No.-Residual sugar		PS	5	2.49	0.9	0.699*	1.94	0.5947
Qual. No.-Acidity (wine)		IS	5	6.01	7.9	0.704*	23.22	-2.1676
Qual. No.-Acidity (wine)		MS	10	2.61	5.0	0.336*	5.44	-0.5645
Qual. No.-pH-Value(wine)		IS	5	6.01	3.32	0.741*	9.91	-1.1744

The 4 regression lines which were found are laid out in Fig. 3. They are Riesling subclones from the internationally known Riesling clone Weis 21. With an increase of 1 °Oechsle they improve:

in the aroma	about 0.189 points
in the taste	about 0.135 points
in the harmony	about 0.137 points
in the quality no.	about 0.153 points.

These are small improvements which are seldom significant.

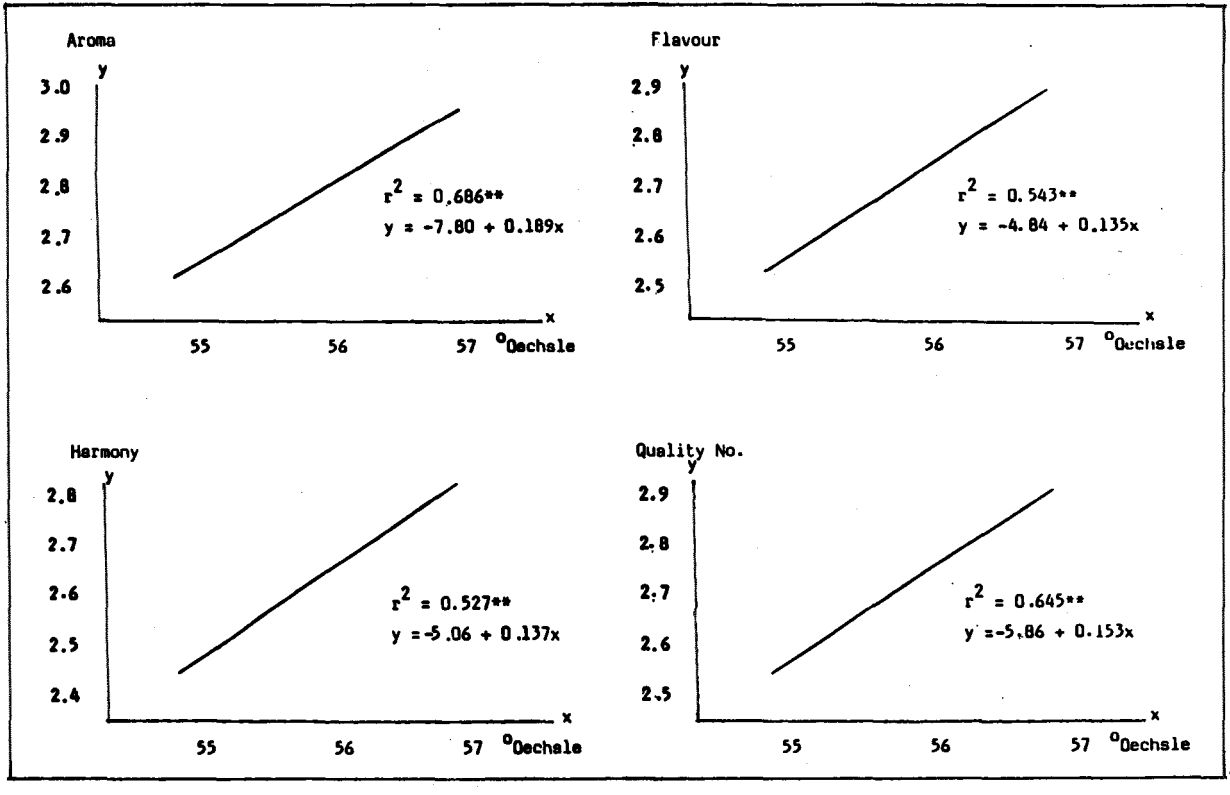


Fig. 3: Regression lines for the relationships between sensory data and must density within the subsequent selection phase II, 1987.

Table 8: Berry weight and other parameters from clones of the variety W. Riesling in the year 1988, as well as sensory data of the wines over 2 vintage years, from the location 'Staatsdomäne Trier-Avelsbach'

Clone	Harvest data			Quantity (n)			Weight (g)				Sensory data
	Grape yield kg/vine	Must-weight °Oe	Total acidity g/l	Shoots per vine	Clusters per vine	Berries per Cluster	Cluster	Rachis	Berry	Diameter (cm)	0 - 20
Weinsberg 29	1.595	81.7	10.5	15.3	23.9	78.7	66.85	2.95	0.64	11.5	13.27
Bernkastel 68	1.793	77.5	11.1	15.3	24.2	75.1	74.09	3.03	0.71		13.11
Neustadt 90	1.859	78.8	10.3	15.3	23.3	86.9	79.63	3.19	0.76		13.00
Trautwein 356	2.194	80.2	10.3	15.3	24.2	99.3	90.86	3.99	0.87		12.97
Heinz 65	2.473	76.4	10.6	15.3	25.2	102.7	98.03	3.59	0.94		12.53
Weis 21	2.595	76.5	12.0	15.4	24.9	107.2	104.24	4.26	1.00	12.3	12.21

Conclusion

It can be deduced from these data that we cannot rely only on must weights for determination of quality in clonal selection. There is no sure relationship between wine quality and must weight differences between clones. Therefore, sensorial wine assessment should be given preference. For this reason, we recommend urgently that in the single selection phases winemaking in 2 replicates should be undertaken. Research with berry size and gas chromatography should not be excluded in such a program because they can be used to indicate the clone quality (Table 8).

Literature cited

FAAS, K. H.; SCHÖFFLING, H.; 1986: Resultate und Nutzung der Klonenselektion. Wein-Wiss. **41**, 377-403.

RAPP, A.; 1989: Aromastoffe des Weines. Vortrag, Fachhochschule Trier, Fachbereich Ernährungs- und Haushaltstechnik, 22.06.1989, Trier.

SCHÖFFLING, H.; 1989: Méthodologie de la sélection clonale de la vigne. Vortrag, 17. Sitzung der Groupe d'Experts, Sélection de la Vigne, Office International de la Vigne et du Vin, 21.06.1989, Paris.

– – ; FAAS, K. H.: Zweihundert Jahre (1787-1987) Erhaltungszüchtung bei Reben in Deutschland. Schriften zur Weingeschichte (im Druck).

– – ; FABER, W.; 1987: Qualität vom Rieslingwein in Abhängigkeit vom Klon. Wein-Wiss. **42**, 28-53.

STELLMACH, G.; 1987: Die neue Rebenpflanzgutverordnung aus der Sicht des Pflanzenschutzes. Dt. Weinbau-Jahrbuch **38**, 39-47.

Clonal variability of several grapevine cultivars (*V. vinifera* L.) grown in the Emilia-Romagna [1])

O. Silvestroni, C. Intrieri, R. Credi [2]), F. Faccioli, B. Marangoni and G. Vespignani

Istituto di Coltivazioni Arboree, Sezione Viticola del Centro Ricerche Viticole ed Enologiche, Università di Bologna, Via Fillippo Re 6, I-40126 Bologna, Italy

Summary: Clonal selection has been performed over the past 2 decades by the University of Bologna to maintain the traditional grapevine cultivars grown in the Emilia-Romagna. Around 1980 budwood canes from several biotypes of the cvs Lambrusco di Sorbara, Lambrusco Salamino, Lambrusco Grasparossa, Lambrusco Maestri and Fortana were collected from old vineyards and used to establish a preliminary trial.

The vines were tested for their virus status and compared for yield, grape quality, leaf characters and phenological phases in order to evaluate the biotype variability and clonal repeatability within each cultivar.

L. Salamino, L. Grasparossa and L. Maestri showed very low degrees of genetic determination for yield and quality, while Fortana and L. Sorbara exhibited quite high degrees. The results in both cases were independent on the virus status of the vines. While for cvs L. Salamino, L. Maestri and L. Grasparossa selection can be made only on the basis of virus status, good selection potentials were found with cvs L. Sorbara and Fortana. Fortana also exhibited marked differences in leaf morphology and phenological phases. Further investigations are needed to better characterize the diversity among biotypes of this variety, since the delimitation between cultivars and clones remains questionable.

Key words: variety of vine, clone, Italy, variability, genetics, selection, biometry, virosis, yield, must quality, morphology, phenology.

Introduction

A necessary premise to clonal selection is variability among biotypes of a given variety. The main issue is genotypic variance, which can be transmitted by vegetative propagation and separated from environmental effects in planned trials. The proportion of the phenotypic variance which is due to permanent differences between individuals (genotypic variance) – which can be easily calculated – is called degree of genetic determination or clonal repeatability (Falconer 1981).

In Italy studies on the degree of genetic determination among biotypes have been performed recently for some cultivars of the Veneto Region and the results have indicated favourable conditions for selection (Calo *et al.* 1987). Similar research has been conducted in Emilia-Romagna since 1980 on several of the main grapevine cultivars of the area.

This paper focuses on the red cvs Lambrusco di Sorbara, Lambrusco Salamino, Lambrusco Grasparossa, Lambrusco Maestri and Fortana, whose biotype variability and degree of genetic determination were evaluated for productivity and must composition. An attempt to relate biotype heterogeneity to morphological characters and sanitary status of the vines is also reported.

Materials and methods

Budwood canes from 7 biotypes of L. di Sorbara, 6 of L. Salamino, 10 of L. Grasparossa, 5 of L. Maestri and 5 of Fortana were collected from old vineyards located in the cultural areas of the varieties and used to establish a preliminary trial (Intrieri 1976).

[1]) Research funded by the Italian Ministry of Agriculture and Forestry and by ESAVE, Emilia-Romagna Viticulture and Enology Extension Service.

[2]) Istituto di Patologia Vegetale, Università di Bologna, Italia.

The buds were grafted on a virus-free rootstock (SO 4) and in 1980 3 blocks of 4 vines per biotype were planted for each cultivar in a field in Modena area (30 km northwest of Bologna). In the subsequent years the biotypes were indexed by grafting to woody indicators, i. e. *Vitis rupestris* cv. St. George, LN 33, to detect grapevine fanleaf virus (GFV), grapevine fleck, grapevine stem pitting (LR), grapevine leafroll (GLR) and corky bark. In addition, enzyme-linked immunosorbent assay (ELISA) was also used to detect GFV infection (MARTELLI 1979).

Since 1983 the number and weight of bunches per vine have been recorded yearly at harvest. In 1986 and 1987 berry samples were taken and the juice analyzed for pH, titratable acidity and soluble solids concentration. In addition, the time of bud burst and flowering were recorded and the leaf traits were investigated for 5 biotypes per cultivar. 10 leaves from the medial part of the shoot were sampled after berry shatter as proposed by ALLEWELDT and DETTWEILER (1986) and data were collected as reported in Fig. 1.

Yield and must composition data were subjected to analysis of variance (ANOVA) and variance partitioning was calculated as reported in Table 1. Clonal repeatability as the ratio between genotypic and phenotypic variance (after OTTAVIANO 1968) was also determined.

To evaluate the variability in yield quantity and quality, multivariate analysis of variance (MANOVA) was performed after standardization on crop, bunch weight, must pH, soluble solids

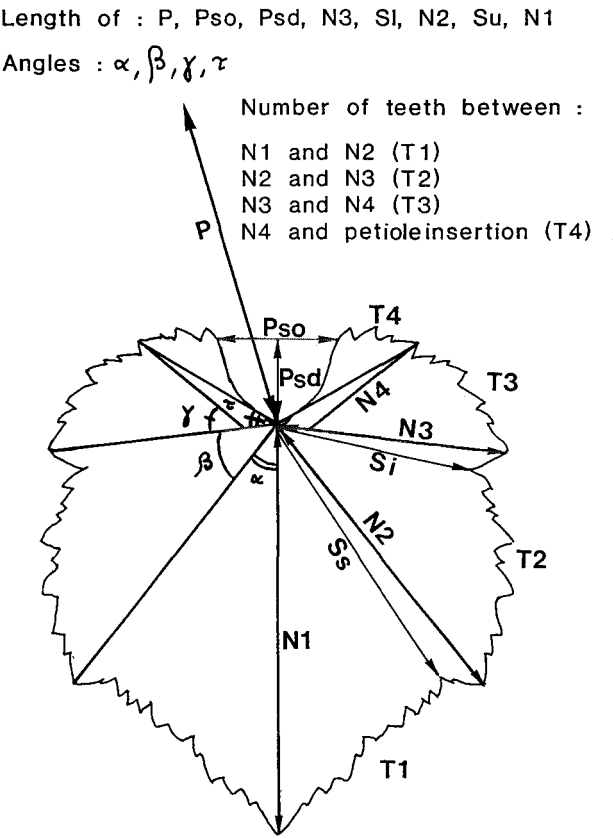


Fig. 1: Leaf characters measured for ampelographic descriptions.

Table 1: Analysis of variance and its partition

Source of variation	Degree of freedom	Variance	Partition of variance
Biotype	c - 1	V1	Ve + nVge + neV
Year	e - 1	V2	Ve + nVge + ncV
Biotype x Year	(c-1) (e-1)	V3	Ve + nVge
Error	c e (n-1)	V4	Ve

Environmental variance (Ve), Genotypic variance (Vg)
Genotype x Environment interaction variance (Vge)

and titratable acidity. MANOVA (Camussi *et al.* 1986) was also applied to describe leaf morphology, using the following standardized variables: length of petiole (P), of main vein (N1) and of lateral veins N2 and N3; distance from petiole insertion to lower (Sl) and to upper sinuses (Su); petiole sinus opening (Pso) and depth (Psd); number of teeth between vein N1 and N2 (T1), N2 and N3 (T2), N3 and N4 (T3), N4 and petiole insertion (T4); length, width and length/width ratio of teeth between N1 and N2; angles between N1 and N2 (α), N2 and N3 (β), N3 and N4 (γ), N3 and petiole insertion (τ); N2/N1 and N3/N1 length ratios; Sl/N3 and Su/N2 length ratios; petiole/main vein length ratio (Fig. 1).

Results

Virus status

The tests on woody indicators and ELISA showed a satisfactory health status in Fortana and L. Maestri, which had 1 infected biotype each. In contrast, virus status was critical in

Table 2: Grapevine fanleaf virus (GFV), grapevine fleck, stem pitting (LR) and grapevine leafroll (GLR) infections

Cultivar	Collected biotypes No.	Infected biotypes GFV No.	Fleck No.	LR No.	GLR No.	Disease-free biotypes No.
L. di Sorbara	7	0	2	2	1	2
L. Salamino	6	1	0	3	0	2
L. Grasparossa	10	2	2	10	8	0
L. Maestri	5	0	0	0	1	4
Fortana	5	0	1	1	0	4

Table 3: Significance of biotype effects (probability of F ratios) on yield and must composition at harvest

Cultivar	Yield	Bunch weight	Soluble solids	pH	TA
Lambrusco di Sorbara	.064	.041	.003	.470	.101
Lambrusco Salamino	.775	.005	.810	.484	.319
Lambrusco Grasparossa	.276	.262	.146	.498	.596
Lambrusco Maestri	.332	.265	.368	.017	.014
Fortana	.000	.000	.000	.000	.008

L. Grasparossa which had no virus-free biotypes. 2 virus-free biotypes were found for L. Salamino and 2 for L. Sorbara (Table 2). Corky bark was not present in any of the biotypes indexed.

Yield and must composition

L. Salamino, L. Grasparossa and L. Maestri showed a lack of variability among biotypes in yield, must soluble solids, pH and titratable acidity as can be readily inferred from the high probability of F values reported in Table 3. On the other hand, Fortana evidenced a wide variability in yield and must composition, while biotypes of L. Sorbara were different in bunch weight and must soluble solids concentration.

The biotype x year interaction was negligible for all 5 cultivars, indicating that the collected biotypes were similarly affected by the environmental conditions (Table 4). The high clonal repeatability in Fortana and L. Sorbara indicates good selection potential (Table 5). MANOVA confirmed that variability was lacking among biotypes of L. Salamino, L. Grasparossa and L. Maestri, but clearly indicated its presence in Fortana as well as in L. Sorbara (Figs. 2-5).

Table 4: Significance of biotype x year interaction (probability of F ratios) and must composition at harvest

Cultivar	Yield	Bunch weight	Soluble solids	pH	TA
Lambrusco di Sorbara	.115	.400	.938	.470	.408
Lambrusco Salamino	.970	.304	.919	.046	.396
Lambrusco Grasparossa	.034	.752	.016	.367	.084
Lambrusco Maestri	.370	.366	.399	.037	.026
Fortana	.583	.064	.499	.823	.006

Table 5: Clonal repeatability i. e. genotypic variance as percentage of the total phenotypic variance ($h^2 = V_g/V_p$) for yield and must composition at harvest

Cultivar	Yield	Bunch weight	Soluble solids	pH	TA
Lambrusco di Sorbara	(16)	58	93	(53)	(46)
Lambrusco Salamino	(36)	70	(3)	-	(10)
Lambrusco Grasparossa	-	(51)	-	-	-
Lambrusco Maestri	(8)	(22)	(7)	22	17
Fortana	92	98	96	93	29

Phenological phases and leaf morphology

Differences in phenological phases were found only within Fortana, in which 2 biotypes bud-burst and flowered 1 week earlier.

