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Selfed and Crossed Proportions of Avocado Progenies Produced by Caged Pairs of Complementary Cultivars

Chemda Degani

Department of Subtropical Horticulture, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel

Shmuel Gazit

Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel

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Abstract. **Using a malate dehydrogenase isozyme system, it was possible to identify the pollen donor parent in progenies of avocado *(Persea americana* Mill.) obtained by enclosing 2 cultivars of complementary flower type in a screenhouse with a beehive. The observed percentage of cross-pollination ranged from 7% to 92%. Isozymes are demonstrated to be a practical way of identifying hybrids.**

The avocado is an important economic crop and the subject of intensive breeding programs aimed at the improvement of fruit quality and productivity. Such improvement can be obtained by hybridization, designed to combine complementary traits of 2 cultivars or selections. Hand pollination is reported to result in very low fruit set (1, 2, 3, 5, 6). Enclosure of 2 desirable parents in a screenhouse with a beehive provides a more practical method of hybridization (2, 3, 6). Selection of the parents should recognize the synchronous dichogamy of avocado flowers. One cultivar is of "type A," where under ideal conditions each flower is functionally female in the morning and functionally male the following afternoon; the other one is of "type B," where each flower is female in the afternoon and male the following morning. This flowering dichogamy is rarely absolute and self-pollination often takes place; thus, fruits produced may result from either cross- or self-pollination (1, 2, 3, 6).

Offspring from self- and cross-pollinations may be differentiated by using appropriate isozymes as genetic markers due to the fact that the electrophoretic pattern usually reflects the genotype. Since most alleles coding for the different isozymes are known to be codominant, the pollen donor may be identified in F, if the parental genotypes are known. Several isozyme systems have been analyzed in avocados using mesocarp and leaves (7, 9). Leaves are preferred for parentage analysis since their analysis permits the screening of young seedlings.

The present study was undertaken to explore the use of isozymes for identification of the pollen parent and thus the determination of the relative proportions of self- and cross-pollinated offspring from paired, enclosed cultivars.

Hybridization was attempted by caging 2 complementary cultivars in a screenhouse with a beehive during flowering. The following 3 different systems were used: (1)

caging of 2 mature trees of complementary cultivars ('Ettinger' x 'Anaheim'); (2) caging of a single mature tree with a large central branch, comprising 10-20% of the tree canopy, grafted to a complementary cultivar 2 years earlier ('Ettinger x 'Rosh Hanikra', 'Ettinger' x 'Tova', and 'Tova' x 'Ettinger'); and (3) caging of a single mature tree with 2 grafted plants in large containers ('Horshim' x 'Anaheim', 'Anaheim' x 'Horshim', 'Ettinger' x 'Pinkerton', and 'Pinkerton' x 'Ettinger').

Horizontal starch gel electrophoretic assays were performed on leaves stored at 0° to 4°C for 1 to 2 weeks. Mature, healthy leaves of the actual parents and progeny were sampled from the orchards of Rosh Hanikra and the Akko Regional Experiment Station. Samples for isozymic analysis were prepared by grinding 6x6 mm pieces of leaves in an extraction buffer consisting of 0.1 M potassium phosphate (pH 7.5), 0.1 M 2-mercaptoethanol, and 12% soluble polyvinyl pyrrolidone (M. W. 40,000). The extracts were absorbed on 4 x 6 mm Whatman 3mm paper wicks. The gel buffer was 16 mM Tris-citrate (pH 6.9) and the electrode buffer was 49 mM Tris-citrate (pH 6.9). Gels were run at 1.1 mA/cm^2 gel cross section (g).

The staining mixture for malate dehydrogenase (MDH) consisted of 2.5 ml 0.2 M Tris-HCl (pH 8.0), 20 ml H_2O, 5 ml 2 M malic acid (pH 7.0), 10 mg nicotinamide adenine dinucleotide, 10 mg nitro blue tetrazolium chloride (NET), and 2.5 mg phenazine methosulfate (PMS). Starch and all staining reagents were from Sigma.

