HortScience 21(5):1187-1188. 1986.

Genetic Selection During the Abscission of Avocado Fruitlets

Chemda Degani, Anat Goldring, Shmuel Gazit, and Uri Lavi

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

Additional index words. Persea americana, leucine aminopeptidase

Abstract. **Evidence is presented suggesting that genetic selection could be an important factor in avocado fruitlet abscission. 'Ettinger' embryos *(Persea americana* Mill.) at different stages of fruit development were classified according to their leucine amino-peptidase (LAP) electrophoretic pattern in the Lap-2 locus. Analysis of several fruitlet populations showed significant deviations from the expected Mendelian ratio. The genotypic ratios at the different stages indicate genetic selection during fruitlet abscission.**

A typical mature avocado tree has about one million flowers during each flowering season (10); yet, it usually yields only a few hundred mature fruits. Inadequate pollination and fertilization may be responsible for low fruit set (2), but, in most instances, we see excellent set culminating in low yield due to excessive fruitlet abscission. Very little is known about the regulation of fruit abscission in avocado. Most of the work that has been carried out on this subject dealt with physiological and anatomical aspects (1, 3, 15). Some recent studies (2, 7) showed that survival of young fruitlets is dependent on the pollen donors.

Isozymes as genetic markers have been used to detect different genotypes resulting from controlled pollinations (6). Any deviation from the expected segregation ratios should be attributed to genetic selection.

The present study started with a population of self-pollinated 'Ettinger' offspring obtained from caged trees (6). The population of seedlings was cultured in the orchard of the ARO Akko Regional Experiment Station as part of our breeding program. Horizontal starch gel electrophoretic assays were performed on mature leaves from those seedlings (6). Samples of embryos and endosperms of young fruitlets were prepared for isozymic analysis by grinding them in an extraction buffer (6), and absorbing the extracts onto 4x6 mm Whatman 3MM paper wicks. Large fruitlets (100 g) were assayed according to Torres (16). The gel buffer was 20 mM Tris-citrate (pH 7.6) and the electrode buffer was 0.4 M boric-NaOH (pH 8.7). Gels were run at 3.9 mA-cm^{-2} gel cross section for 3.5 hr. The staining mixture for leucine aminopeptidase (LAP) consisted of 2.5 ml 0.1 M potassium phosphate (pH 6.0), 60 ml H_2O, 100 mg fast black K salt, and 15 mg L-leucine-naphthylamide HC1 dissolved in 4 ml *N,N* dimethyl formamide.

Received for publication 30 Sept. 1985. Contribution from the Agricultural Research Organization, The Volcani Center, P.O.B. 6, Bet Dagan, Israel. No. 1538-E, 1985 series. This work was supported by grant No. 1-625-83 from the United States-Israel Binational Agricultural Research and Development Fund (BARD). We thank R. Elbazri for her expert technical assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

The LAP isozyme system and its genetic control in avocado have been described by Torres and Bergh (17), who suggested that Lap-2 is a monomeric system having fast (F) and slow (S) as the most common alleles. Among the cultivars grown in Israel, 34 have been examined so far, and all have the genotype FF at Lap-2, except 'Ettinger' and 'Regina', which are both FS heterozygotes.

Thirty-seven selfed seedlings of 'Ettinger', obtained by caging individual 'Ettinger' trees with a beehive within net tents, were shown to be a pure selfed population by assaying with enzyme systems for which 'Ettinger' is homozygous—malate dehydrogenase (SS), acid phosphatase (FF), and glutamate oxaloacetate transaminase (FF). Segregation analysis with the enzyme systems phosphoglucomutase and triosephosphate isomerase, for both of which 'Ettinger' is heterozygous (FS) showed that the observed ratio did not deviate significantly from the expected 1:2:1. On the other hand, when this population was assayed for the LAP-2 system, 34 seedlings had the genotype FS, 3 showed FF, and none SS. This ratio, on basis of a χ^2 test, significantly deviated from the expected ratio *(P* < 0.05). Apparently this unexpected distribution was the result of genetic selection. It was not possible, at that time, to point out the exact stage at which the selection took place—at gamets production, at fertilization, or after the zygotes were formed.

In Summer 1983, 3 different populations of 'Ettinger' fruitlets were assayed for the genotype pattern at Lap-2. The results, presented in Table 1, show that fruitlets with the genotype S S were formed but probably abscise later on.

Table 1. Distribution of Lap-2 genotypes in embryos of young 'Ettinger' fruitlets.