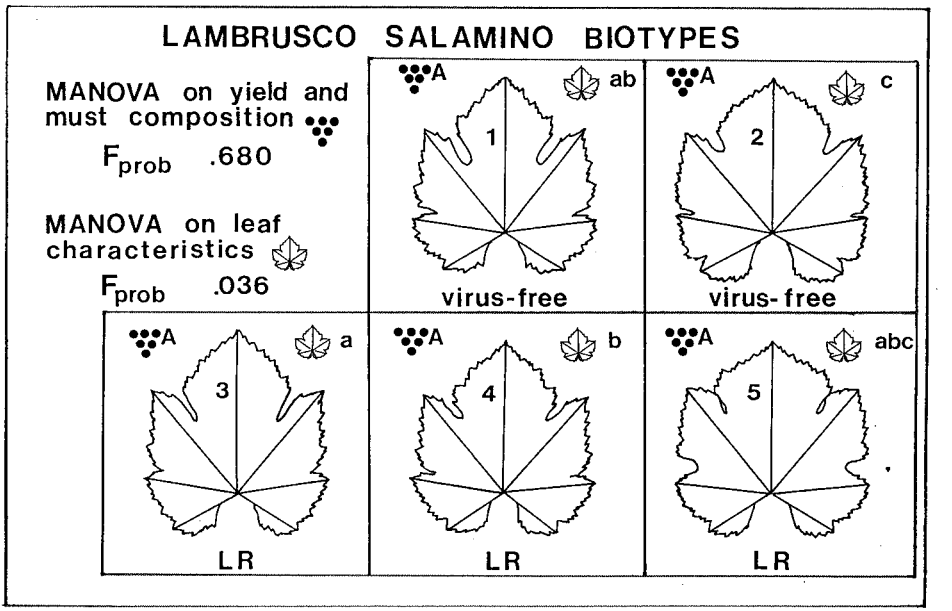


Fig. 2: Drawings of the mean leaf traits of 5 biotypes of cv. Lambrusco Salamino and results of MANOVA on yield and must composition and leaf characteristics. For each biotype the virus status is also reported.

Although some differences among biotypes within a cultivar were always evidenced, a very low variability in leaf characteristics was found in L. Maestri; MANOVA revealed variations within L. Sorbara, L. Grasparossa, L. Salamino and Fortana (Figs. 2-5). Differences among L. Grasparossa and L. Salamino biotypes were not as prominent as within Fortana and L. Sorbara, which were divided into 3 and 4 groups, respectively.

Discussion and conclusions

High degree of genetic determination for yield and must composition was found in Fortana and L. Sorbara. In contrast, negligible variability and hence low degree of genetic determination for the same characters was found within cvs L. Salamino, L. Maestri and L. Grasparossa, although differences among biotypes were evident for virus status. In addition, while leaf trait investigations indicated significant differences among L. Salamino and L. Grasparossa biotypes, they were unable to characterize or to identify them. We may speculate that the restricted growing areas of these cultivars and a prior mass-selection for yield carried out by local nurseries might have strongly reduced an eventual heterogeneity. As already suggested (Calo *et al.* 1987), a rough selection may have eliminated low cropping biotypes regardless of their virus status, so that infected vines with satisfactory yield and must quality might also have been propagated.

As regards the cvs L. Sorbara and Fortana, the trials indicated differences among biotypes in crop and juice composition, however independently on their virus status, which could not completely account for the recorded variability. Leaf trait differences among biotypes of L. Sorbara and Fortana were also found. With L. Sorbara these variations did not correlate with the previous findings on yield quality and quantity and virus status; with Fortana variations in the ampelographic characters were larger and associated with differences in yield and must quality.

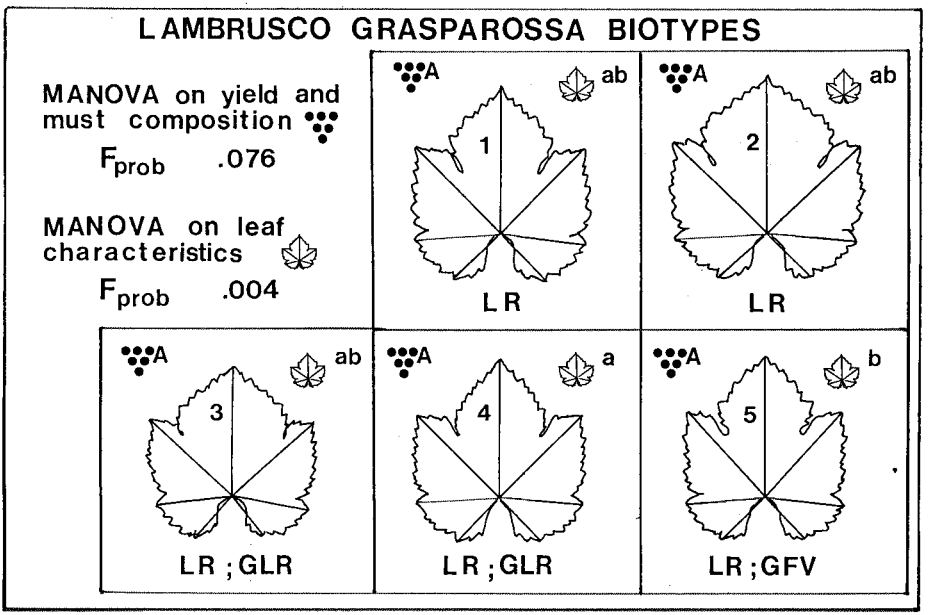


Fig. 3: Drawings of the mean leaf traits of 5 biotypes of cv. Lambrusco Grasparossa and results of MANOVA on yield and must composition and leaf characteristics. For each biotype the virus status is also reported.

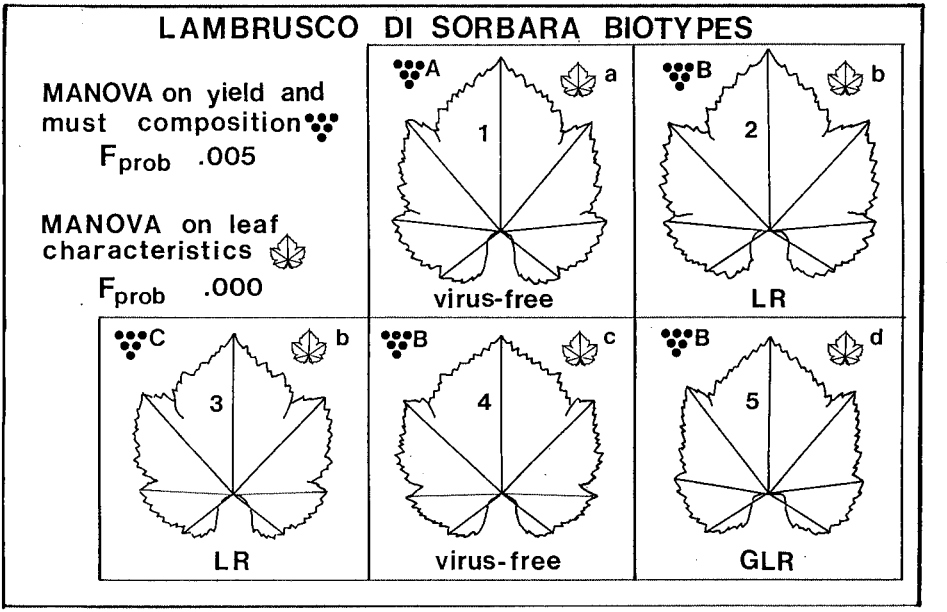


Fig. 4: Drawings of the mean leaf traits of 5 biotypes of cv. Lambrusco di Sorbara and results of MANOVA on yield and must composition and leaf characteristics. For each biotype the virus status is also reported.

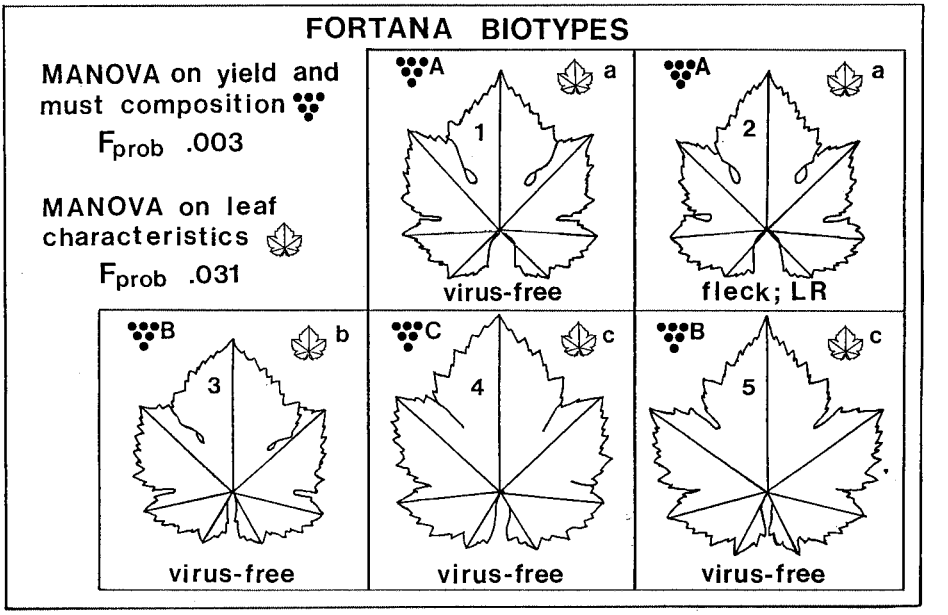


Fig. 5: Drawings of the mean leaf traits of 5 biotypes of cv. Fortana and results of MANOVA on yield and must composition and leaf characteristics. For each biotype the virus status is also reported.

The biotypes of L. Salamino, L. Maestri, L. Grasparossa showed low potentials for clonal selection, which can only be made by choosing the initially disease-free biotypes or the disease-free biotypes after heat-treatment. In contrast, the cultivars L. Sorbara and Fortana showed a high degree of genetic determination and their clonal selection can be performed for yield and quality as well as for virus status. It should also be noted, however, that Fortana exhibited marked differences in leaf morphology and phenological phases, leading to a well-differentiated polyclonal variety, as already reported for other cultivars like Pinot noir (BOURSIQUOT *et al.* 1989) and Arneis (MANNINI *et al.* 1986).

In situations such as this, when crop, grape quality and ampelographic differences are in evidence, further investigations are needed to better characterize the diversity among biotypes since the delimitation between cultivar and clone remains questionable.

References

ALLEWELDT, G.; DETTWEILER, E.; 1986: Ampelographic studies to characterize grapevine varieties. Vignevini **13** Suppl. al (12), 56-59.

BOURSIQUOT, J. M.; VIGNAU, L.; BOULET, J. C.; 1989: Ricerche sull'utilizzazione dell'ampelometria. Riv. Viticolt. Enol. **42**, 37-52.

CALO, A.; COSTACURTA, A.; CANCELLIER, S.; GIUST, M.; 1987: Variabilità di alcune caratteristiche produttive di vitigni del Veneto. Schweiz. Landw. Forsch. **26**, 345-350.

-- ; EGGER, E.; BORGO, M.; COSTACURTA, A.; CANCELLIER, S.; NIERO, M.; 1987: Considerazioni su alcune relazioni esistenti tra aspetti sanitari e caratteristiche produttive in alcuni vitigni del Veneto. Schweiz. Landw. Forsch. **26**, 341-344.

CAMUSSI, A.; MÖLLER, F.; OTTAVIANO, E.; SARI GORLA, M.; 1986: Metodi Statistici per la Sperimentazione Biologica, 404-427. Zanichelli, Bologna.

FALCONER, D. S.; 1981: Introduction to Quantitative Genetics, 112-133. Longman Editor, London.

INTRIERI, C.; 1976: La selezione clonale della vite in Emilia-Romagna. Concetti informatori e primi risultati. Atti Accad. Ital. Vite Vino **28**, 127-141.

MANNINI, F.; GERBI, V.; SCHNEIDER, A.; 1987: Grapevine clonal selection in Piedmont: agronomical and enological aspects. Schweiz. Landw. Forsch. **26**, 273-295.

MARTELLI, G. P.; 1979: Identification of virus diseases of grapevine and production of disease-free plants. Vitis **18**, 127-136.

OTTAVIANO, E.; 1968: Analisi genetica della variabilità continua. In: Trattato di Genetica Agraria Speciale, 7-9. EdAgricole, Bologna.

Clonal selection of rootstocks by means of determination of hard bast layers

V. PALENIK, D. POSPISILOVA and A. HAMADEJOVA

Research Institute for Viticulture and Enology, Matúskova 25, 83311 Bratislava, Czechoslovakia

Summary: Determination of the number of closed circles of hard bast plates on matured shoot cuttings, which is used for wood maturity control, was applied as selection method to four rootstocks: *Vitis berlandieri x V. riparia* Kober 5 BB, Teleki 5 C, Craciunel 2 and V. *riparia x V. rupestris* Schwarzmann. For each selected vine stock the linear regression of wood maturity was calculated, considering the distance from the base of the cane. From the linear regression equation the average length of the matured wood part was calculated for each stock.

The results of 3-year investigations show that the degree of rootstock wood maturity is not only dependent on the meteorological conditions of the years but is also genetically conditioned.

Key words: rootstock, clone, selection, wood, maturity, lignification, hard bast, sclerenchyma, phloem.

Introduction

The relation between the larger number of hard bast circle layers on rootstock cuttings and vine nursery yields of first-class grafted vines has been generally known for a long time (e. g. KRAUS 1979; KISIL 1986). We have oriented our efforts to determine if the above mentioned relationship may be applicable for clonal selection in populations of rootstock varieties.

Material and method

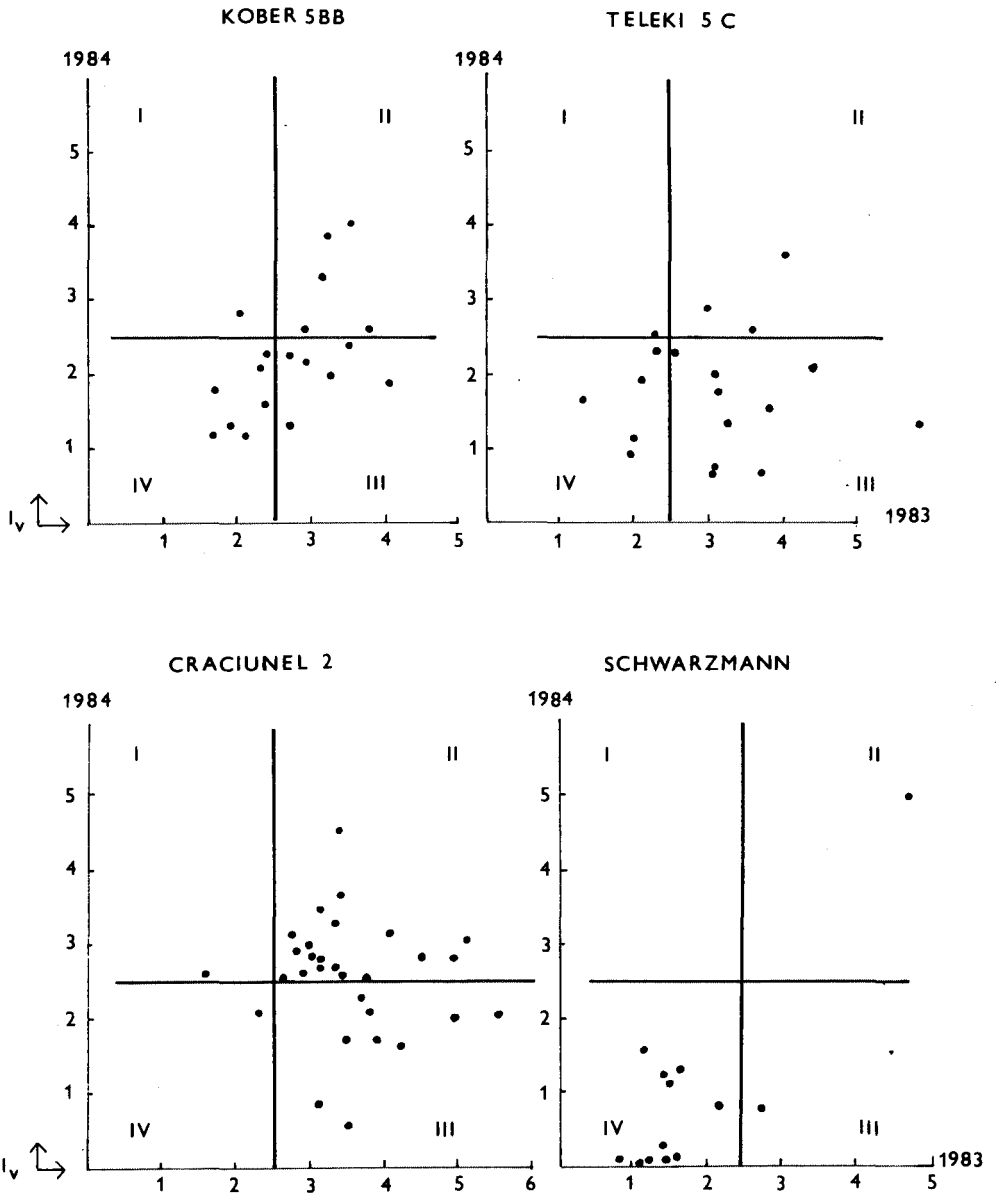
During a 3-year experiment, observations of closed hard bast circles of vine stocks selected according to different criteria (wood : pith tissue ratio, length of matured cane, number of cuttings, etc.) were recorded on four rootstocks – *Vitis berlandieri x V. riparia* Kober 5 BB, Teleki 5 C, Craciunel 2 and *V. riparia x V. rupestris* Schwarzmann – in one locality. The hard bast layers were counted behind each sympodium of all canes on separate vine stocks. Observations were recorded on the hollow as well as on the dorsal side of cane. The number of circles of both sides – 3/1, 3/2, 2/0, etc. – were reciprocally multiplied (3, 6, 0) and these data characterizing the maturity stage 'p' in the corresponding cane cross section were then statistically evaluated. For each selected vine stock the linear regression of the maturity stage 'p' was calculated in relation to the distance from the base of cane (PALENIK 1984).