The MDH isozyme system and its genetic control in avocado have been described by Torres and Bergh (9), who suggested that *Mdh-1* is a dimeric system having fast (F) and slow (S) as the most common alleles.

Table 1. *Mdh-1* genotypes of avocado parents.

Cultivar	Flower type	*Mdh-1*
Ettinger (Et)	B	SS
Horshim (Ho)	B	SS
Rosh Hanikra (RH)	A	FF
Anaheim (An)	A	FF
Pinkerton (Pi)	A	FS
Tova (To)	A	FS

Table 1 presents *Mdh-1* isozyme profiles of the cultivars used as parents in this study. The isozyme profiles of 'Ettinger', 'Pinkerton', and 'Anaheim' are identical to those previously reported (9); genotypes of the other 3 cultivars are reported here for the first time. The offspring were analyzed by the MDH alleles and the rates of cross- and self- pollination were calculated (Table 2).

'Ettinger' x 'Rosh Hanikra'. 'Ettinger' and 'Rosh Hanikra' are homozygous for *Mdh-1,* SS and FF respectively. Hence all hybrids should be of the heterozygous type FS, while self-pollinated 'Ettinger' seedlings should be SS and self-pollinated 'Rosh Hanikra' seedlings FF. Considerable self-pollination was observed in the attempted reciprocal matings, with considerable difference between the reciprocals. When 'Ettinger' was the female parent, 80% of the progeny were the result of self-pollination in contrast to 26% in the reciprocal (Table 2).

'Ettinger' x 'Anaheim'. This combination is similar to the previous one in that

'Anaheim' is homozygous FF and all hybrids should be heterozygous FS, and self-pollinated seedlings should be SS or FF, depending upon the female parent. As in the previous case, when 'Ettinger' was the female parent, the rate of self-pollinated progeny was very high (71%); but, with 'Anaheim' as the female, the self-pollinated progeny was only 10%.

Table 2. *Mdh-1* genotypes of avocado progenies.

Parents		No. seedlings	Expected (%)						Observed (%)		
			Self-pollination progeny			Cross-pollination progeny					
Female	Male		FF	FS	SS	FF	FS	SS	FF	FS	SS
Et	RH	98			100		100			20	80
RH	Et	76	100				100		26	74	
Et	An	48			100		100			29	71
An	Et	20	100				100		10	90	
Ho	An	36			100		100			25	75
An	Ho	14	100				100		93	7	
Et	Pi	61			100		50	50		8	92
Pi	Et	92	25	50	25		50	50	12	55	33
Et	To	113			100		50	50		39	61
To	Et	132	25	50	25		50	50	2	45	53

'Horshim' x 'Anaheim'. This is another case where one of the parents is SS for *Mdh-1* ('Horshim') while the other is FF ('Anaheim'). A high rate of self-pollinated progeny was observed in both combinations in this case; 75% and 93% with 'Horshim' and 'Anaheim', respectively.

'Ettinger' x 'Pinkerton'. The parent genotypes are SS ('Ettinger') and FS ('Pinkerton'). The expected genotype of the hybrids is therefore 50% SS and 50% FS. With 'Ettinger' as the female parent, only 8% of the seedlings were FS. Assuming that the percentage of SS hybrids is the same, about 84% of the progeny came from self-pollination. Twelve percent of the progeny was FF (which results from self-pollination) in the reciprocal mating, indicating a self-pollination rate of about 48% (since the theoretical ratio of FF:FS:SS progeny for selfing of FS genotype is 1:2:1).

'Ettinger' x 'Tova'. The parent genotypes are SS ('Ettinger') and FS ('Tova'). When 'Ettinger' was the female parent, 39% of the progeny were FS, suggesting a self-pollination rate of about 22%. The 2% FF progeny indicated 8% self-pollination in the reciprocal combination.

