

Breeding Tetraploid Avocados¹

R. S. BRINGHURST,
*University of California, Davis, California*²

Polyploid tissue has been induced in many crop plants by the colchicine technique. Successful treatment of fruit trees has been reported in recent years in apples (4), peaches (2), and pears (3). This is to report colchicine-induced polyploidy in the avocado in the Fuerte clone and in Mexicola seedlings. All *Persea* species examined are diploid with 24 somatic chromosomes (1).

MATERIALS AND METHODS

Newly germinated seedlings of the Mexicola variety and young grafted scions of the Fuerte variety growing in containers in the greenhouse were treated with colchicine as described by Dermen (2). Succulent seedling shoots 3 to 15 cm long and shoots from grafted scions were treated with aqueous 0.01 to 1.0 per cent colchicine solutions containing approximately 5 mg of dry Santomerse per 100 cc of water as a wetting agent. In some of the treatments the growing-point was split with a razor blade to facilitate penetration. The tip was wrapped with absorbent cotton and a wick of the same substance was extended into a colchicine reservoir consisting of a 3 to 5 cm microvial made from 5 mm o.d. glass tubing. The microvial was held in place by wire support or friction tape. High humidity was maintained by enclosing the treated area in an inverted glass jar or a plastic bag or by placing the plants in a greenhouse section where a near-saturated atmosphere was maintained. Treatments were continued for periods of from one to four days, after which the reservoir and the wick were removed and the cotton wrapping was thoroughly washed with water. Shoots were then left to grow under conditions of relatively high humidity and high temperature. Cotton wrapping was removed after a week or more.

Plants immediately affected by the treatment were characterized by retarded growth, curvature of the shoot, and a thick, distorted leaf growth.

RESULTS

Mexicola Seedlings:—Survey experiments involving treatment of 200 open-pollinated Mexicola seedlings during 1951 and 1952 indicated the following: Solutions of .01 per cent were ineffective; solutions of 0.1 per cent were effective only if the growing point was split and the treatment continued for two days or more; solutions of 0.5 per cent were effective if the growing point was split or unsplit, as long as the treatments were continued for two or more days; solutions of 1.0 per cent were effective under all conditions but splitting of the growing point frequently resulted in severe die-back of the shoot, particularly when treatments were continued for two days or more.

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Nineteen of the treated Mexicola seedlings were selected as having morphological characteristics indicative of polyploidy after the development of mature leaves. The leaves were abnormally broad and thick. Four of the plants had leaves bearing only small stomata. Large stomata were present on leaves of the other 15 plants. Some of them outgrew this condition. On one plant the first three leaves above the treated area had large stomata and the remaining 13 leaves had small stomata. In other plants the condition recurred intermittently up the shoot. The following sequence of large and small stomata occurred in the leaves of one plant, starting with the first leaf above the point of treatment; two large, two small, two large, 16 small, one large, one small, one large and two small. In other plants large stomata were present in all of the leaves. Some leaves had large stomata on half the blade and small stomata in the other. Affected leaf areas can be easily detected by the widened blade, darker color when held in light, and the thickness of the leaf blades (5).

A single raceme of flowers developed on one Mexicola seedling 15 months after the colchicine treatment. Leaves with only large stomata alternated with leaves with only small stomata up the shoot of this plant. The flower buds were large and very blunt when compared with buds on an adjacent Mexicola plant in the greenhouse. The corolla, pistil, anthers, and pollen were proportionately larger. Comparison of the pollen size with that of the parent Mexicola is given in Table 1. Mean grain size was significantly larger on the treated plant.

TABLE 1.—CLASSIFICATION, MEAN DIAMETER, AND RANGE OF DIAMETERS OF POLLEN GRAINS FROM TETRAPLOID, CHIMERAL, AND DIPLOID ANTHERS OF A COLCHICINE TREATED MEXICOLA SEEDLING, A COLCHICINE-TREATED FUERTE SCION AND APPROPRIATE CONTROLS.