The values obtained for the single rootstocks in the years 1983 and 1984 are shown in the figure. Similarly, in the table the selected vine stocks are included in maturation groups, considering the entire cane length, for each possible 2-year comparison, i. e. 1984-1983, 1985-1983, 1985-1984.

Results and discussion

Already the first look at the graphical illustration (Fig.) indicates the applicability of this method for rootstock selection. The fact that rootstocks Kober 5 BB and T 5 C are clonal populations while Craciunel 2 is a previously selected clone, is reflected by the larger diversion of Kober 5 BB and Teleki 5 C vine stocks, while in Craciunel 2 the majority of vine stocks was placed in quadrant II (the highest wood maturity). Only one vine stock of the variety Craciunel 2 is placed

in quadrant IV (very weakly matured wood). For the example of rootstock Schwarzmann, the stated method also appeared to be positive. From the diagram it is apparent that the experimental location with heavy soils is not suitable for this rootstock, which is generally known and confirmed by applying this method. The majority of vines of this rootstock are placed in quadrant IV (the most weakly matured wood). Only one vine stock in each year showed conclusively above-average values of wood maturation.



Lignification of single vines, l_v (m).

Also, the data in the table confirm these statements for the 3-year period of the selection process. The percentage of vine stock diversion in the individual quadrants points to the fact that in

Lignification of rootstocks. Frequency of single vines in the quadrants of two-dimensional variance analysis

Cultivar	Compared years	Frequency in quadrants				Number of vines	
		I	II	III	IV	3x the best lignific. above 2,5 m	3x the worst lignific. under 1,5 m
Kober 5BB	1984-1983	5/43	4/38, 5/12, 5/32, 6/9, 8/36	4/18, 4/36, 5/5, 5/31, 5/23, 6/22	4/8, 4/60, 6/2, 6/39, 8/22, 8/34, 8/55		
	1985-1983	4/60, 6/2	5/12, 6/22	4/18, 4/36, 4/38, 5/5, 5/31, 5/23, 5/32, 6/9, 6/39, 8/36	4/8, 5/43, 8/22, 8/34, 8/55	1	4
	1985-1984	4/60, 6/2, 6/22	5/12	4/36, 5/32, 5/36, 5/43, 6/9	4/8, 4/18, 4/36, 5/5, 5/23, 5/31, 6/39, 8/22, 8/34, 8/55		
% of vines		10,53	14,03	36,84	38,60	1,67	6,67
Teleki 5C	1984-1983	-	4/10, 5/39, 10/50	4/33, 4/42, 4/59, 5/34, 6/5, 6/65, 6/69, 6/74, 7/61, 8/63	4/14, 6/49, 6/64, 8/18, 8/34, 8/58		
	1985-1983	4/14	4/33, 4/42	4/10, 4/59, 5/34, 5/39, 6/5, 6/65, 6/69, 6/74, 7/61, 8/63, 10/50	6/49, 6/64, 8/18, 8/34	0	4
	1985-1984	4/33, 4/42	-	4/10, 4/14, 5/39, 10/50	4/59, 5/34, 6/5, 6/49, 6/64, 6/65, 6/69, 6/74, 7/61, 8/18, 8/34, 8/63		
% of vines		5,45	9,09	45,45	40,0	0	7,27

Cultivar	Compared years	Frequency in quadrants				Number of vines	
		I	II	III	IV	3x the best lignific. above 2,5 m	3x the worst lignific. under 1,5 m
Craciunel 2	1984-1983	6/8	4/40, 4/52, 5/4, 5/8, 6/30, 6/69, 7/6, 7/13, 7/18, 7/27, 7/33, 7/39, 7/67, 9/58, 9/70, 10/3, 10/12,10/61	5/70, 6/68, 7/34, 7/69, 10/13,10/28, 10/46,10/70,11/7	4/49		
	1985-1983	-	17/13, 10/12,10/46	4/27, 4/40, 4/52, 5/4, 5/8, 5/70, 6/30, 6/68, 7/6, 7/18, 7/27, 7/33, 7/34, 7/39, 7/67, 7/68, 9/70, 10/13, 10/28,10/61,10/70, 11/7	4/49, 6/8	2	1
	1985-1984	10/46	7/13, 10/12	4/40, 5/4, 5/8, 6/8, 6/30, 6/69, 7/6, 7/18, 7/27, 7/33, 7/39, 7/67, 9/70, 10/13,10/61	4/49, 5/70, 6/68, 7/34, 7/68, 10/13, 10/28,10/70,11/7		
% of vines		4,71	27,05	54,12	14,12	2,35	1,17
Schwarzmann	1984-1983	-	6/24	4/24	4/8, 7/5, 7/19, 7/22, 7/23, 8/11, 8/12, 9/24, 9/30, 15/5, 15/31		
	1985-1983	-	6/24	-	4/8, 4/24, 7/5, 7/19, 7/23, 8/11, 8/12, 9/24, 9/30, 15/5, 15/31	1	8
	1985-1984	-	6/24	-	4/8, 4/24, 7/19, 8/11, 8/12, 9/24, 9/30, 15/5, 15/31		
% of vines		-	8,57	2,86	88,57	8,57	22,86

case of the rootstock Craciunel 2 the quadrant IV is very rarely frequented in comparison with the other ones.

Another proof of the applicability of the examined method in clonal selection is the fact that the same vine stocks of two extreme groups (the best and the worst wood maturation) are represented in each of the experimental years. Some of the vine stocks oscillate between neighbouring quadrants. This means that wood maturation is conditioned not only by season but also genetically. Obviously, all clonal selection including that of *V. vinifera* varieties is based on this principle. But the most frequently used selection methods for determination of rootstock wood maturation consist of subjective evaluations which sometimes lead to controversial results.

In accordance with these facts, this objective method, which determines not only wood maturity in successive years but also the genetic disposition of individual vine stocks with regard to wood maturation, contributes to successful selection work. Such an objective selection method, which considers the most important selection criterion of rootstocks – wood maturation – and which can be applied already in the first selections, saves the breeder a great portion of labourious work.

References

KISIL, M. F.; 1986: Ekologiceskije uslovija i produktivnost' vinogradnogo rastenija [Ecological conditions and vine productivity]. Vinodelije i Vinogradarstvo SSSR (1), 19-22.

KRAUS, V.; 1979: Vinohradnictvi. II. Základni agrotechnika révy vinné [Viticulture. II. Basic Agrotechnique]. Vysoká skola zemedelská, Brno.

PALENIK, V.; 1984: Urcovanie vyzretosti dreva vinica parametrom 'p' [Determination of wood maturation by means of the parameter 'p']. Zahradnictvi 11, 205-214.

Flavour compounds of clones from different grape varieties

G. Versini[1]), A. Rapp[2]), C. Volkmann[3]) and A. Scienza[1])

[1]) Istituto Agrario Provinciale, I-23010 San Michele all'Adige, Italy

[2]) Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, F. R. Germany

[3]) Universität Karlsruhe, Institut für Lebensmittelchemie, D-7500 Karlsruhe, F. R. Germany

Summary: The selection of highly productive and characteristic clones is essential for the maintenance and improvement of the viticultural and commercially valuable qualities of certain grape varieties. In the future, this very difficult and time consuming selection of adapted clones will be supplemented with more precise analytical techniques. In this regard, GC analysis will be most valuable.

Analytically, German white wines can be divided into three groups by the content of only a few monoterpene compounds ('terpene profiles'): 'Riesling', 'Muscat' and 'Sylvaner' or 'Weißburgunder' types. Furthermore, the use of linear discriminant analysis on some components allows the differentiation within types (e. g. for 'Riesling'-tpye: Riesling, Müller-Thurgau, Bacchus, Kerner, Ehrenfelser, Scheurebe).

Till now no significant differentiation could be made from flavour compounds of Riesling clones, although clear differences can be perceived between wines from Traminer clones. Wines with a low sensory appraisal typically have a low content of monoterpene compounds.

Differences can also be recognized in the aroma profiles of Chardonnay clones, allowing an analytical differentiation between aromatic and non-aromatic types. Certain clones, however, show differing compositions of flavour compounds when grown in different areas (clones with an 'unstable' aromatic character).

Also during ripening of the berries, differences occur within individual Chardonnay clones, resulting in a few clones having recognizable ripening curves for monoterpene compounds.

Key words: must, wine, flavour, terpene, analysis, sensory rating, statistics, selection, clone, variety of vine, Germany, Italy.

For enological and economic reasons, the selection of productive clones with a typical varietal aroma is of considerable importance to keep and improve the good qualities of a grape variety.

The aim of our investigations is to support the difficult and lengthy procedure of clone selection, involving must and wine quality evaluations, by reproducible analytical methods.

To judge the varietal character of musts and wines GC analysis should provide important informations. Some aspects concerning the successful analytical differentiation of grape and wine varieties are:

- a complete enrichment of the aroma compounds without artefacts
- a complete and quantitatively reproducible separation of the aroma compounds
- and it is especially necessary to determine 'key substances'; these are substances which occur in very different concentrations correlating to a single variety.

Monoterpenes are well-known to play an important role in the characterization of wine varieties (Rapp 1988). At present, about 50 monoterpene compounds can be identified in must and wine. Based on quantitative data from only 12 monoterpenes, German white wines can be divided into three groups: the 'Muscat group', the 'Riesling group', and the 'Silvaner-Weißburgunder group'. In the terpene patterns ('terpene profiles') clear differences exist between the varieties with a Muscat-related aroma, those with a fruity Riesling-related aroma and those with a neutral bouquet (Rapp 1982; Rapp *et al.* 1983; Rapp and Mandery 1986; Rapp 1988).

Application of statistic methods to the analytical data, as for example discriminant analysis, allows a clear differentiation of wine varieties. Wines of the varieties Riesling, Müller-Thurgau and Silvaner could be separated with the content of a few monoterpene compounds (Rapp and Günter 1985; Rapp 1988). In recent investigations, we could even differentiate Riesling, Ehrenfelser, Kerner, Bacchus and Scheurebe, which all derive from Riesling and often exhibit similar bouquets.

Considering these results, it is also likely that wines of the same variety but of different clones can be characterized by means of GC analysis.

Comparing the terpene patterns of several Riesling clones – all grown at the same location and treated equally – we could not find any significant difference (Fig. 1). Furthermore, no

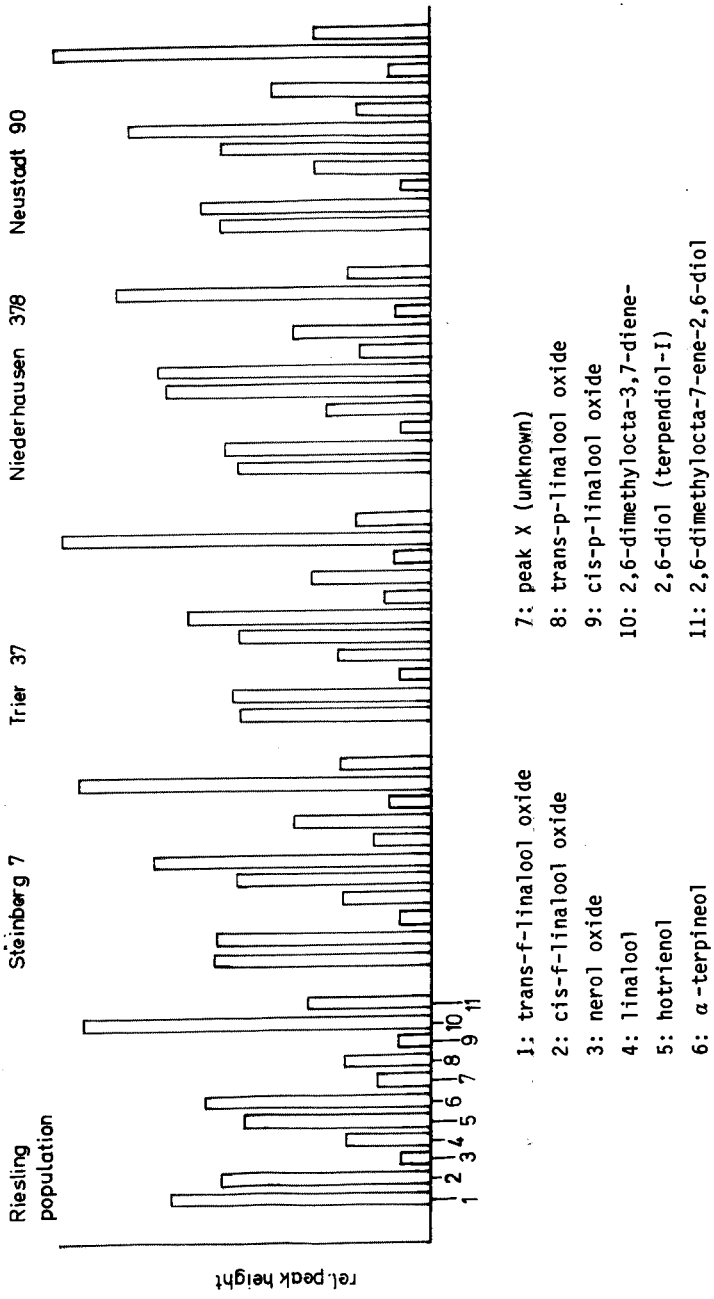


Fig. 1: 'Terpene profiles' of several Riesling clones.

significant correlations exist between terpene concentrations and sensory differences. Evaluation of the complete analytical data revealed that compounds other than monoterpenes showed

Table 1: Monoterpene compounds (relative peak height) of different Traminer clones (LLFA Neustadt, 1987)

	FR 46-107	N 23	N 25	N 22	Gm 1	K 33	Lb 14	C 48
trans-f-linalooloxide	63	61	201	203	219	186	243	216
linalool	341	367	947	894	814	995	1336	965
α -terpineol	166	160	435	407	377	477	585	483
citronellol	79	99	279	272	219	314	284	216
nerol	43	53	242	226	176	232	216	148
2,6-dimethylocta-3,7-diene-2,6-diol	63	34	248	215	219	232	239	185
rank: first place / last place	- / 13	3 / 13					15 / -	

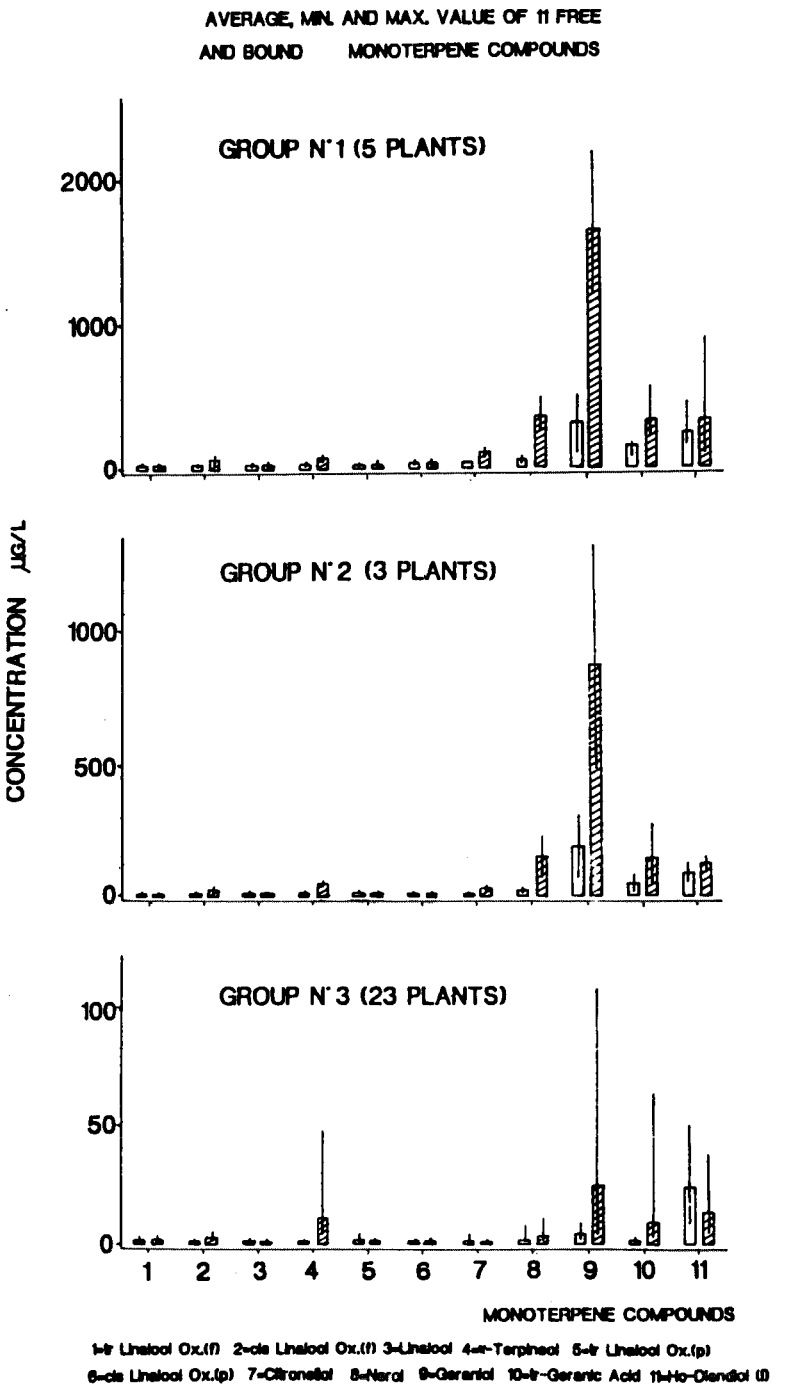


Fig. 2: 'Terpene profiles' from musts of 3 Traminer vines groups (Trentino, vintage 1987). The groupe were detected by cluster analysis.

