Breeding Tetraploid Grapes[1]

By H. P. Olmo, *University of California, Davis, California*

Tetraploid forms of several commercial grape varieties were first described in California as early as 1914. Growers often refer to such individual vines with prodigious habit of growth and poor fruitfulness as "bulls", "males", or "giants". Nebel, in 1928, identified two such varieties as the tetraploid forms of the Muscat of Alexandria and Sultanina (Thompson Seedless) and found 76 chromosomes in root tip cells. Since 1934 increased interest has been attached to such varieties and at the present time a collection of more than thirty tetraploid varieties has been accumulated at the California Experiment Station. These have been obtained by searching in large vineyards for canes or vines that are spontaneous tetraploids or by colchicine treatment of shoot tips.

The possibility of utilizing tetraploidy in the production of new grape varieties received further impetus when it was discovered in 1937 that the tetraploid Muscat of Alexandria was equivalent to the variety Muscat Canon Hall, cultivated in English greenhouses as a market variety. In 1938, a group of varieties was imported from Japan that were suspected of being tetraploid because of the large berry size. These were later identified as tetraploid forms of the Delaware, Niagara, Catawba and Koshu (5). The notice and successful commercial cultivation of these tetraploids by the Japanese was probably fostered by the intensive methods of grape culture in use in that country, whereby every vine is observed and trained with more than usual care. The most widely grown "Eastern" grape in California, the Pierce, has recently been found to be tetraploid. This is the first instance of a tetraploid grape variety that is successful as a commercial sort in field plantings.

It is interesting to note that tetraploid varieties have often been referred to as arising as "chance" seedlings of a given variety, because of their overall resemblance to it. Thus, the Muscat Canon Hall was reported to be a seedling of Muscat of Alexandria (5), the Eaton and King were presumed seedlings of Concord (2). This would indicate that if these varieties did first originate as chimeras, it usually required that a whole vine be propagated before the difference in the forms became readily apparent.

In some varieties, the somatic mutation involving chromosome doubling, although presumably originating in a single cell, is able to repeatedly produce totally tetraploid tissue. Thus we have studied six separate occurrences of the Thompson Seedless tetraploid mutation and all of these clones are totally tetraploid and always give the same results in hybridization with other tetraploids, besides being indistinguishable morphologically. No sectorial or periclinal chimeras have been observed.

When tetraploid canes are found on an otherwise diploid vine, they

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have often been observed to arise at a pruning wound or near an area where a primary bud or shoot has been injured or killed. Thus the mutant shoots arise from dormant bud initials that are probably more deeply imbedded in the cane or trunk. We do not know whether it is the sudden reactivation of this tissue that causes doubling or whether some tissue sectors were already tetraploid and are thus given a chance for emergence by the suppression of the competing diploid areas. Individual berries wholly tetraploid sometimes occur in otherwise diploid clusters. Spontaneous doubling of chromosome number can thus occur in the floral meristem as well as in shoot apices. Another interesting observation supported by grower opinion indicates that the number of "giant" or tetraploid vines has been increasing in California vineyards since the practice of field budding has become more general. This consists in planting a rootstock in place and then inserting a single chip bud of the desired scion variety in early fall. The top of the established rootstock vine is cut off in the spring and the single scion bud makes very vigorous growth. Instead of growing a vine from a cutting or rooting containing several buds, this practice in effect segregates out more effectively any buds that might be tetraploid.

It appears that one must be cautious in determining whether or not a given tetraploid is wholly of this nature. The existence of slightly different morphological varieties from the same parent indicates that some of these giant sports are chimeras. The King and Eaton are in this category. Randall (6) in 1941 obtained unusual results in measuring the pollen size of a Cornichon tetraploid. A bimodal curve for pollen size was obtained, which indicates the presence of both haploid and diploid grains. In 1944 examination of young growing shoot tips of this vine indicated that a portion of the inner cell layers were actually diploid, but the chimera could not be proved to be wholly periclinal.

Occasionally, selfed seedlings or hybrid seedlings between two tetraploid clones are recovered that are diploid. This indicates either that such clones are partially chimerical or that certain cells are capable of undergoing a somatic chromosome reduction, leading to gametes with the haploid number. The alternative of having two haploid gametes arise from irregular distribution of chromosomes and meeting by chance, seems to be a more remote possibility.

The first attempts at breeding tetraploid grapes were made in 1936 by crossing the tetraploid Muscat of Alexandria and the tetraploid Thompson Seedless. For the past 15 years this work has continued and more than three thousand tetraploid hybrids have been grown. The present report will treat only of comparisons between some of the diploids and their derived tetraploid forms and briefly review some of the aspects of the breeding problems with tetraploids.