Pollen source	Pollen class	Mean diameter	Range
		microns	
Mexicola seedling, treated.....	2n	56 ± 6	43-79
Mexicola, untreated.....	n	43 ± 2	39-49
Fuerte treated.....	2n	54 ± 3	45-77
Fuerte treated.....	n + 2n	50 ± 7	40-77
Fuerte treated.....	n	42 ± 2	40-49
Fuerte untreated.....	n	41 ± 1	40-47

The range and consequently the variation in grain size also tended to be greater on the treated plant. The large grains were viable, and would take stain and germinate on avocado stigmas as rapidly as grains from flowers of an untreated Mexicola plant. The rate of the pollen tube penetration of the style was not studied. The plant was too juvenile to set fruit.

Fuerte Clone:—Growth on two of six Fuerte scions gave evidence of polyploidy following treatment. Some of the leaves had only small stomata and others only large. Flower buds began to develop on two shoots from one of the scions six months after treatment and the first flowers opened seven weeks later (Fig. 1). Shoot A had three racemes and shoot B had eight. All the flowers on shoot A were large while on shoot B two of the racemes had only small flowers, five bore large and small flowers and one bore large flowers only (Fig. 2).

Each raceme was labeled and every flower collected as it opened. Pollen from every flower was measured. All of the pollen was large in each of the 27 flowers from the three racemes on shoot A (Fig. 1) and was judged to be diploid pollen originating from tetraploid tissue. On shoot B (Fig. 1) two racemes had small flowers bearing only haploid pollen, racemes the second and seventh numbering from the base. Five

racemes had both small and large flowers that yielded only haploid or only diploid pollen in most cases respectively, racemes the first, third, fourth, sixth, and eighth. The fifth raceme had all large flowers bearing only diploid pollen.



FIG. 1. Fuerte scion seven months after colchicine treatment. A. Tetraploid shoot, diploid pollen only. B. Chimeral shoot, diploid and haploid pollen.

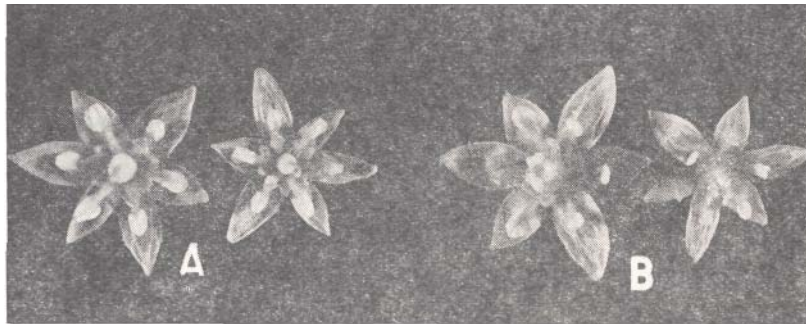


FIG. 2. Tetraploid and diploid Fuerte flowers. A. First opening flowers. B. Second opening flowers. Tetraploid flowers from treated plant, left, compared with diploid flowers from untreated plant right. A and B, $\times 2$.

Chimeral Flowers and Anthers: —It was difficult to classify certain of the flowers from shoot B, as large or small on the basis of their appearance because they were somewhat asymmetrical in shape. Some of the anthers were small, and some were large and the size of the flower increased with an increase in the number of large anthers. For the most part, large anthers bore only diploid pollen and small anthers only haploid pollen as revealed by examination of pollen from each anther (Table 1).

Certain anthers were also intermediate in size, suggesting chimeral structure. Pollen samples from most of the intermediate anthers contained a mixture of haploid and diploid grains in varying proportions. The mean diameter, range, and standard error of the pollen grains from the mixaploid anthers are compared with those from the tetraploid and diploid anthers in Table 1. The standard error for pollen from mixed anthers was

large compared to those obtained for anthers bearing only haploid pollen or only diploid pollen. The diameter range of individual pollen grains from mixed anthers overlaps the range of that for the tetraploid and the diploid anthers. Grains from the chimeral anthers could be clearly divided into two groups, one with a mean diameter of 42 μ and the other with a mean diameter of 54 μ . The bimodal distribution is shown in Table 2 in comparison with the single-modal distribution of grains from anthers bearing only haploid or only diploid pollen.

