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Fruit and Leaf Isozymes as Genetic Markers in Avocado[1]

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ABSTRACT. Leaf samples of over 100 avocado (*Persea americana* Mill.) cultivars and 8 other Persea species were analyzed for isozyme variation of peroxidase (PX), malate dehydrogenase (MDH), leucine aminopeptidase (LAP) and phosphoglucose mutase (PGM). MDH, LAP and PGM isozymes of leaf and mesocarp were identical. Isozymes of alcohol dehydrogenase (ADH) and glutamate oxaloacetate transaminase (GOT) were detected in mesocarps but were not available from leaves. Leaf PX isozymes were specified by 3 genes having 11 alleles and MDH isozymes by 1 gene with 3 alleles. To date, 6 enzyme systems specified by 12 genes with 37 codominant alleles are available as genetic markers of possible use in problems of avocado systematics, measurements of outcrossing rates, documentation of parentages and screening of seedlings in breeding programs.

The methods and the rationale for using variant molecular forms of enzymes, isozymes, as genetic markers in the avocado to overcome the difficulties inherent in its perenniality and breeding system (10) have been described (11, 12, 13). Isozymes are ideal markers because they are colinear with the gene, commonly codominant in effect and relatively unaffected by the environment. An earlier study (13) utilized isozymes from the fleshy mesocarp of the avocado but certain problems are best addressed using leaf tissue because leaves are available sooner than fruit. For example, the breeder may be concerned with screening young seedlings for parentage long before fruiting (5-8 years) so that space and other resources are not needlessly utilized (11); the same problem

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obtains in citrus (14) and presumably other woody, long-lived, slow-growing perennials. Avocado seeds would provide even earlier analyzable material from controlled crosses but it has not yet been possible to resolve their enzyme systems.

The previous report on avocado isozymes provided the first single-gene analysis for this species (13). It was found that 4 mesocarp enzyme systems were specified by 10 genes with 20 alleles: ADH is dimeric and specified by 2 genes, *Adh-1* and -2; LAP and PGM are monomers each coded by 2 genes, *Lap-1* and -2 and *Pgm-1* and -2; the isozymes of *Got-1* and *Got-2* are dimers; *Got-3* is apparently monomorphic as no variation has been found thus far. Isozymes of *Got-4* were studied earlier but they are not included here because of recently observed variations which called into question their assumed subunit structure. The study involved about 30 cultivars including several of major commercial importance in California and Florida. Substantial polymorphism was found and encouraged a search for additional markers.

The present article presents genetic studies of the leaf isozymes of PX and MDH, shows that leaf and mesocarp isozymes of LAP, PGM and MDH are identical and lists the isozyme profiles of over 100 *Persea taxa*.

Methods and Materials

Fruit of Florida cultivars were from the Agricultural Research and Education Center, Homestead; leaves were not available. California materials were from the University of California, Riverside or the South Coast Field Station and each is identified by a UCR field number (Table 1). Samples for leaf PX were prepared by crushing three 8 × 8 mm pieces of healthy, mature leaves with slip joint pliers in .07 ml 0.1 M potassium phosphate buffer (pH 7.5) in a plastic weighing boat. The crushing buffer for leaf LAP, PGM and MDH were as above, but included 12% soluble PVP (M. W. 40,000); the addition of 10 mM 2-mercaptoethanol improved resolution. PVP interfered with zymograms of PX. Mesocarp MDH required no crushing buffer. The starch gel electrophoretic conditions for leaf and mesocarp LAP and PGM and for mesocarp ADH and GOT were as described (13). A 17 mM tris-citrate gel buffer pH 7.9 and a 0.4 M sodium borate electrode buffer pH 8.7 were used for PX. The gel buffer for leaf and fruit mesocarp MDH was 16 mM tris-citrate (pH 6.9) and the electrode ubuffer was 48 mM triscitrate (pH 6.9). MDH gels were run at ca. 1 ma/cm^2 of gel cross section for 6-8 hr. The staining mixture for PX was 40 mg of 3-amino-9-ethylcarbazole, 2 ml dimethyl-formamide, 4 ml 1 M sodium acetate buffer (pH 5.0), 1.6 ml of 0.1 M $CaC1_2$, 93 ml of H_2O and 0.4 ml of 3% H_2O_2. For MDH, the staining mixture included 6 ml of 1 M tris-HCl (pH 8.8), 50 ml H_2O, 8 ml 0.01 M nicotinamide adenine dinucleotide, 0.8 ml 0.01 M phenazine methosulfate, 4 ml 0.01 M nitro blue tetrazolium chloride, and 3 ml 2 M D,L-malic acid neutralized to pH 7.0 with NaOH.

For MDH dissociation-recombination, 4 g of mesocarp were ground in 8 ml 0.1 M potassium phosphate buffer (pH 6.0), with 0.4 g sucrose and 0.1 M 2-mercaptoethanol. The slurries were strained and centrifuged at 10,000 × g at 2°C for 15 min. Aliquots of 0.4 ml of each separately prepared extract were combined and 46 mg NaCl was slowly added while stirring. The mixture was frozen for 6 days, thawed 90 min and dialyzed 12 hr against 400 vol (pH 7.5) 0.1 M tris-HCl with 0.4 M sucrose and 0.1 M 2-

mercaptoethanol. The centrifuged supernatants were then electrophoresed and stained for MDH.