To obtain some measure of the fruit characteristics of diploid vs. tetraploid forms, a sample of 50 clusters was obtained at random from an interplanted block of five varieties in the first fruiting season of 1951. Commercial spacing was used and the vines trellised; both diploids and tetraploids were trained and pruned in the same way. Reference to Table 1 indicates that the fruit clusters of the tetraploids weighed considerably less than the diploids. An exception is the wine grape Sauvignon blanc, which reversed this trend. The cluster length of three of the tetraploid varieties was significantly less than for the diploid prototype, was not significantly different in the Sauvignon blanc, but was longer in the Zinfandel. The width of the clusters was significantly less in only the first two varieties. The stem structure was ordinarily thicker, but its total weight was either less or greater than in the corresponding diploid.

Table 1. Cluster characteristics of diploids and tetraploids.

		Weight in grams	Length in cms	Width in cms	Weight of stem grams	Number of berries[a]	Compactness[b]	Average weight of berry grams	Average number seeds per berry[a]
Alicante Bouschet	2n	228±95	16.1±3.0	11.6±2.8	8.2±3.5	117	3.1	1.87	1.89
	4n	125±62	14.0±3.5	8.8±1.9	5.1±2.6	43	4.0	2.68	1.31
	Diff:	103±13.4	2.1±0.65	2.8±0.48	3.1±0.62				
Niagara	2n	139±55	11.9±1.8	8.1±1.9	3.4±1.6	59	3.5	2.29	2.56
	4n	95±33	10.6±2.2	6.3±1.0	3.0±1.4	28	3.9	3.16	1.56
	Diff:	44±2.45	1.3±0.26	1.8±0.27	0.4±0.30*				
Sauvignon blanc	2n	85±37	10.2±2.5	8.0±1.8	2.9±1.4	74	3.4	1.10	1.12
	4n	107±45	9.4±2.8	8.5±2.1	6.7±2.9	45	3.2	2.19	1.14
	Diff:	22±4.50	0.8±0.53*	0.5±0.39*	3.8±0.45				
Sultanina	2n	497±155	30.6±5.1	12.6±2.0	10.0±3.9	414	3.2	1.18	—
	4n	374±233	19.2±6.1	11.7–2.6	16.7±8.7	98	3.9	3.64	—
	Diff:	123±39.6	11.4±1.12	0.9±0.46*	6.7±1.35				
Zinfandel	2n	164±13	14.7±1.9	7.7±2.6	9.6±5.2	102	4.0	1.43	1.33
	4n	115±70	16.5±4.7	7.8±3.7	13.2±6.4	29	4.7	1.61	1.14
	Diff:	49±21.1	1.8±0.72	0.1±0.46*	3.6±1.17				

*By t test, difference not significant at 5 per cent level; other differences highly significant.
[a]Berries with one or more hard seeds.
[b]1 = very compact, 2 = compact, 3 = well-filled, 4 = loose, 5 = straggly.

The tetraploids all had fewer berries per cluster, but the berries weighed much more. This increase in berry weight was not of equal proportion for all varieties, but was characteristic of the variety itself. The weight of berry of the Thompson Seedless tetraploid was actually more than double its diploid prototype. The average number of hard seeds developed per berry was reduced by tetraploidy with the exception of the Sauvignon blanc, where no significant difference was apparent.

It is evident that one can hardly generalize on the changes produced by tetraploidization. This depends on the variety genotype involved. Since the differences between tetraploids are thus due to changed genetic balance, it has been possible by breeding to obtain tetraploid varieties differing markedly in vigor, fruitfulness and fruit quality. The undesirable characteristics of most tetraploids, such as brittleness of canes and clusters, too few canes to provide shading of the fruit from sunburn, straggly clusters, and low total yield can thus be eliminated by crossing and selection, while still maintaining the larger berry size that is so desirable.

The type of tetraploid cross that produces the most vigorous and fruitful progenies are those which increase gene heterozygosity. Inbreeding has been an extremely detrimental process, while outcrossing to unrelated tetraploids has resulted in greater vigor and fruitfulness. The Muscat tetraploid when selfed (Fig. 1) gave in the F_1 progeny a large number of weak seedlings that died after field transplanting, and even a greater percentage that never fruited. Selfing the tetraploid Muscat has been more depressing on growth and fruitfulness than selfing the diploid. The reasons for this are not understood. When the Sultanina tetraploid is crossed with the Muscat tetraploid, vine vigor and fruitfulness is in large measure restored.