TABLE 2.—BIMODAL DISTRIBUTION OF FUERTE POLLEN GRAINS IN CHIMERAL ANTHERS COMPARED WITH SINGLE MODAL DISTRIBUTION OF POLLEN FROM DIPLOID AND TETRAPLOID ANTHERS.

Pollen classification	Percentage in each class grouping (microns)		
	40-45	46-51	52-77
2n.....	1	10	89
2n and n.....	48	13	39
n (treated plant).....	95	5	0
n (untreated plant).....	98	2	0

Anthers bearing only large, small, or mixed pollen on chimeral flowers were grouped in sectors and in some instances only a single anther differed from all the rest in the pollen borne. The four locules of individual anthers from chimeral flowers also frequently bore different pollen. A typical anther had diploid pollen only in two locules, mixed pollen in one locule, and haploid pollen in the fourth locule.

Diploid Fuerte pollen originating from tetraploid pollen mother cells took stain as readily as did normal haploid Fuerte pollen and germinated just as rapidly on stigmata of flowers from diploid avocado varieties. Germination started within 10 minutes at temperatures of 70 to 80 degrees F. Attempts to set fruit with diploid pollen were not successful.

Propagating Tetraploid Clones:—In order to establish a tetraploid clone of the Fuerte variety, the dominant shoot B of Fig. 1, which was chimeral, was suppressed and growth was forced on the side-branch shoot A of Fig. 1, which bore only tetraploid flowers. Shoot A is shown in Fig. 3: right, five months after flowering ceased; leaves on this shoot are wide and thick in comparison with the normal Fuerte clone (Fig. 3, left). The guard cells of the stomata of all leaves examined have been correspondingly larger on shoot A. Flowers developing from shoot A during the following season were all large, indicating that the tetraploid tissue has persisted. Scion material has been worked into an orchard tree in order to grow it under field conditions.

Several of the Mexicola seedlings that gave evidence of successfully induced polyploidy are also thriving in the field.

The tetraploid nature of tissue from the treated Mexicola seedlings and the treated Fuerte specimen was verified by leaf smear preparations in which approximately 48 chromosomes could be counted.

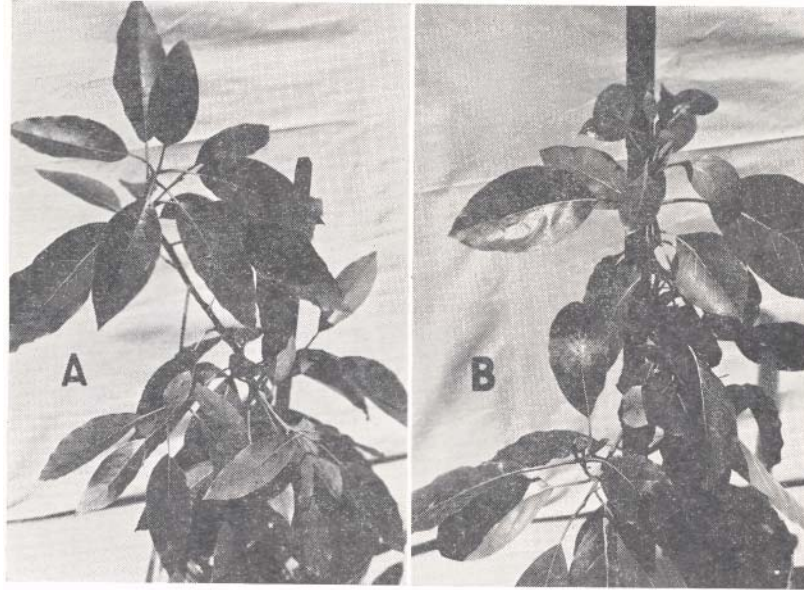


FIG. 3. Diploid and tetraploid Fuerte scions. Left. Normal diploid shoot from untreated scion. Right. Tetraploid shoot A of Fig. 1 five months later. Note the broad leaves of the tetraploid.