Our original aim was to differentiate between self- and cross-pollinated offspring within a population of seedlings produced in an enclosed system containing a pair of complementary avocado cultivars. The *Mdh-1* analyses for the 10 seedling populations (Table 2) allowed full individual assignment of progeny only when the 2 parents were both homozygotes of different genotype (FF and SS). Partial individual assignment could be made when one of the parents was heterozygous. That is, with the male parent heterozygous, half the hybrids could be identified; with the female parent heterozygous, ¼ of the selfs could be identified. In all cases, the overall rate of self-pollination vs. cross-pollination could be calculated or estimated.

Although hybrids were expected to constitute the majority in the 10 populations as

Gazit (4), our findings (Table 2) show a tremendous variation among populations of different parental combinations. The variation between reciprocal matings was equally large. The percentage of hybrids varied between 7% and 92%. About 54% of the 690 seedlings produced in our "controlled pollination" systems are hybrids.

The only economical way of producing avocado hybrids presently known is by using bees, or some other large insect, in a closed system to perform the millions of pollinations needed to obtain the thousands of seeds required for an effective breeding program (1, 2, 3, 6). For many years it has been known that avocado cultivars can set good crops without the benefit of cross pollination (1, 2, 3, 4, 6). Nevertheless, because of the unique synchronous dichogamy of the avocado flower and in the light of results from some pollination experiments (1, 4), it has been postulated that when 2 complementary cultivars flower in close proximity a high rate of cross pollination will ensue, and the majority of the seeds produced will be of hybrid origin. In the present study, it was possible to test this assumption in several reciprocal crosses and the results showed that, frequently, the assumption was not valid under our conditions. The conclusion drawn from this study is that in the future, when only hybrids are wanted, it will be desirable to analyze all seedlings produced by insect pollination in order to discard the self-pollinated offspring.

The fact that in paired, enclosed complementary cultivars a high incidence of self-pollinated mature fruits was found, reaching 93% in 1 case, suggests that high rate of hybridization may be obtained when 2 cultivars belonging to the same flowering group are in close proximity.

The progenies analyzed by us were produced under artificial conditions, i.e., enclosed pair of complementary cultivars with a beehive. There is a host of differences between conditions prevailing in this artificial system and those in the orchard; the more pronounced one is the exceedingly high concentration of pollinating bees. Thus, our results may not necessarily reflect the rate of selfed vs. crossed progeny in the orchard. Indeed, in the case of 'Pinkerton', Torres and Bergh (8) found higher rates of cross-pollination under orchard conditions than those observed in the present study.

At this stage we are not certain of the reason for the wide variability in the final rate of hybridization. This variability may be effected in any stage of pollination, fertilization, or fruit abscission. A mature avocado tree has hundreds of thousands of flowers. The number of mature fruits carried by the tree is usually only a few hundreds. The extent of fruitlet abscission may be enormous. The genotypes of the embryo and endosperm may play an important role in differential selection for survival. We speculate that some pollen donors will enhance the set and survival of their progeny more than other pollen donors. This may be the main reason for the great variability in the percentage of crossed progeny. This suggestion is supported by the fact that in our study 'Ettinger' consistently excelled as a pollen donor, producing a large percentage of hybrids when used as a pollinator for 'Rosh Hanikra', 'Anaheim', 'Pinkerton', and 'Tova' (74%, 90%, 52%, and 92% respectively). At the same time, 'Ettinger' in the reciprocal combinations usually produced a large proportion of selfed progeny (Table 2). Apparently the 'Ettinger' pollen is more effective and/or confers a better chance of survival on its progeny, whether selfed or crossed. This explanation is in accord with

a previous observation that 'Ettinger' was a very effective pollinator for 'Tova' cultivar, as indicated by a large increase in yield (4). The present analysis of the seedlings, showing that 92% of them were cross-pollinated, supports this conclusion.

Our evidence for the inherent superiority of certain pollinators in producing hybrid seeds has implications for increased commercial production by cross-pollination in avocado (1, 2, 3, 4). We may speculate that pollinators which produce a high rate of hybrids also may increase yields under certain environmental conditions. In this way, the isozyme technique may be used also to select promising pollen donors to be tested as pollinators in the orchards.

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