Table 2: Gewürztraminer wines (1985 and 1986): Linear correlations between score evaluation and concentration of some monoterpene compounds

Compound		Coefficient	
nerol	smell	0.4841	0.040
nerol	taste	0.8681	0.000
geraniol	smell	0.8041	0.000
geraniol	taste	0.6237	0.009
linalool	smell	0.2450	0.199
linalool	taste	0.1323	0.326

differences between the various Riesling wines. Basically, the components concerned are fermentation compounds which can influence the wine bouquet. Hence it is not possible to judge the varietal character of the different Riesling clones tested (VOLKMANN 1989).

In further investigations, the aroma compounds – especially monoterpenes – of 8 Traminer clones were determined. Table 1 shows clearly that analytical and sensory data are well correlated. In sensory evaluation, the clone Lb 14 received a first place ranking 15 times, the clone FR-46-107 received a last place ranking 13 times. In accordance with these sensory results, the monoterpene concentrations of clone Lb 14 are very high, while those of clone FR-46-107 are low. It could be shown that clones with a typical Traminer aroma have higher geraniol and nerol concentrations than less aromatic clones (VOLKMANN 1989). For these monoterpenes, high correlation coefficients up to 0.9 between score evaluation and terpene concentration were calculated (Table 2) (AURICH *et al.* 1989).

Using the results of these investigations, we tried to identify various types of Traminer clones out of a population by analysing the aroma composition of the musts. This population was an unknown mixture of different clones of French origin. Free and bound terpene concentrations of these musts harvested from 31 plants were determined. The analysis was carried out at comparable degree of ripeness and each time under the same conditions.

The results show clear differences between the monoterpene concentrations whereas the 'terpene profiles' are comparable. Based on the quantitative data of 11 monoterpene compounds and on cluster analysis, two aromatic groups with 5 and 3 plants, and one neutral group with 23 plants were found. The terpene concentrations in the aromatic groups were about 10 times higher than in the neutral group no. 3 (Fig. 2).

We then compared the population of unknown clones with the analytical data (Fig. 3) of some Traminer clones known to have ampelographic and sensory differences. Savagnin clones, for instance, are known as Traminer clones with only slight aromatic flavour properties, whereas Gewürztraminer is much more aromatic.

Discriminant analysis of unknown and known clones together – that means that a function was calculated to differentiate the three groups of unknown clones and the same function was used for the Savagnin and Gewürztraminer clones – revealed that the Savagnin clones belonged to one of the clustered groups of unknown neutral clones and the Gewürztraminer clones should be classified into the aromatic group (Fig. 4).

It can be concluded that a characterization of Traminer clones based on the analytical data of a number of monoterpene compounds is possible.

Chardonnay clones also possess different sensory characteristics. They range between neutral, as for example the clones 116 and 130 SMA, and quite aromatic, as for example the Chardonnay clones Musqué and 77. An analytical differentiation should be possible.

The results of GC analysis (Table 3) actually showed that there were significant differences between the monoterpene concentrations of aromatic and less aromatic clones (Versini *et al.* 1988). Table 3 refers to certain Chardonnay clonal grapes from Trentino. The relatively high contents of (E)- and (Z)-2,6-dimethyl-octa-2,7-diene-1,6-diol have to be pointed out especially in bound forms and even in clones defined as 'neutral', such as the 130 SMA and 116. Usually the amount of the (Z)-compound was larger than that of (E).

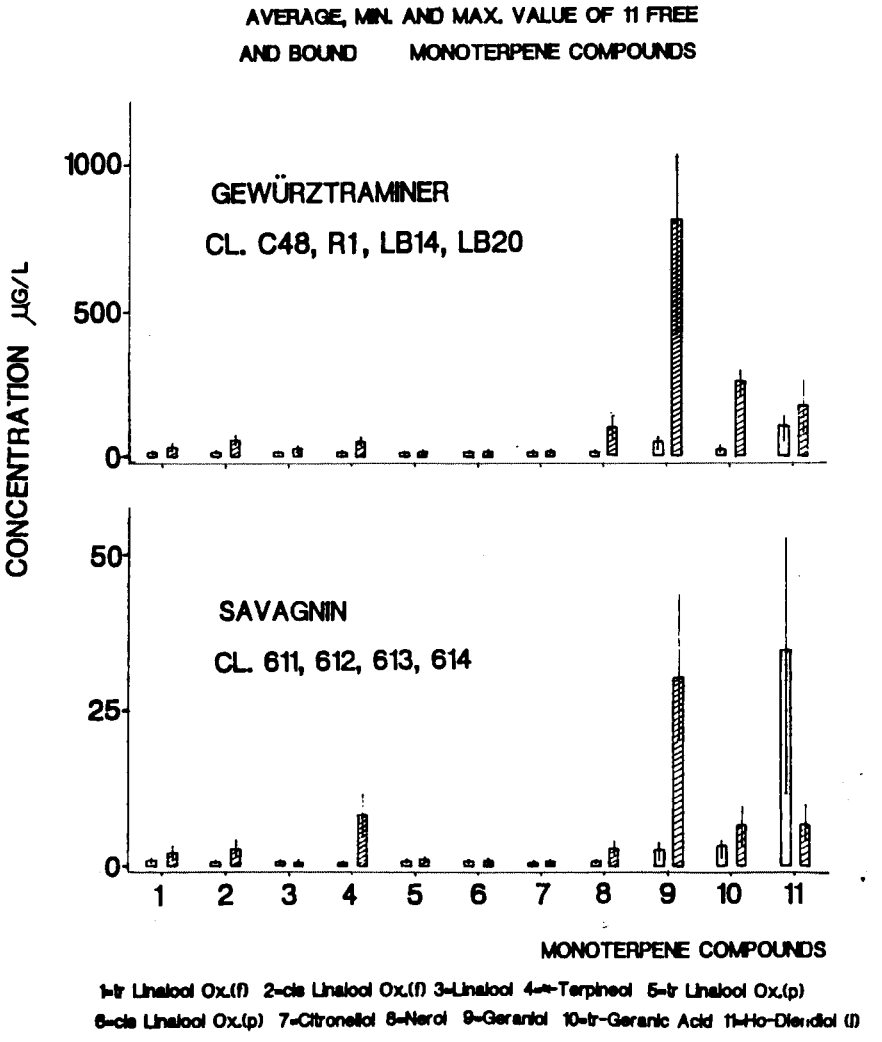


Fig. 3: 'Terpene profiles' from musts of Gewürztraminer and Savagnin clones (Trentino, vintage 1988).

Again a population of unknown clones (18 plants) was taken and the concentrations of free and bound monoterpenes in the grapes were determined. One neutral and two aromatic groups were found by cluster analysis. The monoterpene concentrations of the aromatic groups B and C were about 10 times higher than those of the neutral group A. Again a clear correlation between terpene contents and aromatic flavour can be seen (Fig. 5).

To characterize different clones by analytical methods it is necessary to use only those aromatic compounds which depend as little as possible on vintage. Investigations on the dependence of the evolution of free and bound monoterpene concentrations on the degree of ripeness were carried out over 3 years with Chardonnay clones. The contents of monoterpene compounds were analysed several times during the ripening period (Versini *et al.* 1989).

Fig. 6 shows that the content of free trans-pyranoid-linalooloxide in the more aromatic clone 77 is much higher than in the neutral clone (130 SMA), but the shapes of the curves are comparable. There is an increase at the beginning of the ripening period and a decrease after a certain time. Also the linalool concentrations (Fig. 7) are much higher in the aromatic clone 77 than in the more neutral clones (130 SMA, 116). Evaluation of linalool showed differing tendencies between clones. For the neutral clone 130 SMA, the concentrations of free and bound linalool

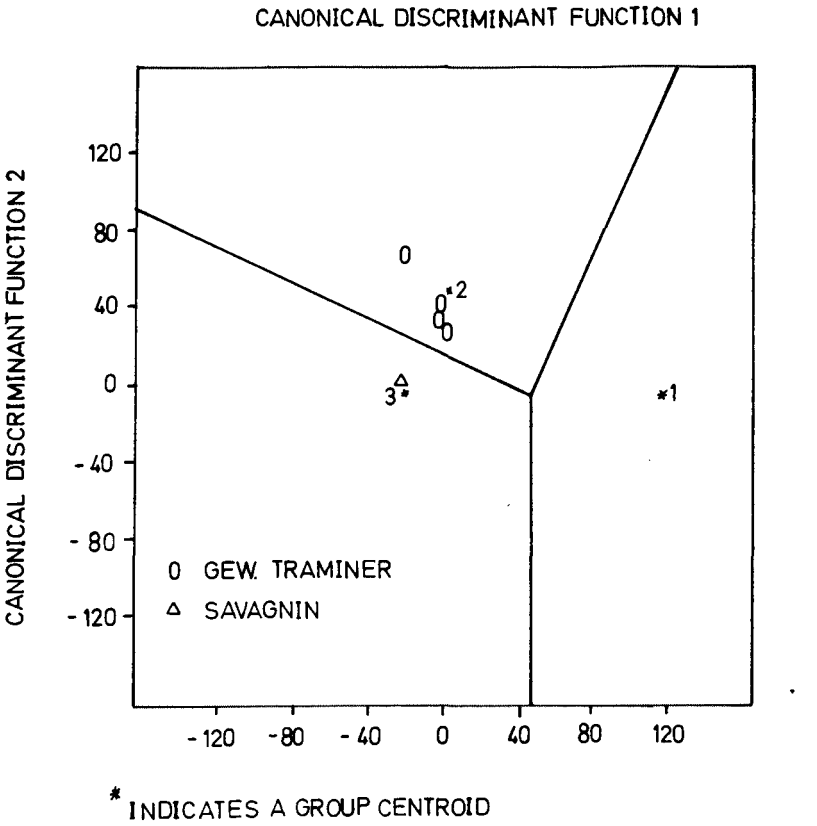
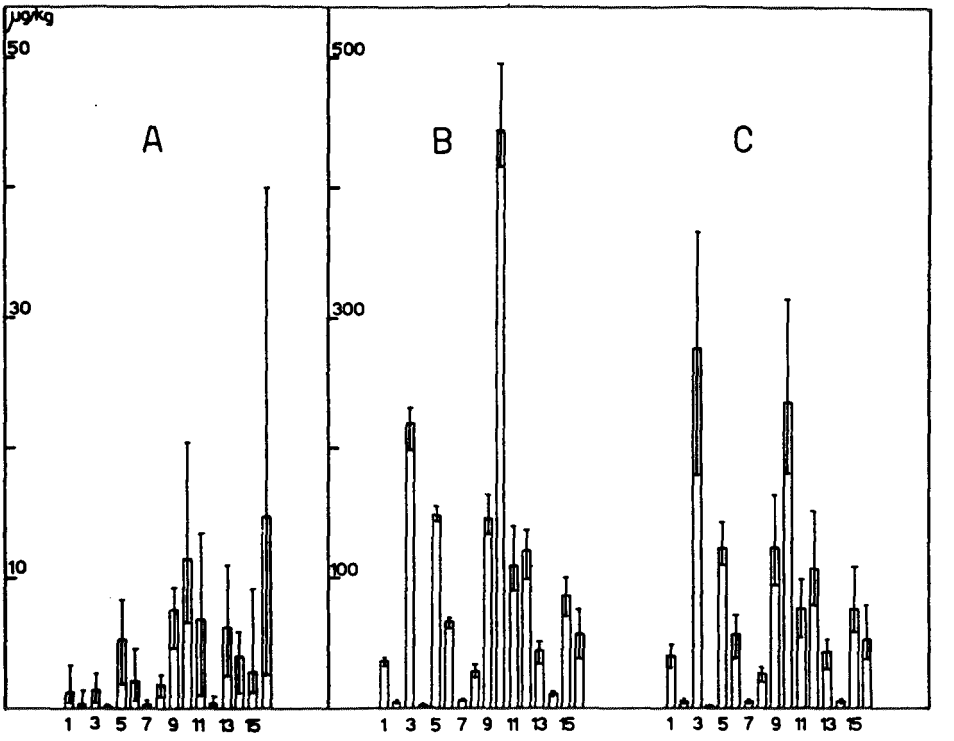


Fig. 4: Discriminant analytical differentiation of 31 Traminer plants in 3 groups, fixed by clustering, using 11 free and bound monoterpene compounds. Inside classification of clonal Gewürztraminer (0) and Savagnin (△).

Table 3: Free (f) and bound (b) components (μg/kg of berries) in Chardonnay grapes (Trentino 1986)

Compounds	Clone Musqué 9-9-'86		Clone 77 12-9-'86		Clone 130 SMA 9-9-'86		Clone 116 9-9-'86	
	(f)	(b)	(f)	(b)	(f)	(b)	(f)	(b)
trans-f-linalooloxide	11	26	3	26	7.5	1.9	54	2.0
cis-f-linalooloxide	2.7	2.0	0.5	1.3	0.4	0.3	4	0.3
linalool	40	383	137	123	1.6	4	3	17
trans-p-linalooloxide	36	6.5	85	5.5	3.9	0.7	1.2	0.3
cis-p-linalooloxide	12	3.1	31	1.3	0.9	0.3	0.5	0.2
nerol	87	67	12.5	10	2.6	7	2.2	2.8
geraniol	325	114	49	19	14	13	9	6
2,6-dimethylocta-1,7-diene-3,6-diol	181	36	221	24	-	-	-	-
(E)-2,6-dimethylocta-2,7-diene-1,6-diol	84	334	108	264	15	72	11	62
(Z)-2,6-dimethylocta-2,7-diene-1,6-diol	535	976	85	258	85	400	83	208
nor-isoprenic dicetone	-	36	-	26	-	70	-	40
3-oxo- α-ionol	-	424	-	200	-	640	-	313
2-phenylethanol	550	77	525	243	622	222	732	93

increase during the ripening period. The aromatic Chardonnay clone 77 shows maxima of linalool concentrations, after which the curves of free and bound linalool decrease to very low concentrations. These tendencies were observed in all 3 years.



A: 'Neutral' 9 plants; B: 'Aromatic' 3 plants; C: 'Aromatic' 6 plants.

Considered compounds:

1: tr-furan linalool oxide
2: cis-furan linalool oxide
3: linalool
4: α-terpineol
5: tr-pyran linalool oxide
6: cis-pyran linalool oxide
7: citronellol
8: nerol
9: geraniol
10: tr-geranic acid
11: ho-diendiol (I)
12: ho-diendiol (II)
13: 8-hydroxy-6.7-dihydrolinalool
14: 7-hydroxycitronellol
15: tr 8-hydroxylinalool
16: cis 8-hydroxylinalool

Fig. 5: 'Terpene profiles' of 3 groups of Chardonnay determined by clustering. Average minimum and maximum concentrations of free monoterpene compounds. - A: Neutral group (9 plants), B: aromatic group I (3 plants), C: aromatic group II (6 plants).