DISCUSSION

Persistent tetraploid clones of avocado varieties may be originated by the colchicine technique as evidenced by data presented here.

Increased pollen size has been generally recognized as one reasonably reliable criterion for detecting polyploidy in subepidermal tissue (5). None of the developing buds on the avocados described here were sacrificed for pollen mother cell examination since anthers of all the developing flowers were beyond the meiotic stage before the increased bud size was obvious. Chromosome counts in somatic tissue supported the assumption of tetraploidy. Pollen could be accurately separated into two size classes as shown in the tables. Pollen from fifteen other avocado varieties was measured several times and the grain size was essentially the same as that reported here for haploid grains from the diploid Mexicola and Fuerte varieties.

The chimeral distribution of diploid and haploid subepidermal tissue extending into the individual locules of the anthers may be similar to the lily chimeras reported by Emsweller and Stewart (6). They found diploid and haploid pollen mother cells undergoing division side by side in the same anther. Olmo (9) referred to a similar bimodal curve for pollen size in grapes as indicative of the presence of both haploid and diploid grains.

An increase of fruit size through induced tetraploidy could be of economic importance in the avocado. Small fruit size is a limiting factor in market acceptance of Mexican avocado varieties, such as Mexicola. The few fruiting hybrids between Mexican varieties and commercial sorts have also borne small fruit. Mexican varieties are characterized by a delightful spicy flavor not found in any avocado fruit currently marketed in quantity. Size of fruit has frequently increased markedly following chromosome doubling in other

fruit crops (5). It is possible that tetraploid avocado derivatives from varieties such as Mexicola might bear fruit with size increase proportionate to that recorded here for flowers, pollen, etc. Tetraploid Mexican selections might be directly useful as varieties, since varieties such as Mexicola are very hardy and can be successfully cultured in areas where commercial types including Fuerte are eliminated by cold.

Increased ploidy in the avocado might decrease the fruit set. Sporadic bearing behavior is the chief weakness of the Fuerte variety although it has persisted as the most important variety in the California avocado industry. Factors underlying this problem are not clearly understood but temperature relationships are probably important (7, 8). The Mexicola variety in contrast to Fuerte is a consistent producer of abundant fruit under a wide variety of conditions and some reduction in fruitfulness would not be a serious problem.

An even more important use of tetraploid avocados might be in the development of good tetraploid varieties through breeding. Olmo (9) pointed out that there were fertility differences in autotetraploid grapes and that vigorous and fruitful tetraploid selections can be found among hybrids resulting from crosses between tetraploid varieties.

In considering the potential bearing behavior of tetraploid avocados it is significant that the diploid pollen from the tetraploids studied here is as functional in germination experiments as is haploid pollen. Virtually all of the large grains were well filled and would take the stain or would germinate on diploid or tetraploid avocado stigmas as rapidly and completely as would comparable haploid pollen. The rate of penetration of the pollen tube through the style tissue was not determined. Germination as reported here has reference only to the rate at which the pollen tube developed and commenced penetration of the stigma.

The fact that no fruit set was obtained in the limited pollinations made with the diploid pollen is not significant. The pollinations were made during the winter under unfavorable conditions, as evidenced by the fact that pollinations involving haploid pollen on the same trees at the same time were also unsuccessful. This is not surprising since the best average success reported for avocado pollination during a favorable period is only 8 per cent (1).

SUMMARY

Persistent tetraploid tissue has been induced in the Fuerte avocado clone and in seedlings of the Mexicola variety by the colchicine technique. Large flowers bearing only large pollen have been obtained and this pollen was judged to be diploid. The diploid grains show evidence of high viability. Chimeral tissue extended into the flowers of one Fuerte plant. Haploid and diploid grains occurred together in the same locule of certain anthers. Tetraploid Fuerte scions from non-chimeral shoots have been propagated. The possible use and economic value of tetraploid avocado material is discussed.

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