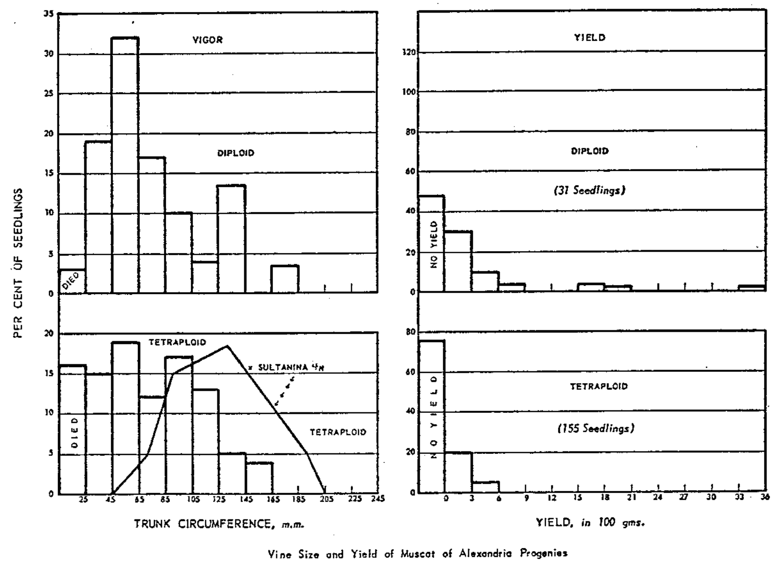

FIG. 1. Vine size and yield of F_1 progenies from selfing diploid and tetraploid Muscat of Alexandria.

The results of crossing and selfing four diploid *vinifera* varieties and their autotetraploid forms in all possible combinations have been reported by Randall (7). All of the flowers were emasculated, hand pollinated, and bagged. The average set of seeded berries was much lower in the tetraploids than in the diploids, and the ovule set even more strongly depressed. However, it is important to note that the tetraploid varieties set almost as many seeded berries as the diploids if pollen from a diploid was applied. These results support growers' observations that giant vines may sometimes give well-filled clusters when found singly in a vineyard surrounded by the usual diploid varieties, but give very poor yields when grown in a more isolated position in a home garden. The necessity of artificial cross-pollination for the greenhouse variety Muscat Canon Hall, was recognized long before this variety was discovered to be a tetraploid. Artificial pollination of rather unfruitful tetraploids with pollen of diploid varieties is thus a method of obtaining better filled clusters, but has only been practical when the added quality of larger berry size compensates for the extra

cultural expense. Interplanting of diploid varieties to serve as pollinators might be commercially feasible to increase the fruit set of some tetraploid varieties.

A smaller percentage of functional ovules are present in the tetraploids, and a lower average number of seeds per berry. Since there is such a high correlation between the number of seeds per berry and its ultimate size (6), an important advance could be made in the breeding of tetraploids if the number of functional ovules could be increased, or even a return to the average of the diploid level accomplished. The seeds obtained from the cross 4n × 2n averaged 80 per cent "floaters" (Table 2). Seeds that float on water are partly hollow, only

Table 2. Summary of type crosses without regard to variety (Randall, 1941).

	Number of flowers pollinated	Per cent seeded berry set	Per cent ovule set	Average number seeds/berry	Per cent floaters
2n × 2n	25,271	27.88	13.20	1.90	23.49
2n × 4n	13,777	21.37	8.52	1.59	56.04
4n × 4n	31,472	18.38	6.22	1.35	41.13
4n × 2n	13,018	25.96	8.91	1.37	80.25

a portion of the endosperm having filled the seed cavity. They rarely germinate. In the reciprocal cross more viable seeds are obtained, perhaps because the endosperm has a lower chromosome number and grows more in harmony with the embryo and integuments. It is difficult to get critical evidence on this point. The seeds obtained by selfing or crossing autotetraploids are more apt to show endosperm irregularities.

The poor fruitfulness of autotetraploids has often been ascribed to gametic infertility, because of expected variation in chromosome distribution, either numerical or structural. However, the facts set forth earlier, namely that some autotetraploids were even more fertile than the diploid progenitors, led us to re-examine the relationship between chromosome behavior and fruitfulness. In 1946 meiotic pairing was studied in the tetraploid Concord and Thompson Seedless. An average of four to five quadrivalents were found with occasional univalents and trivalents. Individual vines of tetraploid populations from both selfed and intercrossed F_1 populations of the Muscat of Alexandria and Zinfandel were selected that showed a high and a low degree of self-fruitfulness. A study of the meiotic chromosome behavior of these selections has just been completed by Alley (1). His work rather conclusively demonstrates that the wide differences in the fertility characterizing individual plants cannot be due to differences in univalent loss or multivalent formation at first metaphase.

Despite variability in pairing between the four chromosome sets, there is some strong regulatory mechanism that results in the formation of many functioning gametes with the new diploid chromosome balance. The wide differences in fertility level are thus genetically controlled, and not primarily to be attributed to competition in pairing or irregular distribution of chromosomes.

Summary

The origin and history of several tetraploid varieties of grapes are reported. Comparisons of cluster and berry characters of diploid varieties and their derived autotetraploid forms indicate that the effect of doubling the chromosome number is related to the particular genotype concerned.

Preliminary results show that inbreeding on the tetraploid level in the grape appears more detrimental to plant growth and fruitfulness of the F_1 progeny than in the diploid, while outcrossing between different tetraploid varieties results in a restoration of vigor and fruitfulness in the F_1 progeny.

Since the particular characteristics of autotetraploids are subject to gene control, and hence segregation, it is possible to breed new varieties that have large berry size and yet do not retain the undesirable features of the raw tetraploids.

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