The results show that an analytical characterization based on monoterpene compounds is also possible for different Traminer and Chardonnay clones, but not for the Riesling clones which

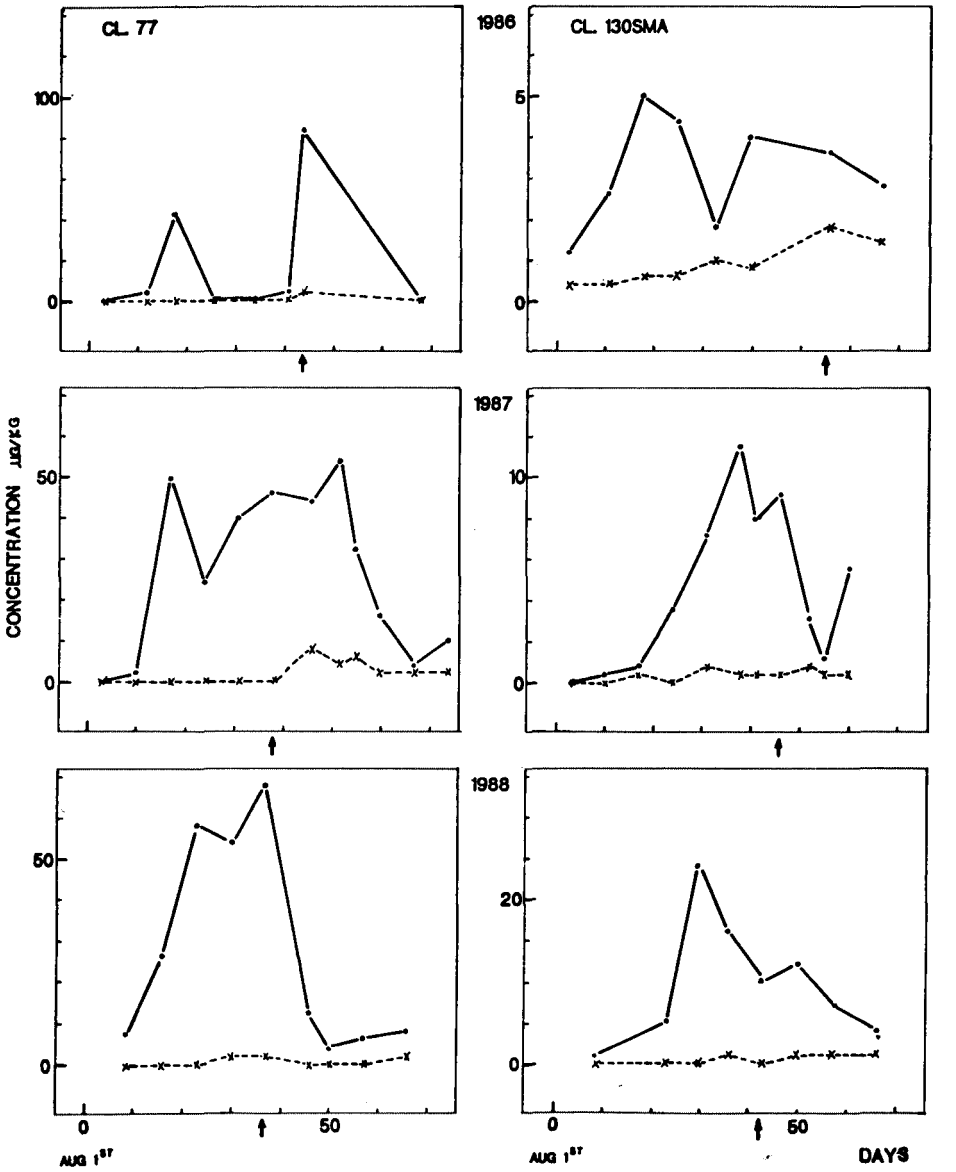


Fig. 6: Chardonnay clones: Evaluation of trans-pyranoid-linalool-oxide during ripening (free = full line, bound = broken line).

have been investigated. For an accurate characterization, the GC analysis should be carried out at comparable degrees of ripeness.

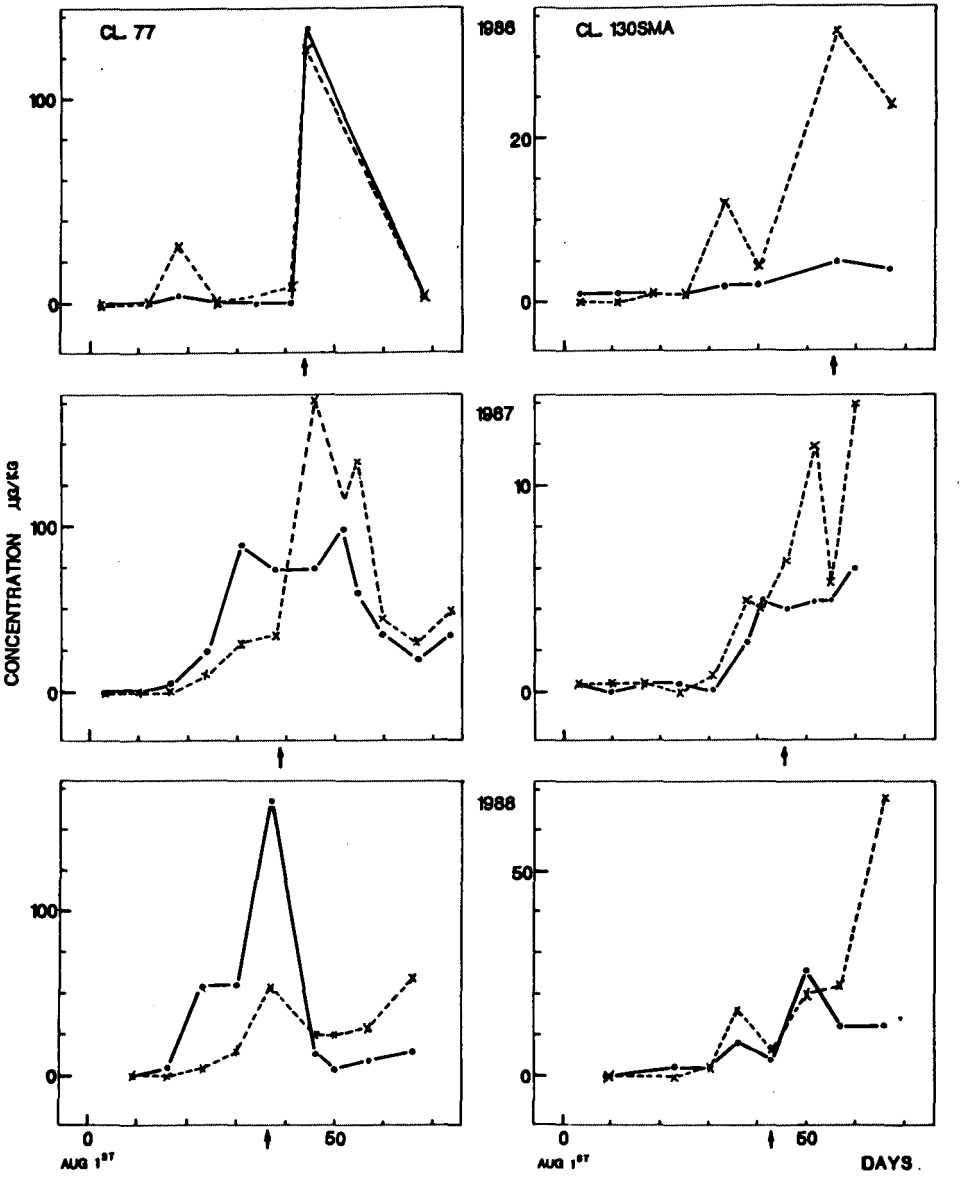


Fig. 7: Chardonnay clones: Evaluation of linalool during ripening (free = full line, bound = broken line).

References

AURICH, M.; VERSINI, G.; DALLA SERRA, A.; 1989: Influenza delle epoche di vendemmia e della macerazione sulle caratteristiche di tipicità dei vini Traminer aromatico dell'Alto Adige. In: SCIENZA, A.; VERSINI, G. (Eds.): Le Sostanze Aromatiche dell'Uva e del Vino, 223-232. San Michele.

RAPP, A.; 1988: Wine aroma substances from gas chromatographic analysis. In: LINSKENS, H. F.; JACKSON, J. F. (Eds.): Modern Methods of Plant Analysis. 6. Wine Analysis. 29-66. Springer-Verlag, Berlin, Heidelberg, New York.

– –; GÜNTERT, M.; 1985: Beitrag zur Charakterisierung des Weines der Rebsorte Weißer Riesling. II. Untersuchung der Aromastoffzusammensetzung deutscher Weißweine der Rebsorten Weißer Riesling, Müller-Thurgau und Silvaner. Vitis **24**, 139-150.

– –; KNIPSER, W.; ENGEL, L.; HASTRICH, H.; 1983: Capillary-chromatographic investigations on various grape varieties. In: CHARALAMBOUS, G.; INGLETT, G. (Eds.): Instrumental Analysis of Foods, 435-454. Academic Press, New York.

– –; – –; HASTRICH, H.; ENGEL, L.; 1982: Possibilities of characterizing wine quality and vine varieties by means of capillary chromatography. Symp. Proc., Univ. of California, Davis, 304-316.

– –; MANDERY, H.; 1986: New progress in vine and wine research. Experientia **42**, 873-884.

VERSINI, G.; DALLA SERRA, A.; DELL'EVA, M.; SCIENZA, A.; RAPP, A.; 1988: Evidence of some glycosidically bound new monoterpenes and norisoprenoids in grapes. In: SCHREIER, P. (Ed.): Bioflavour 87, 161-170. Walter de Gruyter, Berlin, New York.

– –; RAPP, A.; SCIENZA, A.; DELLA SERRA, A.; DELL'EVA, M.; 1989: Nuovi componenti monoterpenici e norisoprenici complessati identificati nelle uve. In: SCIENZA, A.; VERSINI G. (Eds.): Le Sostanze Aromatiche dell'Uva e del Vino, 71-92. San Michele.

VOLKMANN, C.; 1989: Untersuchung flüchtiger Inhaltsstoffe des Trauben- und Weinaromas. Diss. Univ. Karlsruhe.

Effect of vine vigour of *Vitis vinifera* cv. Nebbiolo clones on wine acidity and quality [1])

A. Schneider [2]), F. Mannini [2]), V. Gerbi [3]) and G. Zeppa [3])

[2]) Centro Miglioramento Genetico Vite, CNR, Via P. Giuria, 15, I-10126 Torino, Italy

[3]) Istituto Microbiologia e Industrie Agrarie, Università, Via P. Giuria, 15, I-10126 Torino, Italy

Summary: The grapevine cv. Nebbiolo grown in northern Italy produces high-quality red wines, of which Barolo and Barbaresco are the best known. During a clonal selection project, clones of this variety were assessed for their agronomical and enological value. Different degrees of vegetative vigour were found among them, and this was related to modifications of must and wine composition, with particular respect to the acidity. Over 4 years of observations, vigorous clones produced musts and wines of higher pH, regardless of the amount of titratable acidity. This was associated with a higher malic acid content in the juice and with a higher concentration of potassium in the wine. In addition, wines from vigorous clones showed an unbalanced ratio of colour components. They ranked at the lowest score in the sensory evaluation tests.

Key words: selection, clone, variety of vine, Italy, vigour, growth, shading, yield, must quality, wine quality, acidity, potassium, organic acid, colour, sensory rating.

Introduction

In the span of the last 10 years, in many grape-growing areas a progressive increase in pH of grapes and musts with the consequent production of lower quality wines has been noticed. With the rise in pH is always associated an increase in the content of potassium in the fruit and, sometimes, a decrease in the ratio of tartaric/malic acid. It is evident that early harvest of the grapes or drastic corrective interventions in the musts may not be considered workable solutions when the objective is to obtain wines of high quality.

Numerous causes, of physiological, agronomic and genetic nature, have been proposed to explain the occurrence of this phenomenon. Among these is noted the increased level of potassium fertilization of soil. It has been observed that high availability of K^+ in the soluble pool of the soil raises the absorption of this element by the plant, provoking an accumulation at the foliar level. However, K^+ accumulation occurs especially in soils deficient in this element (Christensen 1975), and in the majority of cases does not cause the same significant K^+ increase in the musts (Murisier *et al.* 1982; Morris *et al.* 1983; Dundon *et al.* 1984).

An increase in berry pH has also been associated with the use of rootstocks and/or scions having an elevated capacity of absorption, translocation and accumulation of K^+ in the fruit (Ough *et al.* 1968; Champagnol 1988). Much proof exists of the different K^+ uptake, translocation and metabolism aptitudes of rootstocks and scions (Hale 1977; Morard *et al.* 1981; Boulay 1982; Scienza *et al.* 1984; Hayes and Mannini 1988).

Even if the increased production per ha has influence on the numerous quality components of the grape, in the case of the pH increase it does not seem to play a direct role. What seems to have a preponderant role is the modification of the microclimate to which the plant is subjected. Acting directly upon the physiology of the plant, the microclimate will influence the metabolism of K^+, malic acid, tartaric acid, and the ion balance in general (Smart 1982; Smart *et al.* 1985). An increase in shading of the leaves, for example, has been positively associated with an increase of K^+ and of pH of the must (Smart 1982; Wolpert *et al.* 1983; Morrison 1988). An increase in leaf shading always accompanies an increase in the vigour of the plant, independently of other cultural

[1]) Contribution no. 210 of the Centro Miglioramento Vite, CNR, Torino.

Table 1: Canopy variable measurements of cv. Nebbiolo clones (1989)

Canopy variable	Clone 111	120	230	141
Total leaf area (m^2)/main shoot	10.9	9.6	8,5	6.9
Total lateral leaf area (m^2)/main shoot	6.9	5.8	4,9	3.7
Mean main shoot length (cm)	286	287	304	320
Mean lateral shoot length (cm)	46	42	32	33
Total leaf area (m^2)/vine	96.3	90.1	83,9	62.3
Total leaf area /canopy surface	2.67	2.50	2,32	1.73

conditions. High vigour may thus influence the metabolism of the plant during ripening, causing substantial modifications in the composition of the fruit. Among the factors which may lead to an increase in vigour, including rational pest control, irrigation and better nutritional state of the soil, the choice of rootstock and scion must not be forgotten. For the latter, the use of virus-free clonal selections is continuously increasing and is often identified with plants of good vigour.

In the course of clonal selection of cv. Nebbiolo, different levels of vegetative vigour have been observed among clones. Reported in the present work are the results of clone productivity and enological characteristics from 4 years of experimentation. In addition, the components of the vegetative vigour of the plants have been analysed.

Materials and methods

Vines of 4 clones of *Vitis vinifera* L. cv. Nebbiolo grafted on Kober 5 BB MI-K-9, free from the most harmful viruses, were grown in a comparison vineyard on a steep slope located in La Morra (north-west of Italy), a temperately continental area (mean annual temperature: 12.4 °C; annual rainfall: 813 mm).

The vineyard was planted in 1978 on a randomized block design in east-west rows, at 3.8 m x 1.0 m spacing in loamy soil of subalkaline pH (7.5) and total carbonate content of 33 %.

Vines were single cane-pruned on a multiwire trellis; the lowest wire carrying a 14-15 bud cane is positioned at 0.5 m from the ground and the highest one, on which the apical tips were twisted, at 2.0 m. Pest, disease and weed control, fertilization and green pruning were in accordance with local practices.

For 4 years, starting when the vines were 6 years old, pruning weight, yield, and juice composition were recorded. Must analyses included percent sugar, titratable acidity, pH, tartaric acid (colorimetric method according to Vidal and Blouin 1978), and malic acid contents (enzyme assays).

Table 2: Vegetative vigour, yield and juice composition of cv. Nebbiolo clones (averages 1983-86)

Clone	111	120	230	141	F Clones	F Years	F CxY
Pruning wt (kg/vine)	1.8	2.0	1.5	1.4	* *	*	n.s.
Yield (kg/vine)	4.3	4.9	4.8	3.2	* *	* *	n.s.
Bunch wt (g)	273	301	334	271	* *	* *	n.s.
Sugar (%)	22.8	23.1	22.3	23.3	* *	* *	*
Titratable acidity (‰)	11.2	10.6	9.8	10.0	* *	* *	* *
pH	3.08	3.10	3.06	3.04	* *	* *	n.s.
Tartaric ac. (‰)	7.1	7.0	8.3	8.4	* *	* *	n.s.
L-malic ac. (‰)	5.7	5.5	3.8	3.4	* *	* *	* *

Wines were made on a small-scale basis (0.5 hl) from each clone, with 6-7 d of skin contact. The following spring, the usual chemical wine analyses were carried out following the official Italian methods. The colour of wines was evaluated for intensity and tint according to SUDRAUD (1958) and total anthocyan pigments were determined by means of the pH difference method (RIBÉREAU-GAYON *et al.* 1972). Sensory evaluations were performed by a panel of 11 experts using both a rank (SALGUES 1977) and a score test (MANNINI *et al.* 1988) at each tasting session.