Source of fruitlets	Date	No. fruitlets	Fruitlet wt (g)	Frequency (%) SS	FS	FF	P^z
A single isolated tree (self pollination ?)	25 May	25	0.35–0.70	8	76	16	0.05
Uncaged orchard tree (open pollination)	1 June	47	0.3–0.7	4	68	28	0.005
A single caged tree (self pollination)	7 June	21	0.5–2.0	0	86	14	0.005

[z]Significance of deviation from expected ratio based on χ^2 test.

In 1984, 3 'Ettinger' trees in the Hebrew Univ.'s Experimental Orchard at Rehovot were caged before the beginning of flowering (at the end of February). Beehives were put inside the cage on 15 Mar. At different times during fruit development, fruitlets were sampled from the trees and gathered from the ground. Embryos from all samples were assayed for genotype distribution in the LAP-2 system. Beginning in early April, very young fruitlets (50-100 mg) were examined, but we could find LAP-2 enzymatic activity only at the end of April. The results, presented in Table 2, show a deviation from the expected segregation ratio in fruitlets picked from the trees in June and July, unlike the segregation ratio in the April and May samples. No correlation was found between fruitlet weight and Lap-2 genotype. It should be noted that once Lap-2 is expressed, its pattern remains unchanged during development, as shown by the fact that leaves of different ages sampled from the same 'Ettinger' tree exhibit the same isozymic pattern. Furthermore, analysis of a population of 'Ettinger' selfed seedlings during 3 successive years show consistent isozymic bandings.

The small size of the samples (Table 2) was due to low rate of fruitlet setting. In

addition, the majority of the young fruitlets sampled contained a degenerated embryo and therefore could not be assayed. For these reasons and because we were unable to sample fruits at the picking season, we repeated the experiment in 1985 using 5 caged 'Ettinger' trees (Table 3). Here again the numbers of fruitlets or fruits analyzed in each sample were relatively small, but the results were consistent with those obtained in the previous year (Table 2). In both experiments, there was no significant difference between genotype segregation in fruitlets picked from the tree compared to abscissed fruitlets in each date tested.

Table 2. Distribution of Lap-2 genotypes in embryos of 'Ettinger' self-pollinated fruitlets, 1984.

	Fruitlets picked from tree					Abscissed fruitlets				
	No.	Frequency (%)				No.	Frequency (%)			
Date	fruitlets	SS	FS	FF	P[z]	fruitlets	SS	FS	FF	P[z]
April–May	61	28	59	13	0.1	68	23	65	12	<0.01
June–July	36	14	72	14	<0.01	---	---	---	---	---

[z]Significance of deviation from expected ratio based on χ^2 test.

Table 3. Distribution of Lap-2 genotypes in embroys of 'Ettinger' self pollinated fruitlets, 1985.

	Fruitlets picked from tree					Abscissed fruitlets				
	No.	Frequency (%)				No.	Frequency (%)			
Date	fruitlets	SS	FS	FF	P[z]	fruitlets	SS	FS	FF	P[z]
May	39	21	69	10	0.01	98	19	66	15	<0.01
July–October	25		92	8	<0.01					

[z]Significance of deviation from expected ratio based on χ^2 test.

In Oct. 1984, 48 mature fruits from an isolated 'Ettinger' tree were assayed—45 of them had the genotype FS in Lap-2, 3 had FF, and none were SS—again showing a significant deviation from the expected ratio *(P* < 0.005).

Genetic selection during fruitlet abscission is the most probable explanation for all these results. Genetic selection was demonstrated by the high frequency of FS heterozygotes, the low frequency of FF homozygotes, and the lack of SS homozygotes among the progeny of this FS heterozygous cultivar. This conclusion is supported by the fact that the SS genotype was found at the stage of small fruitlets and never among mature fruits. The selection against fruitlets with FF genotype apparently starts somewhere close to fruit set (Tables 2 and 3). The selection against fruitlets with SS genotype probably occurs late in the season (after May), but we failed to determine the exact time. The genes responsible for such selection are not known. The Lap-2 locus could possibly be involved in the heterozygote advantage, or, more likely, serve as a genetic marker linked to other advantageous loci.

It is noteworthy that Vrecenar-Gadus and Ellstrand (18) found that the 'Bacon' cultivar has the FS genotype at *Lap-2,* but the genotype ratios among the selfed progeny did not deviate significantly *(P* = 0.4) from the expected 1:2:1 ratio. Our studies and those of Torres and Bergh (17) agree that 'Bacon' is homozygous (FF) for Lap-2. Of possible significance is the fact that Vrecenar-Gadus and Ellstrand used the Tris-EDTA-borate buffer system as opposed to our Tris-citrate buffer system. The only other heterozygous cultivar in the LAP-2 system available to us is 'Regina', from which we do not yet have selfed or open-pollinated progeny.

