

Independent Assortment of Four Isozyme Loci in the 'Bacon' Avocado (*Persea americana* Mill.)

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Abstract

Four enzyme loci (*Lap-2*, *Pgm-2*, *Tpi-1*, and *Mdh-1*) were examined for evidence of genetic linkage in the 'Bacon' avocado. Allele segregation ratios were obtained by starch gel electrophoresis of the cotyledon tissue of 100 fruits. Statistical analysis of these ratios indicated that these loci assort independently.

Introduction

A linkage group is an association of genes that are physically located on the same chromosome (Gardner, 1975). Genetic linkage can be detected by a variety of methods. Traditionally, joint segregation of morphological characters was considered evidence of genetic linkage. A similar approach can be employed with the use of isozyme markers (Peirce and Brewbaker, 1973). Each electrophoretic phenotype usually corresponds to a particular genotype. Therefore, by progeny testing for joint allelic segregation, probable linkage groups can be easily identified (e.g., Wendel and Parks, 1982).

Identification of these linkage groups is of particular value to the crop breeder. The presence of linkage between favorable and unfavorable characters or genes may make it difficult to arrive at the optimum phenotype. Conversely, joint segregation of favorable genes may enhance the breeder's success (Tanksley and Rick, 1980).

Isozyme loci can be particularly useful for marking linkage groups (Tanksley and Rick, 1980). Since these biochemical characters are usually expressed in a co-dominant fashion, they can be assayed in each generation. Crops with long generation times, such as trees, may benefit most from use of isozyme markers in a crop breeding program because the breeder does not have to wait until maturity of the offspring to evaluate his results (Torres, 1984). However, few isozyme studies have been published on tree crops (see Torres, 1983).

This paper analyzes the linkage relationships between 4 allozyme loci in the 'Bacon' avocado. Previous studies (Torres and Bergh, 1978; Torres *et al*, 1978; Torres, 1984) have identified isozymes in avocado mesocarp, leaf, and cotyledon tissue, but direct study of linkage groups has been confined to the tightly linked glutamate oxaloacetate transaminase loci *Got-1* and *Got-2* (Torres *et al*, 1978). Therefore, an investigation of this nature should provide a basis for both further genetic research and future breeding programs.

Materials and Methods

Several cultivars of the avocado are grown in California. We chose the cultivar 'Bacon' for this study due to the heterozygosity of 4 easily resolved isozyme loci in the parental tissue, phosphoglucosmutase (*Pgm-1*), malate dehydrogenase (*Mdh-1*), leucine amino peptidase (*Lap-2*), and triosephosphate isomerase (*Tpi-1*). [*Lap-2* of 'Bacon' is phenotypically homozygous in another gel system (Torres *et al.*, 1978).]

A large commercial planting of 'Bacon' trees was selected on the Irvine Ranch in Orange County, California. This orchard encompasses approximately 2 acres, large enough that the fruit in the center of the orchard probably results from 'Bacon' x 'Bacon' fertilization (Bergh, 1977). On October 12, 1983, 100 avocado fruits were collected from trees from the center of the grove. The fruits were stored in a refrigerated room until the day prior to electrophoresis.

A slice of tissue approximately 1 mm x 10 mm x 10 mm was shaved from the interior of the cotyledons. The tissue was immediately placed in a ceramic mortar and covered with liquid nitrogen. The sample was ground to a fine powder and approximately 0.5 ml of crushing buffer was added. The crushing buffer was based on the 0.10 M tris-maleate pH 7 buffer of Soltis *et al.* (1980), with the salt molarities reduced by one-half, and the polyvinylpyrrolidone (PVP) concentration lowered to 12%. Once the avocado-buffer mixture had thawed to a slurry, filter paper wicks were introduced into the mortar. The moistened wicks were inserted in 12% starch gels and horizontal electrophoresis was performed at 2°C.

Three types of gel-electrode systems were used. A tris-EDTA-borate system (Heywood, 1980) was run for 4.5 hr at 50 milliamps, sliced, and stained for LAP and PGM. A morpholine-citrate system (O'Malley *et al.*, 1980) was run for 4 hr at 50 milliamps, sliced, and stained for MDH. Finally, a tris-citrate system pH 7 (modified from Ellstrand *et al.* (1983)) was run for approximately 4 hr at 50 milliamps and stained for TPI.