In 1989, measurements of vine leaf area, shoot length and amount of lateral growth were carried out in order to better assess different levels of clonal vegetative vigour. For each clone, the average main and lateral shoot leaf area and length were measured in July from 6 shoots for each of the two 3-vine replications. This was done in a non destructive way by measuring length (l) and width (w) of each leaf on the wine shoot, and by adjusting the value l x w on the basis of the regression equation coefficients resulting from 100 leaf samples each of the clones collected from neighbouring vines and measured by an areameter (SMITH and KLIEWER 1984).

The distribution of the foliage and canopy shading was also measured on the basis of the leaf layer number by means of the point quadrant technique (MUELLER-DOMBOIS and ELLENBERG 1974).

Results

The 4 clones considered in this study differed markedly for their vegetative vigour as shown by the canopy component values reported in Table 1 and by winter pruning weight (Table 2). Vines of clones 111 and 120 showed higher pruning weight compared with clones 230 and 141. Likewise the measurements of total leaf area, which included the main and the lateral shoots of the vine, gave decreasing values from clone 111 and 120 to clone 230 and 141, suggesting a gradient of plant leaf surface through the 4 clones. In contrast, the length of the main shoots showed the opposite growth trend; shorter shoots being born by the vigorous large-leaved clones 111 and 120, which had, in addition, more extensive lateral growth.

The crop level was lower only in the weakest clone, Nebbiolo 141, with an average yield/vine of 25 % less than the other genotypes. The bunch weight varied among the different clones but independently of their vegetative vigour and crop.

Regarding fruit composition, sugar accumulation was always considerable (higher than 22.3 %), as is necessary for producing the superior aged Barolo and Barbaresco wines made with Nebbiolo grapes.

The titratable acidity in the juice, however, was higher in the vigorous 111 and 120 clones, when compared to the weaker 141 and 230 clones, despite the pH, whose values were slightly

Table 3: Chemical and sensory analyses of wines from cv. Nebbiolo clones (averages 1983-86)

Clone	111	120	230	141
Alcohol (%)	13.3	13.3	12.7	13.2
Extract (‰)	24.2	25.5	23.6	24.1
Ash (‰)	2.5	2.9	2.2	2.2
Alkal. N.	12.2	11.3	10.4	10.0
pH	3.75	3.83	3.53	3.53
Titratable acidity (‰)	5.3	5.3	5.6	5.5
K^+ (‰)	1.27	1.33	0.80	0.89
Tartaric ac. (‰)	1.3	1.2	1.7	1.7
Origin. L-malic ac.(‰)	4.5	4.1	2.9	2.9
Total phenols (‰)	1.8	1.9	1.9	2.0
Total anthocyans (‰)	0.081	0.082	0.100	0.092
Colour intensity ($E_{420nm} + E_{520nm}$) x 10^3	352	363	536	601
Colour tint (E_{420nm}/E_{520nm})	0.91	0.96	0.77	0 .77
Sensory score (%)	62	59	74	72
Sensory ranking sum * (1985-86)	27	35	21	19

* The higher is the ranking sum, the lesser the wine is appreciated.

higher. These results coincide with different contents of the major organic acids in the musts: the amount of malic acid was higher and of tartaric acid slightly lower in the vigorous clones, whereas the opposite situation was found in the weaker ones.

Table 4: Correlation coefficients (r) between colour (intensity and tint) and ion balance components of wines from cv. Nebbiolo clones

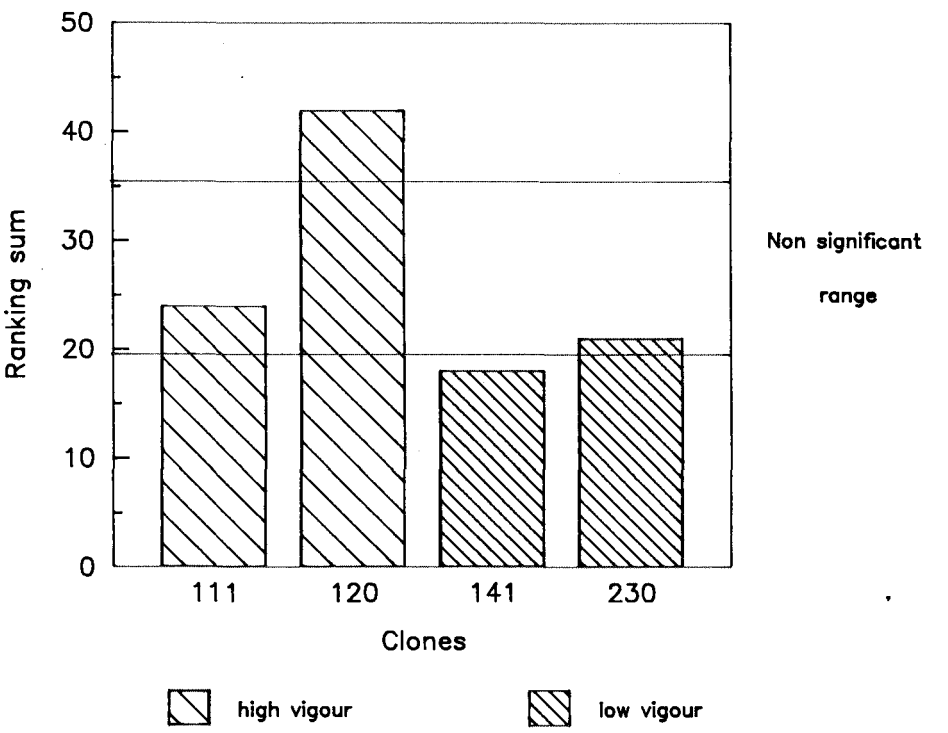
Parameters	Colour components	
	Intensity	Tint
Titratable acidity	+ 0,906 * *	- 0,559 n.s.
[H+]	- 0,810 * *	- 0,682 * *
Ash	- 0,677 * *	+ 0,847 *
Alcalinity N.	- 0,800 * *	+ 0,954 * *
K+	- 0,676 *	+ 0,869 * *
Tartaric acid	+ 0,900 * *	- 0,799 * *
Original malic acid	- 0,251 n.s.	+ 0,222 n.s.

Wine composition, in accordance to the juice analysis results, shows important differences between the more and the less vigorous genotypes, particular with respect to the ion balance and related parameters (Table 3). While extract values and alcohol percentages were similar (though ethanol was slightly lower in clone 230), pH, alkalinity number (ash alkalinity/ash), potassium, ash, and the original l-malic acid contents (calculated from the amount of l-lactic acid resulting from its biological transformation) were markedly higher in the wines from the vigorous clones 111 and 120. Tartaric acid content, in contrast, was slightly lower.

The effect of acidity components on wine colour is demonstrated by the correlations reported in Table 4. It is likely that the ion balance has a major effect on wine colour, thus affecting the visual evaluation in wine sensory analysis.

Wine colour intensity and tint were more favourable in the less vigorous clones, with a higher proportion of red colour components than yellow ones, although total phenol and anthocyanin amounts showed small differences between the 2 groups of clones. It is not by chance that wines from Nebbiolo 111 and 120 (vigorous) were judged lacking for aspect and taste more than for flavour and always ranked at the lowest score.

The ranking test indicated a similar evaluation trend. The results, referring to wines from the 1985 vintage, as shown in the figure, indicate that the wine produced from the moderately vigorous clone 141 was judged significantly better than the wine from vigorous 120.



Results of ranking sum test on 1985 vintage wines from clones of cv. Nebbiolo. The higher the histogram, the lesser the wine is appreciated.

Discussion

Among the clones of cv. Nebbiolo considered in this study, those showing a considerable vegetative vigour always performed very well in terms of yield and sugar accumulation, but produced wines of lower quality, mainly due to the higher amount of K^+ and the lower tartaric/malic ratio, which have a combined effect on both pH and colour of the wine.

In addition to a greater quantity of pruning wood, higher values of total leaf area were recorded in the vines of the vigorous clones. Having the training system of the same type, dimension and geometry for all the clones, this higher total leaf area resulted in a higher ratio of total vine leaf area/exterior canopy surface for the vigorous clones. In other words, their canopy was more crowded, as also confirmed by the fact that the main shoots were shorter despite the presence on them of larger main and lateral leaves and longer lateral shoots. Since the leaf layer number was rather consistent for all the clones (average of 7.4 at 1.8 m from the ground and 4.7 at 1.0 m), the more vigorous ones showed a higher proportion of shaded leaf surface on the total leaf surface caused by leaf overlapping.

Although physiological implications are not yet clear, the influence of leaf shading on grape composition has been already proved (SMART 1982; MORRISON 1988), and our findings confirm the effects on pH, potassium and tartaric/malic ratio in the fruit.

In this experiment, only leaf interior canopy shading was involved, while no differences on bunch shading occurred among the clones, because of the traditional practice of leaf removal in the bunch zone for improving maturity.

Leaf shading, depending on clone vigour, has a genetic origin and this entails important implications for viticultural production. The selection of genotypes of high vigour, which is often coincident with virus-free status, may affect wine quality, not simply as a direct consequence of yield excess and delayed maturity as is sometimes reported, but as a consequence of fruit composition modifications, of which the anion-cation balance and related parameters are the more involved.

Literature cited

BOULAY, H.; 1982: Absorption différenciée des cépages et des porte-greffes en Languedoc. Progr. Agric. Vitic. **99**, 431-434.

CHAMPAGNOL, F.; 1986: L'acidité des moûts et des vins. Facteurs physiologiques et agronomiques de variation. Progr. Agric. Vitic. **103**, 361-374.

– –; 1988: Rôle du potassium dans la physiologie de la vigne. Progr. Agric. Vitic. **105**, 431-435.

CHRISTENSEN, P.; 1975: Long-term responses of 'Thompson seedless' vines to potassium fertilizer treatment. Amer. J. Enol. Viticult. **26**, 179-183.

DUNDON, C. G.; SMART, R. E.; MCCARTHY, M. G.; 1984: The effect of potassium fertilizer on must and wine potassium levels of Shiraz grapevines. Amer. J. Enol. Viticult. **35**, 200-205.

HALE, C. R.; 1977: Relation between potassium and the malate and tartrate contents of grape berries. Vitis **16**, 9-19.

HAYES, P. F.; MANNINI, F.; 1988: Nutrient levels in Sauvignon Blanc grafted to different rootstocks. Proc. 2nd Intern. Symp. Cool Climate Viticulture and Oenology, Auckland, NZ, 43-44.

MANNINI, F.; SCHNEIDER, A.; GERBI, V.; LENZI, R.; 1988: Quadro ampelografico e selezione clonale in Liguria. Atti Accad. Ital. Vite e Vino **39**, 157-171.

MORARD, P.; TORRES, P.; ANDRÉ, L.; 1981: Influence des porte-greffes sur la nutrition minérale de la vigne (variété Grenache). Progr. Agric. Vitic. **98**, 604-608.

MORRIS, J. R.; SIMS, C. A.; CAWTHON, D. L.; 1983: Effect of excessive potassium levels on pH, acidity and color of fresh and stored grape juice. Amer. J. Enol. Viticult. **34**, 35-39.

MORRISON, J. C.; 1988: The effect of shading on the composition of Cabernet Sauvignon grape berries. Proc. 2nd Intern. Symp. Cool Climate Viticulture and Oenology, Auckland, NZ, 144-146.

MUELLER-DOMBOIS, D.; ELLENBERG, H.; 1974: Aims and Methods of Vegetation Ecology, 84-90. Ed. John Wiley & Sons, N.Y.

MURISIER, R.; RYSER, J. P.; AERNY, J.; 1982: Fumure potassique et acidité des moûts. Rev. Suisse Viticult. Arboricult. Horticult. **14**, 33-36.

OUGH, C. S.; LIDER, L. A.; COOK, J. A.; 1968: Rootstock-scion interactions concerning wine making. I. Juice composition changes and effects on fermentation rate with St. George and 99-R rootstocks at two nitrogen fertilizer levels. Amer. J. Enol. Viticult. **19**, 213-227.

RIBÉREAU-GAYON, J.; PEYNAUD, E.; SUDRAUD, P.; RIBÉREAU-GAYON, P.; 1972: Sciences et Techniques du Vin. Ed. Dunod, Paris.

SALGUES, M.; 1977: La pratique de la dégustation du vin et son interprétation statistique. Progr. Agric. Vitic. **94**, 78-87, 112-123, 137-139.

SCIENZA, A.; CASASSA, M. T.; VISAI, C.; CONCA, E.; 1984: Il controllo genetico della nutrizione potassica nella vite. Riv. Viticolt. Enol. **37**, 682-691.

SMART, R. E.; 1982: Vine manipulation to improve wine grape quality. Proc. Symp. Grape Wine Cent. Davis, Ca, USA, 362-375.

-- -- ; 1985: Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A review. Amer. J. Enol. Viticult. **36**, 230-239.

-- -- ; ROBINSON, J. B.; DUE, G. R.; BRIEN, C. J.; 1985: Canopy microclimate modification for the cultivar Shiraz. I. Definition of canopy microclimate. Vitis **24**, 17-31.

SMITH, R. J.; KLIEWER, W. M.; 1984: Estimation of Thompson Seedless grapevine leaf area. Amer. J. Enol. Viticult. **35**, 16-22.

SUDRAUD, P.; 1958: Interprétation des courbes d'absorption des vins rouges. Ann. Techn. Agric. **7**, 203-208.

VIDAL, M.; BLOUIN, J.; 1978: Dosage colorimétrique rapide de l'acide tartrique dans les moûts et les vins. Rev. Fr. Oenol. **16**, 39-46.

WOLPERT, J. A.; HOWELL, G. S.; MANSFIELD, T. K.; 1983: Sampling Vidal Blanc grapes. I. Effect of training systems, pruning severity, shoot exposure, shoot origin, and cluster thinning on cluster weight and fruit quality. Amer. J. Enol. Viticult. **34**, 72-76.

Brief ampelographic characterization of indigenous grapevine cultivars subjected to clonal selection in Turkey

Y. S. Agaoglu [1]), H. Çelik [1]) and E. Gökçay [2])

[1]) Department of Horticulture, Faculty of Agriculture, University of Ancara, 06110 Ankara, Turkey

[2]) Viticultural Research Institute, Manisa, Turkey

Summary: A unique national clonal selection programme is still being conducted on 24 indigenous table (15 white, 6 black, 3 red), 16 wine (7 white, 9 red) and 4 raisin (2 white seedless, 2 white seeded) grape cultivars in 9 agricultural regions of Turkey. As the results of this programme, 127 candidate clones belonging to 13 cultivars have been selected.

This paper also includes a brief ampelographic description of the indigenous Turkish grape cultivars subjected to clonal selection, based mainly on fruit characteristics, growth, productivity and ripening periods in their primary locations.

Key words: selection, clone, variety of vine, table grape, wine grape, raisin, Turkey, ampelography, fruit, growth, yield, maturation.

Introduction

Viticulture is still one of the most important horticultural enterprises in Turkey with 590,000 ha of area and 3,500,000 t of production in 1988, in spite of a serious phylloxera invasion, especially in central and south-east regions.

Today, 42 table (33 indigenous, 9 introduced), 32 wine (20 indigenous, 12 introduced) and 4 raisin (2 seedless, 2 seeded – all indigenous) standard grape cultivars are being grown in different localities of Turkey.

Studies on the clonal selection of grape cultivars to select virus-free superior clones with vigorous growth, high yield and quality, resistance to low temperatures and certain fungal diseases (downy and powdery mildew, grey mold and dead-arm) were started in 1964. In 1979, a national clonal selection programme with a three-step (mass selection, clone collection (A), and clone comparison (B)) standardized method (Agaoglu 1981) were prepared and former studies have been adapted to this method (Anonymous 1979).

Materials and methods

The national clonal selection programme is still being carried out on 59 grapevine cultivars (44 indigenous, 5 introduced). All indigenous cultivars with the exception if Isikli, Bilecik Irikarasi and Osmancik are standard-selected varieties.

In the present paper, brief ampelographic characters of Turkey's indigenous grape varieties subjected to clonal selection were described using the methods of Galet (Morton 1979) and IBPGR (Anonymous 1983). In addition to these descriptions, some definitive information on type and region of the cultivars and stage of selection were also presented.