Genetic selection, expressed as deviation from the expected ratio of different

genotypes, is widely known in different crops including forest trees. For example, it was found in Eucalyptus by Phillips (13) and in conifers by Mitton (12). It is our suggestion that the mechanism of fruit drop in avocado could be based upon genetic selection. We are not aware of such phenomenon being found in any fruit trees. Although it is unlikely that selection is operating on the Lap locus itself, it is noteworthy to mention deviations from the expected ratio found in this locus among seedlings of forest trees. Rudin (14) found such deviations in *Pinus sylvestris* and Lundquist (11) found selection against a certain lap allele in *Picea abies*.

The selective fruitlet abscission, found in our studies, culminates in almost total lack of Lap-2 SS and FF genotypes. It is logical to assume that similar genetic selection might exist at other loci as well. If so, it might be possible to explain by such genetic selection a great portion of the massive fruitlet abscission in avocado. One can assume that such a tool will have evolutionary implications. The vast number of flowers on an avocado tree (10^6) allows about 10^4-fold selection rate. In other words, only the fittest 100-200 fruits will survive.

It seems worthwhile to continue this study along 3 lines: identification of the stage(s) at which the selection takes place; investigation of the physiological mechanism of this selection; and a search for similar cases of genetic selection in the abscission of fruitlets of other fruit trees.

Literature Cited

1. Adato, I. and S. Gazit. 1977. Role of ethylene in avocado fruit development and ripening: I. Fruit drop. J. Expt. Bot. 28:636- 643.
2. Argaman, A. 1983. Effect of temperature and pollen source on fertilization, fruit set and abscission in avocado. (In Hebrew). MS Thesis, Faculty of Agriculture, Hebrew Univ. of Jerusalem, Rehovot, Israel.
3. Blumenfeld, A. and S. Gazit. 1974. Development of seeded and seedless avocado fruits. J. Amer. Soc. Hort. Sei. 99:442-148.
4. Clegg, M.T. and R.W. Allard. 1972. Patterns of genetic differentiation in the slender wild oat species *Avena barbota.* Proc. Natl. Acad. Sci. USA 69:1820-1824.
5. Conkle, M.T. 1971. Inheritance of alcohol dehydrogenase and leucine aminopeptidase isozymes in knobcone pine. Forest Sci. 17:190-194.
6. Degani, C. and S. Gazit. 1984. Selfed and crossed proportions of avocado progenies produced by caged pairs of complementary cultivars. HortScience 19:258-260.
7. Gafni, E. 1984. Effect of extreme temperature regimes and different pollenizers on the fertilization and fruit set processes in avocado. (In Hebrew). MS Thesis, Faculty of Agriculture, Hebrew Univ. of Jerusalem, Rehovot, Israel.
8. Gazit, S. 1977. Pollination and fruitset of avocados, p. 88-92. In: J.W. Sauls, R.L. Phillips, and L.K. Jackson (eds.). Proc. First Intl. Trap. Fruit Short Course: The avocado. Univ. of Florida, Gainesville.
9. Hamrick, J.L. and R.W. Allard. 1977. Microgeographical variation in allozyms frequencies in *Avena barbota.* Proc. Natl. Acad. Sci. USA 69:2100-2104.

10. Lahav, E. and D. Zamet. 1975. Abscission of flowers, fruitlets and fruits in avocado. (In Hebrew). Alon Ha'Notea 29:556-562.

11. Lundquist, K. 1974. Inheritance of leucine aminopeptidase isozymes in Picea abies K. Hereditas 76:91-96.

12. Mitton, J.B., Y.B. Linhart, J.L. Hamrick, and J.S. Beckman. 1977. Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. Theor. Applied Genet. 57:5-13.

13. Phillips, M.A. and Brown, A.H.D. 1980. Mating system and hybridity in *Eucalyptus pauciflora.* Austral. J. Biol. Sci. 30:337- 344.

14. Rudin, D. 1977. Leucine-aminopeptidase (LAP) from needles and macrogametophytes of *Pinus sylvestris* L.: Inheritance of allozymes. Heriditas 85:219-226.

15. Sedgley, M. 1980. Anatomical investigation of abscissed avocado flowers and fruitlets. Ann. Bot. *46:711-111.*

16. Torres, A.M. 1984. Isozymes from avocado cotyledons. J. Her. 75:300-302.

17. Torres, A.M. and B.O. Bergh. 1980. Fruit and leaf isozymes as genetic markers in avocadoes. J. Amer. Soc. Hort. Sci. 105:614- 619.

18. Vrecnar-Gadus, M. and N.F. Ellstrand. 1984. Independent assortment of four isozyme loci in the 'Bacon' avocado *(Persea americana* Mill.). Calif. Avocado Soc. Yrbk. 68:173- 177.