Enzyme staining recipes for LAP and PGM were those of Ellstrand (1984). The TPI stain was modified from Pichersky and Gottlieb (1983). The actual composition was 25 ml 0.2 M tris-HCl pH 8 buffer, 15 ml H₂O, 19 mg EDTA, 100 mg sodium arsenate, 5 mg dihydroxyacetonephosphate (DHAP), 100 units glyceraldehyde-3-phosphate dehydrogenase, 15 mg NAD, 5 mg NBT, and 10 mg PMS. The stain for MDH consisted of 25 ml 0.2 M tris-HCl pH 8 buffer, 15 ml H₂O, 5 ml 2.0 M malate acid pH 7, 20 mg NAD, 10 mg NBT, and 10 mg PMS. All gels were incubated in the dark at 37°C for 1-2 hr.

After incubation, the gels were fixed and scored. Each individual was scored as FS, SS, or FF at each locus. These abbreviations refer to the relative mobilities of the isozymes coded for by the corresponding alleles (F = fast; S = slow) (see Torres *et al.*, 1978).

Table 1. Observed vs. expected genotype ratios for four loci in the selfed progeny of 'Bacon' avocado.

Locus	Genotypes		Chi square (2 df)	p
	Observed	Expected		
	SS:FS:FF	SS:FS:FF		
<u>Mdh-1</u>	20:54:26	1:2:1	1.36	0.50
<u>Tpi-1</u>	19:52:29	1:2:1	2.16	0.40
<u>Lap-2</u>	19:52:29	1:2:1	2.16	0.40
<u>Pgm-1</u>	19:57:24	1:2:1	2.46	0.37

Table 2. Linkage analysis for each isozyme pair.

Genotype	Expected ratio	Observed genotypes					
		<u>Lap-2/</u> <u>Pgm-2</u>	<u>Lap-2/</u> <u>Mdh-1</u>	<u>Lap-2/</u> <u>Tpi-1</u>	<u>Pgm-2/</u> <u>Mdh-1</u>	<u>Pgm-2/</u> <u>Tpi-1</u>	<u>Mdh-1/</u> <u>Tpi-1</u>
SS/SS	1	5	3	3	4	4	3
SS/FS	2	11	10	10	12	12	12
SS/FF	1	3	6	6	3	3	5
FS/SS	2	8	8	8	9	10	9
FS/FS	4	31	28	29	29	29	25
FS/FF	2	13	16	15	19	18	20
FF/SS	1	6	9	8	7	5	7
FF/FS	2	16	16	13	13	11	15
FF/FF	1	7	4	8	4	8	4
Chi-square (8 df)		6.28	8.16	5.96	8.44	7.0	8.84
p		0.61	0.45	0.64	0.43	0.54	0.40

Results and Discussion

Observed gene segregation ratios were compared to those expected for randomly segregating genes. The genes for the four isozymes were confirmed to be segregating in Mendelian ratios (Table 1). The frequencies of all possible genotype combinations for every six enzyme pairs were compared to the values expected due to independent assortment. Chi-square tests were used to determine whether or not these frequencies varied significantly from an expected random distribution (Table 2). Nine genotype combinations are possible in each case. For the 100 fruit surveyed, no locus pair

demonstrated any evidence of linkage (range of $P = 0.40-0.64$).

Thus, the genes coding for 4 enzymes (*Lap-2*, *Tpi-1*, *Pgm-1*, and *Mdh-1*) in the avocado assort independently. Although we cannot conclude that these loci might not be very loosely linked, they represent a step toward mapping linkage groups over the 12 pairs of chromosomes in the avocado.

Although no linkage was detected among these 4 loci, linkage has been detected between 2 other electrophoretic loci. Torres *et al.* (1978) were the first to investigate the genetic basis of isozyme inheritance in the avocado. They noted that the *Got-1* and *Got-2* loci are very tightly linked in 'Duke.' Unfortunately, our study was incapable of confirming these observations because the 'Bacon' cultivar is homozygous at these loci.

Further isozyme studies will be an aid to the avocado breeder (Tanksley and Rick, 1980). The information thus obtained will provide several major benefits. Once biochemical markers are discovered linked to favored phenotypes, the economic trait may be targeted almost immediately in the appropriate offspring. For example, N. Ellstrand, M. Wilson, and M. Coffey (in preparation) have found isozyme markers identifying those outcrossed avocado seedlings with enhanced root rot resistance relative to selfed seedlings. Furthermore, heterozygotes are easily identified in an isozyme profile, effectively eliminating the need for time-consuming back-crosses. Finally, when introducing wild genes into existing cultivars, the success of the incorporation can be followed through each cross. Linkage relationships of isozyme and other loci in avocado may thus prove helpful by speeding improvements of this crop.

Acknowledgements

The authors are indebted to B.O. Bergh, T. Holtsford, J. Lee, R. Soost, A. Torres, and J.G. Waines for their critical comments and to Irvine Ranch Corporation for providing plant material for analysis. This work represents, in part, a Master's Thesis in Plant Science at the University of California at Riverside.

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