Results and discussion

As a result of the clonal selection programme on 24 table, 16 wine and 4 raisin cultivars grown in central-north (4), Aegean (11), Marmara-Thrace (11), Mediterranean (2), north-east (1), south-east (7), central-east (4) and central-south (4) agricultural regions of Turkey, 127 candidate clones

Table 1: Clonal selection programme conducted on indigenous grape cultivars in Turkey

Cultivar	Type	Region	Stage of selection	Candidate clones
Amasya beyazı	WT	Marmara	Clone collection(A)	
Beyaz Çavuş	WT	Marmara	Clone comparison (B)	10
Bozcaada Çavuşu	WT	Aegean	Clone collection(A)	
Dımışkı	WT	Southeast	Mass selection	
Erenköy beyazı	WT	Marmara	Clone comparison(B)	10
Hafızali	WT	Marmara	Clone comparison(B)	10
Hafızali	WT	Thrace	Clone comparison(B)	5
Işıklı	WT	Mediterranean	Clone collection(A)	
İpek	WT	Aegean	Mass selection	
Kozak beyazı	WT	Aegean	Clone collection(A)	
Müşküle	WT	Marmara	Clone comparison(B)	15
Osmancık	WT	Aegean	Mass selection	
Parmak	WT	Centralsouth	Mass selection	
Razakı	WT	Marmara	Clone comparison(B)	15
Razakı	WT	Aegean	Clone collection(A)	
Tahannebi	WT	Southeast	Mass selection	
Tarsus beyazı	WT	Mediterranean	Clone collection(A)	
Bilecik İrikarası	BT	Marmara	Clone collection(A)	5
Değirmendere siyahı	BT	Marmara	Clone comparison(B)	8
Gül üzümü	RT	Centralnorth	Mass selection	
Hönüsü	RT	Southeast	Mass selection	
Karaerik	BT	Northeast	Mass selection	
Karagevrek	BT	Centralnorth	Mass selection	
Kozak siyahı	BT	Aegean	Clone collection(A)	
Pembe Gemre	RT	Aegean	Mass selection	
Siyah Gemre	BT	Centralsouth	Clone collection(A)	
Beylerce	WW	Marmara	Clone comparison(B)	10
Dökülgen	WW	Southeast	Mass selection	
Emir	WW	Centralsouth	Mass selection	
Hasandede	WW	Centralnorth	Mass selection	
Narince	WW	Centraleast	Mass selection	
Sungurlu	WW	Centraleast	Mass selection	
Yapıncak	WW	Thrace	Clone comparison(B)	5
Ada karası	RW	Marmara	Clone collection(A)	
Boğazkere	RW	Centraleast	Clone collection(A)	
Çal karası	RW	Aegean	Clone collection(A)	
Dirmit	RW	Centralsouth	Mass selection	
Horoz karası	RW	Southeast	Mass selection	
Kalecik karası	RW	Centralnorth	Clone comparison(B)	23
Karasakız	RW	Aegean	Clone collection(A)	
Öküzgözü	RW	Centraleast	Clone collection(A)	
Papaz karası	RW	Thrace	Clone comparison(B)	5
Yuvarlak Çekirdeksiz (Round Seedless)	WR	Aegean	Clone comparison(B)	6
Sultani Çekirdeksiz	WR	Aegean	Clone collection (A)	
Besni	WR	Southeast	Mass selection	
Rumi	WR	Southeast	Mass selection	

Symbols: WT: White Table, BT: Black Table, RT: Red Table. TOTAL : 127
WW: White Wine, RW: Red Wine, WR: White Raisin.

belonging to 13 cultivars (8 table, 4 wine cultivars, 1 raisin cultivar) have been selected as shown in Table 1. After the investigations of Agaoglu and Çelik (1985), 54 new candidate clones of Hafizali (5), Müsküle (5), Razaki (5), Bilecik Irikarasi (5), Kalecik karasi (23), Papaz karasi (5) and Yuvarlak çekirdeksiz (6) grapevine cultivars were selected.

The stages of clonal selection studies on indigenous Turkish grapevine cultivars are also presented in Table 1.

Table 2: Brief ampelographic characterization of indigenous table and raisin grape cultivars

	Bunch			Berry				Growth and Yield		
Cultivar	Shape	Size	Density	Shape	Size	Color	Seed	Growth-Habit	Yield	Maturity
TABLE										
Amasya beyazı	conical	large	loose	round	large	green-yellow	1-2	very strong	high	medium
Bilecik İrikarası	shoul-conical	large	loose	long obovate	very large	black	1-4	strong	high	very late
Beyaz Çavuş(FF)*	winged-conical	large	loose	elliptic	large	green-yellow	1-2	strong-erect	high	early-medium
Bozcaada Çavuşu(FF)	winged-conical	large	loose	round	large	green-yellow	1-3	strong	high	early-medium
Değirmendere Siyahı	shoul-conical	medium	medium	elliptic	medium	blue-black	2-3	very strong	high	very late
Dimişki	shoul-conical	medium	loose	long elliptic	large	green-yellow	2-3	strong	high	medium
Erenköy Beyazı	short cylindrical	medium	medium	round	medium	yellow	2-3	strong-erect	high	late
Gül üzümü	cylindrical	medium	dense	short elliptic	medium	rose	2	medium-erect	low	medium
Hafızali	shoul-conical	large	loose	long elliptic	large	green-yellow	2	very strong	high	medium-late
Hönüsü(FF)	shoul-pramidial	large	loose	obovate	large	red-violet	1-2	strong	medium	late
Işıklı	cylindrical	large	loose	elliptic	large	green-yellow	2	strong	medium	medium-late
İpek	winged conical	large	medium	elliptic	large	yellow	2-3	strong	high	medium-late
Karaerik	shoul-conical	large	medium	round	large	blue-black	2-3	medium	high	medium
Karagevrek(FF)	shoul-conical	large	dense	elliptic	medium	black	3-4	strong	high	medium
Kozak beyazı	shoul-conical	medium	loose	elliptic	large	green-yellow	2-3	strong-erect	high	medium-late
Kozak siyahı	shoul-cylindr.	large	loose	round	medium	red-violet	2-3	medium-horizontal	medium	medium-late
Müşküle	shoul-conical	large	loose	short elliptic	large	light-yellow	2-3	strong-erect	high	late
Osmancık	conical	large	loose	round	large	green-yellow	2	strong	medium	late
Parmak	shoul-conical	medium	loose	long elliptic	large	green-yellow	2-3	strong-horizontal	high	medium
Pemce Gemre	winged conical	large	loose	round	large	red-yellow	2-3	strong	medium	late
Razakı	shoul-conical	large	loose	long elliptic	large	green-yellow	2-3	strong	high	medium
Siyah Gemre	shoul-conical	medium	loose	ovate	large	violet	2-3	medium	medium	late
Tahannebi (FF)	winged-conical	medium	medium	ovate	large	light-yellow	1-2	strong	medium	early
Tarsus beyazı	winged-conical	medium	medium	round	medium	light-yellow	2-3	medium-erect	medium	very early
RAISIN										
Yuvarlak Çekirdeksiz Sultani	winged-conical	medium	medium	round	small	yellow	-	strong	very high	medium
Çekirdeksiz	winged-conical	large	medium	elliptic	small	yellow	-	very strong	very high	medium
Besni	shoul-conical	large	loose	long elliptic	large	yellow	2-3	strong	high	medium
Rumi	winged-conical	medium	dense	long elliptic	medium	yellow	3-4	strong	high	medium

* Functionally Female

Table 3: Brief ampelographic characterization of indigenous wine cultivars

	Bunch			Berry				Growth and Yield		
Cultivar	Shape	Size	Density	Shape	Size	Color	Seed	Growth-Habit	Yield	Maturity
Adakarası	winged	medium	dense	ovate	small	blue-black	2-3	medium	medium	medium
Beylerce	conical	medium	dense	elliptic	small	light green	2-3	medium	medium	medium
Boğazkere	winged	medium	dense	round	medium	blue-black	2-3	strong	medium	late
Çal karası	shouldered	small	dense	elliptic	small	black	2-3	strong	high	medium
Dirmit	winged	medium	dense	round	medium	red-violet	1-2	strong	high	medium
Dökülgen	winged-conical	medium	dense	round	large	green-yellow	2-3	strong	high	medium-late
Emir	winged-conical	medium	dense	round	medium	green-yellow	2-3	strong	high	medium-late
Hasandede	conical	large	medium	round	medium	green-yellow	2-3	strong-erect	high	medium
Horoz karası	shoul-conical	medium	loose	long elliptic	large	black	2-3	medium	medium	early-medium
Kalecik karası	winged-conical	medium	dense	round	medium	blue-black	1-2	strong-erect	medium	medium
Karasakız	winged-conical	large	dense	round	medium	red	2-3	medium-erect	high	medium
Narince	winged-conical	medium	dense	elliptic	medium	green-yellow	2-3	strong	high	medium
Öküzgözü	winged-conical	large	dense	round	large	blue-black	2-3	strong-horizontal	high	late
Papaz karası	conical	medium	dense	round	small	red-violet	2-3	strong	very high	very late
Sungurlu	conical	medium	loose	round	medium	green	2-3	strong	high	medium
Yapıncak	winged-conical	medium	dense	round	medium	green-yellow	2-3	strong-erect	very high	medium

Table 4: Rational presentation of ampelographic characters of indigenous grapevine cultivars subjected to clonal selection in Turkey

		Conical			Cylindrical			Winged		
		T	W	R	T	W	R	T	W	R
Bunch	Shape	79.2	68.8	100.0	16.7	-	-	-	18.8	-
		Large			Medium			Small		
	Size	62.5	18.8	50.0	37.5	75.0	50.0	-	-	6.3
		Dense			Medium			Loose		
	Density	8.3	81.3	25.0	25.0	6.3	50.0	66.7	12.5	50.0
		Round			Elliptic			Ovate or Obovate		
	Shape	33.3	68.8	25.0	50.0	25.0	75.0	16.7	6.3	-
		Large or Very Large			Medium			Small		
	Size	66.7	18.8	25.0	25.0	56.3	25.0	-	25.0	50.0
Berry		Green or Yellow(White)			Blue or Black			Rose-Red-Violet		
	Color	62.5	43.8	100.0	16.7	37.5	-	20.8	18.8	-
		Seedless			1-2			2-3			3-4		
	Seed	T	W	R	T	W	R	T	W	R	T	W	R
		-	-	50.0	33.3	12.5	-	54.2	87.5	25.0	4.2	-	25.0
		Very Strong			Strong			Medium		
	Growth	T	W	R	T	W	R	T	W	R
		12.5	-	25.0	66.7	75.0	75.0	16.7	25.0	-
Growth and Yield		Very high			High			Medium		
	Yield	-	12.5	50.0	62.5	56.3	50.0	33.3	31.3	-
		Early or Early Medium			Medium or Medium Late			Late or very Late		
	Maturity	16.7	6.3	-	50.0	75.0	100.0	33.3	18.8	-

While the studies are progressing well in Marmara-Thrace and Aegean regions and satisfactorily in south-east and Mediterranean regions, serious retardation problems, especially in central regions, have to be overcome.

Brief ampelographic characters of indigenous grapevine cultivars subject to clonal selection are presented in Tables 2 and 3. Rational (%) presentation of the ampelographic character can also be seen in Table 4.

Turkish table grape varieties generally have large, conical and loose bunches; round or elliptic and large berries with 2-3 seeds; strong growth, high yield, and medium or late maturity. Our world-famous seedless varieties can be characterized by their winged-conical, medium or large bunches with medium density, round (Yuvarlak Çekirdeksiz) or elliptic (Sultani Çekirdeksiz), small, yellow berries, strong to very strong growth, and very high yield.

Indigenous wine grape varieties subject to clonal selection generally have winged or winged-conical, medium-size and dense bunches; round, medium to small berries with 2-3 seeds; strong growth, high yield, and medium time maturity. Kalecik karasi (RW) and Narince (WW) are the superior wine cultivars.

References

AGAOGLU, Y. S.; 1981: Studies on the vine clonal selection in Turkey. 3rd Intern. Symp. Clonal Selection in Vines, 8-12 June 1981, Conegliano (Italy), 121-125.

-- -- ; ÇELIK, H.; 1985: Conservation of germplasm of *Vitis vinifera* L. in Turkey. 4th Intern. Symp. Grapevine Breeding, 13-18 April 1985, Verona (Italy), 40-42.

ANONYMOUS; 1979: National Project on Viticultural Researches. Application Project on Vine Clonal Selection [Turk.]. Ankara.

ANONYMOUS; 1983: Descriptors for Grape. International Board for Plant Genetic Resources (IBPGR), Rome.

MORTON, L. T.; 1979: A Practical Ampelography. Grapevine Identification. (Translated and adapted from P. GALET.) Cornell University Press, Ithaca (USA).

Selection of highly productive grape variations using methods of multidimensional analysis

L. P. TROSHIN

National Institute for Grape and Products of Grape Processing 'Magarach', 31, Kirov Street, Yalta, Crimea, USSR

Summary: A method of clonal grape selection that allows the identification of genotypes with high productivity, based on phenotypes, with a substantial decrease in the duration of the process of selection is proposed.

Multi-stage selection of the best plants is carried out in the same stock nursery during 4-5 years. 5-6 % of the selected plants include mutated and modified variations characterized not only by high productivity, normal vigor, good quality of fruit and good sanitary condition of vines, but also by a stable expression of these desirable characters.

Key words: clone, selection, yield, fruit, must quality, growth, shoot, leaf, biometry, analysis, USSR.

Introduction

Progress in clonal selection achieved in viticulture of different countries of the world displays great potentials of using the vegetative variability of grape cultivars. Clonal selection has been performed in the Soviet Union since the 1930s (MERZHANIAN and ZELENIN 1932) and, as a result, highly productive plants have been selected for a number of recommended cultivars (GOLODRYGA *et al.* 1975, 1976, 1980; KARAJI and KAISYN 1977; SOLDATOV 1984; TULAYEVA 1986; GAVAKETASHVILI 1986; SERGIYENKO *et al.* 1986).

The USSR State Commission for the Testing of Agricultural Crops has officially registered eight of clones accepted for testing (JENEYEV *et al.* 1984). Presently, research is being performed according to the methodology approved at the First International Symposium for Clonal Selection in Western Germany (1984) and aimed at nearly 20 year testing (SCHÖFFLING 1984).

The complex nature and long duration of clonal selection make it necessary to use scientific achievements to increase the effectiveness of plant selection and to accelerate this process. We have developed a method of clonal selection that allows us to effectively identify genotypes of promising highly productive plants after their phenotypes, the duration of the process of selection thus being substantially reduced.

The method is based on computerized techniques for analysis of complexes of quantitative characters using the method of protein electrophoresis. The method includes two new elements: multi-stage selection for productivity and selection of highly productive clones based on a complex of characters (ZHIVOTOVSKY *et al.* 1984).

Genetic improvement of a cultivar is more effective and profitable when the method of multi-stage selection is used. This method should be used to establish basic vineyards that include representatives of all positive clones and clone stock nurseries consisting of the best clones that have been propagated.

Multi-stage selection of the best plants of a cultivar is carried out during 4 or 5 years in the same vineyard, stock nursery or stock nursery of primary selection. The process works as follows: In the first year, 50 % of the best symptomless plants with regard to a complex of desirable characters will be selected. This is the first stage of selection. In the second year, 50 % of the plants selected in the first year (or 25 % of the total amount of plants in the planting site) are selected. This is the second stage of selection. In the third year, 50 % of the plants selected in the second year (or 10-12 % of the initial amount of plants) are selected (the third stage of selection). In the fourth year,

Table 1: Variability of quantitative characters in the vegetative progeny at different stages of selection, cv. Aligoté

Characters	Vegetative progeny					
	First stage of selection			Second stage of selection		
	trunk height: 70 cm	trunk height: 120 cm	average	lower part of the terrace	upper part of the terrace	average
Yield per vine, kg	7.47	8.39	7.93	8.58	9.03	8.80
	49.0	41.7	45.4	37.3	34.6	35.9
Sugar, g/100 cm^3	15.3	15.3	15.3	16.9	17.1	17.0
	15.2	12.8	13.0	12.9	12.0	12.4
Increase in growth, m	12.7	13.2	13.0	19.5	19.3	19.4
	48.2	35.6	41.8	31.8	31.1	31.5
Coefficient of fruiting*	1.70	1.67	1.69	1.92	1.95	1.93
	23.0	19.2	21.1	19.8	15.1	17.5
Average weight of bunch, g	124.1	131.4	127.8	171.1	175.0	173.0
	29.0	23.1	26.0	20.9	18.7	19.8
Yield per shoot, g	196.6	202.3	199.5	310.5	329.9	320.2
	37.4	32.3	34.8	31.0	27.0	29.0
Shoot productivity: sugar, g	30.4	29.9	30.2	55.2	57.9	56.5
	37.9	35.3	36.6	31.5	30.2	30.9

* Total of bunches/total of shoots ratio

Upper lines give average values of the characters.

Lower lines give coefficients of variation of the same characters for the five-year period of observation.

50 % of the plants selected in the third year (or 5-6 % of the plants evaluated during the 3 years of selection of the best symptomless plants) are selected (the fourth stage of selection).

Individual records and observations are carried out according to standard ampelographic programs. If virus or other infectious diseases occur, they are registered annually in the adequate vegetation periods based on triple examination of plants sampled. In autumn of the fourth and the fifth years of selection, cuttings are taken from plants selected due to their positive characters. These cuttings are utilized to establish a clone testing site or a basic vineyard and testing for virus carriage is conducted simultaneously.

5-6 % of the plants selected include mutated and modified variations displaying not only high productivity, good quality of fruit, normal vigor and good sanitary condition of vines, but also stabile expression of these desirable characters.

Materials and methods

During 1966-69, the best Aligoté plants were selected from 10,000 vines at the Steppe Experimental Farm of the Institute 'Magarach'. Cuttings were taken from these plants and used to establish a cultivar stock nursery covering the area of 3.5 ha. 3 years after the nursery stock had been established, improving selection was begun to obtain phenotypically stable, healthy and highly productive plants. Cuttings were also taken from these plants and used to establish another own-rooted stock nursery (3 ha, vine spacing of 3.0 x 1.5 m) which contained the vegetative progeny of the first stage of selection. (This own-rooted stock nursery is situated in the central part of the third terrace of Alma River; trunk height of vines is 70 and 120 cm). The following year, 4 years after the cultivar stock nursery had been established, improving selection was undertaken again based on the pooled 2-year yield per vine, and cuttings from the selected plants were prepared. Grafted cuttings (Kober 5 BB used as rootstock) were grown and used to establish another stock nursery consisting of the vegetative progeny of the second stage of selection (6.1 ha, vine spacing of 3.0 x 1.5 m). (The nursery is situated in the lower and upper parts of the same terrace; trunk height of vines is 70 cm).

Results and discussion

Average yield per vine and coefficients of variation are given in Table 1 (1980-84). The sampling size for each of the 4 variants was constant for the 5 years of the experiment (50); the total amount of plants evaluated was 200; the same plants were always examined.

Especially demonstrative was the decrease in the variability of the complex of characters investigated when the pooled coefficient of variation was used, accounting for both the variability of individual characters and the extent of their interrelationship (Zhivotovsky 1980).

For the cultural characters studied, the pooled coefficients of variation for the vegetative progeny of the first and the second stages of selection were 18.3 and 13.9 %, respectively.

It is well known that any controlled selection, the method of multi-stage selection included, results both in an increase in the expression of a desirable character and in a disequilibrium between this very character and the other ones (Moser 1966; Altukhov 1981). That is why we think it is useful to practice clonal selection both for productivity and a complex of other quantitative characters. This approach makes it possible to reveal plants which differ from the remainder of a given population in a number of characters (not only in desirable characters).

In order to do it, one has to analyze not only cultural characters (yield per vine, sugar, increase in growth, average bunch weight, acidity, etc.), but also morphological ones that may not be directly related to productivity (Table 2). Using factorial analysis (Kendall and Stuart 1976), one can reveal the effect of factors influencing the variability of correlating characters and evaluated as main components that prove to be statistically independent (non-correlated). These 'masked' factors that cannot be measured directly seem to reflect the way genetic mechanisms work. One cannot always interpret these mechanisms, while the phenomenological interpretation of the factors is possible.

Such main components were obtained for the two groups of characters (cultural and morphological ones) based on Aligoté plants (Table 3). As far as cultural characters are concerned, they are interpreted as follows:

I: 'yield potential' is determined by pooled contribution of such factors as totals of buds, shoots and bunches per vine, vigor and productivity itself;

II: 'taste index' is determined by the sugar/acidity ratio;

III: 'dry matter' is determined by contents of dry matter in the juice.

It can be seen from Table 3 that the individual variability of the three largest components makes 80-90 % of the total variability of the characters analyzed. Thus, the method of main

Table 2: Values of cv. Aligoté parameters

Parameters	Arithmetic average	Coefficients of variation	symmetry	excess
1. Petiole length, mm	94.9	9.3	0.22	-0.34
2. Leaf length, mm	166.8	6.0	0.37	0.53
3. Leaf width, mm	177.7	7.2	0.63	0.64
4. Midrib vein length, mm	120.8	6.0	-0.32	0.13
5. Upper lateral vein length, mm	109.6	6.2	0.14	0.76
6. Lower lateral vein length, mm	80.8	7.6	-0.40	0.58
7. Distance from leaf centre to upper sinus, mm	85.9	6.3	-0.03	-0.32
8. Distance from leaf centre to lower sinus, mm	70.8	7.3	0.06	0.02
9. Angles °: alpha	50.0	5.4	0.30	0.02
10. beta	46.6	6.2	0.11	-0.32
11. gamma	43.0	7.5	0.35	0.31
12. Total of eyes per vine	36.0	25.9	0.62	0.29
13. Total of shoots per vine	31.2	24.0	0.68	0.97
14. Total of fruiting shoots per vine	28.5	23.5	0.72	0.96
15. Total of bunches per vine	61.7	25.9	0.52	-0.01
16. Yield per vine, kg	9.85	29.0	0.89	0.74
17. Sugar, g/100 cm^3	16.8	13.6	1.03	0.81
18. Titrable acids, g/dm^3	6.4	13.5	-0.03	1.22
19. Increase in growth, m	22.3	27.2	0.66	-0.10
20. Germinated eyes, %	95.6	3.7	-1.09	1.92
21. Fruiting shoots, %	91.4	7.1	-0.38	-0.68
22. Coefficient of fruiting	1.97	11.0	-0.28	-0.99
23. Coefficient of fruitfulness	2.16	8.4	-0.00	-0.46
24. Bunch weight, g	162.4	17.8	-0.46	-0.19
25. Yield per shoot, g	321.0	24.2	0.73	1.55
26. Shoot productivity: sugar, g	54.3	29.5	0.54	0.68

components makes it possible to reduce the multidimensional space of 8 or 11 characters mostly to the tri-dimensional space. The results of the analysis can then be plotted in the plane of the axes of the respective pairs of the components: I and II, I and III, II and III. In this case, the differences among the plants are much more pronounced than when the plants are compared based on the initial characters. Yet, one must admit that this approach is not always suitable as it is based on the visual estimate of deviations and because of the fact that using but the three main components results in the loss of certain information.

Taking into account the disadvantages of the method of main components, Zhivotovsky (1984) developed a statistic method for estimation of index do, the index of typicity in this paper.

Table 3: Influence of the main components on the variability of quantitative characters, cv. Aligoté

Years	Components, %			
	I	II	III	Total
	8 cultural characters			
1980*	68.1	14.7	7.8	90.6
1981	65.9	12.8	12.0	90.7
1982	71.5	10.8	9.0	91.3
1983	57.6	14.3	13.6	85.5
1984	55.0	20.2	12.3	87.5
	11 morphological characters			
1981	64.5	15.6	6.8	86.9
1982	59.2	14.8	9.9	83.9
1983	56.2	16.5	6.7	79.4
1984	60.4	11.5	9.4	81.3

* Titrable acidity was not taken into account.

The index of typicity is convenient for comparing 'distances' estimated after different sets of characters (Table 4), the 'distance' of a plant from the 'centre' of the distribution of characters in a given population being a measure of its typicity compared to the conventional plant with the average values of characters.

As it is seen from Table 4, one can reveal plants with maximum values of the index of typicity do (plants 36, 35, etc.) and those with minimum values of the index of typicity (plants 18, 28, etc.). Because each of these groups includes plants with different productivity, only the plants with the highest shoot productivity were taken for propagation (Troshin *et al.* 1976). Such are vines 35 and 22 placed into the group of 'extreme' plants and vines 18 and 28 placed into the group of 'central' plants. Genetic differences among the plants were established using the method of protein electrophoresis (Klochneva *et al.* 1989).

Thus, in order to maintain quality of Aligoté cultivar, it is necessary to propagate plants 18 and 28, while to improve the cultivar, plants 35 and 22 have to be propagated, so that one plant could be left for each direction of selection and those of smaller value could be rejected to provide the intraclonal stability in the vegetative progeny. Moreover, the plant taken as typical for a cultivar to be improved should be used as control for the plants that were selected in order to increase productivity of a cultivar.

It is necessary to emphasize that the new clones (35 and 22) do have certain specialities in their integral characters, both of them being highly productive, with an optimum sugar/acidity ratio. The sugar/acidity ratio for plants 35, 22, 18 and 23 in 1984 was 2.47, 2.97, 2.76 and 2.32, respectively.

Conclusions

The method of selecting highly productive clones results in a 3 to 4-fold decrease in the duration of clonal selection in grapevine and a substantial reduction in the size of the experiment. Thus, the process can be considered sufficiently effective and profitable (3,100 roubles/ha).

Table 4: Differentiation of cv. Aligoté variations depending on the index of typicity

Parameters	Phenotypes									
	"extreme"							"central"		
	11 morphological characters									
do, %	148	122	116	113	112	73	75	76	80	80
Numbers of vines	36	4	22	20	9	24	14	16	28	25
Shoot productivity: sugar, g	43	66	82	60	49	60	50	61	61	68
	8 cultural characters									
do, %	134	130	129	122	118	65	68	72	73	75
Numbers of vines	35	45	6	19	22	18	20	34	26	7
Shoot productivity: sugar, g	71	48	60	41	82	56	60	56	52	52
	Pooled									
do, %	122	121	116	111	102	76	78	80	82	82
Numbers of vines	36	35	45	47	22	18	28	24	34	16
Shoot productivity: sugar, g	43	71	48	54	82	56	61	60	56	61

The new method of selection for highly productive clones is being tested now at the Steppe Experimental Farm of the Institute, using Aligoté planting materials. Stock nurseries covering 146 ha have been established. 50 % of these vineyards are currently bearing.

Clonal grape selection with recommended cultivars is underway in the West Premountainous Region of the Crimea (Riesling planting materials) and in the southern part of the Crimea (planting materials White and Rosy Muscats, Alma and Sersial).

Literature cited

ALTUKOV, YU. P.; 1981: Genetic Processes in Populations [Russ.]. Nauka, Moscow.

GAVAKETASHVILI, A. G.; 1986: Problems of grape mass and clonal selection [Russ.]. In: Works of the National Institute for Grape and Products of Grape Processing 'Magarach' [Russ.] **24**, 116-117. Yalta.

GOLODRYGA, P. YA.; SOUYATINOV, I. A.; TROSHIN, L. P.; 1975: Actual problems of grape clonal and genetic selection [Russ.]. In: Works on Applied Botany, Genetics and Breeding [Russ.] **54** (2), 101-112. VIR, Leningrad.

– – ; – – ; – – ; *et al*; 1976: Methodical Recommendations on Grape Mass and Clonal Selection [Russ.]. National Institute for Enology and Viticulture 'Magarach', Yalta.

– – ; TROSHIN, L. P.; 1980: Clonal selection as an effective method to increase yield [Russ.]. Sadovodstvo, Vinogradarstvo i Vinodelie Moldavii (3), 26-29.

JENEYEV, S. YU.; TROSHIN, L. P.; NIKOLENKO, V. G.; 1984: Greater requirements to the assortment and quality of grafted grape seedlings [Russ.]. Vinodelie i Vinogradarstvo SSSR (2), 24-28.

KAISYN, R. B.; 1986: Clonal grape selection in Moldavia [Russ.]. In: Works of the National Institute for Grape and Products of Grape Processing 'Magarach' [Russ.] **24**, 117-119. Yalta.

KENDALL, M. G.; STUART, A.; 1976: The Advanced Theory of Statistics. Design and Analysis, and Time-Series [Russ.]. Nauka, Moscow.

KLOCHNEVA, V. I.; TROSHIN, L. P.; SHURKHAL, L. Z.; *et al.*; 1989: Interspecific and intraspecific variability in grapevine after allozymous loci [Russ.]. In: Col.: Ways of Intensification of Table Grape [Russ.], 71-90. National Institute for Grape and Products of Grape Processing 'Magarach', Yalta

MERZHANIAN, S. A.; ZELENIN, I. L.; 1932: Methods of Breeding in Viticulture [Russ.]. Izd. sadvinob'yedinenia, Moscow.

MOSER, L.; 1966: Weinbau einmal anders. Österreichischer Agrarverlag.

SCHÖFFLING, H.; 1984: Die Klonenselektion bei Ertragsrebsorten. Ingolstadt.

SERGIYENKO, N. K.; ARESTOV, V. P.; MALIUGANOV, V. A.; 1986: On clonal, mass and phytosanitary selection of grapevine in the Don region [Russ.]. In: Works of the National Institute for Grape and Products of Grape Processing 'Magarach' [Russ.] **24** (6), 119-121.

SOLDATOV, P. K.; 1984: Vegetative Variability in Grapevine and its Role in Breeding [Russ.]. Tashkent.

TROSHIN, L. P.; SOUYATINOV, I. A.; SLONOVSKY, V. G.; *et al.*; 1978: Clonal improvement of Riesling variety [Russ.]. Vinodelie i Vinogradarstvo SSSR (7), 26-30.

-- -- ; ZHIVOTOVSKY, L. A.; 1987: Methodical Recommendations on Clonal Grape Selection for Productivity [Russ.]. Yalta.

TULAYEVA, M. I.; 1986: Grape clonal selection in the Ukraine and creation of elite planting materials [Russ.]. In: Works of the National Institute for Grape and Products of Grape Processing 'Magarach' [Russ.] **24**, 86-93. Yalta.

ZHIVOTOVSKY, L. A.; 1980: Pooled indices of the population variability after a complex of quantitative characters [Russ.]. DAN SSSR **260** (6), 1459-1462.

-- -- ; 1984: Integration of Polygenic Systems in Populations [Russ.]. Nauka, Moscow.

-- -- ; TROSHIN, L. P.; DRANOVSKY, V. A.; *et al.*; 1984: A Method of Grape Clonal Selection after a Complex of Characters [Russ.]. USSR Inventor's Certificate N 1417842.

Final remarks

G. ALLEWELDT

Ladies and Gentlemen,
dear friends and colleagues:

The 5th International Symposium on Grape Breeding is coming to an end. With more than 100 oral and poster presentations, the state of breeding research has been demonstrated. Two workshops accompanied the presentations.

To summarize the results of our Symposium at this state is difficult. The breadth of the research presented and your enthusiasm to solve breeding problems is remarkable. For the first time the Organizing Committee and the Board of Chairmen have thought it worthful to recognize the diverse fields of research represented and to summarize some of them as resolutions which will be brought to the attention of the Office International de la Vigne et du Vin and to other national and international organizations. These resolutions are:

Resolution No. 1 - GENETIC RESOURCES

Being aware of the increasing urgency to maintain biological diversity

Recognizing the importance of germplasm for breeding

it is recommended by the participants of the 5th International Symposium on Grape Breeding:

- that governments of all grapegrowing countries promote maintenance of germplasm and encourage research in the areas of grapevine identification and diversity

  and

- facilitate the exchange of germplasm

Resolution No. 2 - AMPELOGRAPHY

Noting the large number of grapevine varieties in the world

and

Considering that the identification of cultivars is indispensable for legislation, viticulture and breeding

Being aware of the differences in ampelographic description which do not allow comparison between findings

Recognizing that uniform description would lead to comparable, reproducible and more objective results

The participants of the 5th International Symposium on Grape Breeding (workshop 'Ampelography') recommend:

- the official acknowledgement of the 'preliminary minimal list'

- its comprehensive application

  and

- a further meeting after 2 to 3 years to discuss experience and results and to make suitable modifications
- the establishment of a training course in ampelography.

Resolution No. 3 - ISOENZYMES

N o t i n g large numbers of different enzymes, separation methods and protocols are used to analyse isozyme banding patterns

B e i n g a w a r e that the use of diverse methods results in lack of comparability of results

R e c o g n i z i n g that the ampelographic methods should not require sophisticated or expensive equipment

The participants of the 5th International Symposium on Grape Breeding (workshop 'Isoenzymes') recommend:

- that all laboratories conducting ampelographic studies use starch gel electrophoresis according to the protocol of PARFITT, D.E. & ARULSEKAR, S., 1986: Inheritance and isozyme diversity of GPI and PGM among grape cultivars. J. Amer. Soc. Hort Sci. **114**, 486-491
- that research to find better separation methods be accompanied by starch gel electrophoresis of GPI and PGM as a control.

Furthermore the participants of the workshop 'Isoenzymes' recommend that all groups working on *Vitis* enzymes exchange results regularly through a newsletter coordinated by the BFAR.

Resolution No. 4. - DISEASE RESEARCH

B e i n g a w a r e of the worldwide concern about damage to the environment through chemical control of pests and diseases

R e c o g n i z i n g the existence of germplasm carrying genes for resistance to most pests and diseases and knowing that these may be combined to yield resistant plants with goal performance and quality

K n o w i n g that there is no genetic basis for the rejection of resistant grapevine varieties

The participants of the 5th International Symposium on Grape Breeding urge the governments of all grape growing countries

- to utilize the natural genetic resistance to pests and diseases in order to minimize the need for chemical control
- to provide a sound basis for large scale, objective cultivar tests.

Resolution No. 5 – SANITARY SELECTION

B e i n g a w a r e of the worldwide spread of systemic diseases caused by viruses, viroids and bacteria

C o n s i d e r i n g the great difficulties of their chemical control

R e c o g n i z i n g that there are promising methods to eliminate such diseases in grapevines

The participants of the 5th International Symposium on Grape Breeding recommend:

- the promotion of research on *in vitro* methods for grapevine health, propagation, and breeding.

I am sure that all of us will take home new ideas, new impressions and new informations. Furthermore, I have the honour to inform yout that the Organizing Committee accepted the invitation of Prof. Dr. R. Pool, Geneva, USA, to organize the 6th Symposium in the United States.