Fruit and leaf extracts of many cultivars were alternated in the same gel to test whether or not leaf and fruit isozymes were under the control of the same genes. Fruit samples were diluted to obtain staining intensities equal to those of leaf extracts.

Table 1. Genotypes of cultivars of *Persea americana* and *Persea* spp.[z]

Cultivars and species	UCR field location	Genotypes											
		Adh-1	*Adh-2*	*Got-1*	*Got-2*	*Lap-1*	*Lap-2*	*Mdh-1*	*Pgm-1*	*Pgm-2*	*Cpx*	*Px-1*	*Px-2*
102	46/35/16	FF	FF	FF	FF	FF	FF	FS	FS	FF	FM?	SS	BB
151-2	23/31/27	FF	FF	FS	FS	FF	FS	FS	FF	FF	FS	SS	BB
Alboyce	23/29/1	FF	FF	FF	FF	FF	FF	FS	FS	FF	FS?	SS	BB
Allcuke	23/29/24	FF	FS	FF	FF	FF	FF	FS	SS	FF	FF	SS	BB
Anaheim	30/2/5	FF	FF	SS	SS	FF	FF	FF	FF	FF	FS	SS	BB
Arturo	23/27/22	FF	FS	FF	FF	FF	FF	FF	FS	FF	SS	SS	BC[y]
Bacon	23/26/3	FF	FF	FF	FF	FF	FF	FS	FS	FF	FS	SS	BB
Big Leaf	3/20/15	FF	FF	FF	FF	FF	FF	SS	FF	FF	FF	SS	BB
Blake	Sta. Barb.	FF	FS	FF	FF	FF	FF	FS	FS	FF	MS	---	---
Bolles	3/2/5	FF	FF	FF	FF	FF	FF	FF	SS	FF	SM	SS	BB
Bolles A	3/11/14	FF	FS	FF	FF	FF	FF	FF	FS	FF	SM	SS	BB
Bolles B	3/11/15	FF	FS	FF	FF	FF	FF	FS	FS	FF	MM	SM	BB
Bonita	23/32/7	FF	FF	SS	SS	FF	FF	FS	FF	FF	SS	SS	BB
Booth 7	Florida	SS	FF	SS	SS	FS	FF	FM	SS	FF	---	---	---
Booth 8	Florida	FS	FF	SS	SS	FS	FF	FM	FS	FF	---	---	---
Carlsbad	30/7/5	FF	FF	SS	SS	FF	FF	SS	FF	FF	FM	SS	BB
Carolyn	30/6/1	FF	FF	FS	FS	FF	FF	---	FF	FF	FS	SS	BB
Cellon's Hawaii	3/17/28	FS	FF	SS	SS	SS	FF	FF	FS	FF	SM	SS	BB
CH_4	6B/41/9	FF	FF	FF	FF	FF	FF	SS	FS	FF	SM	SS	BB
CH_5	6B/41/8	FF	FF	FF	FF	FF	FF	SS	FS	FF	SM	SS	BB
Chafein #5	3/18/20	FF	FS	FF	FF	FF	FF	FS	FS	FF	SM	SS	BB
Chapingo	3/26/2	FF	FF	FF	FF	FF	FF	FF	SS	FF	SM	SS	BB
Chrones	23/30/9	FF	FF	FF	FF	FF	FF	---	FS	FF	FF	SS	BB
Clifton	19/13/2	FF	FF	FS	FS	FF	GS	FS	FS	FF	SM	SS	BB
Coock	23/30/18	FF	FF	FS	FS	FF	FF	FS	FS	FF	FM	FS	FB
Corona	30/6/2	FF	FF	FS	FS	FF	FF	FF	SS	FF	SS	SS	BB
CRI-71	3/20/8	FF	FF	SS	SS	FF	FF	SS	FS	FF	FM	SS	BB
CR4	3/5/6	MM?	FF	SS	SS	FF	FF	FS	SS	FF	FM	MS	BB
DD33	F. 6	FF	FF	FS	FS	FF	FF	FS	FF	FF	SS	SS	BB
Diamond	23/28/6	FF	FF	FF	FF	FF	FF	SS	FF	FF	FF	SS	FF
Dickinson	23/26/17	FF	FF	FS	FS	FF	FF	SS	FF	FF	FS	SS	BB
Duke	23/26/6	FF	FS	FF	FF	FF	FF	FS	SS	FF	MS	MS	AC
Duke 7	11E/17/5	FF	FF	FS	FS	FF	FS	FS	SS	FF	MM	SS	AC
Edranol	30/5/5	FF	FF	FS	FS	FF	FS	SS	FS	FF	FS	SS	BB
Ettinger	23/27/6	FF	FF	FF	FF	FF	FS	SS	FS	FF	FS	SS	BB
Feito	23/28/29	FF	FF	FS	FS	FF	FF	SS	FS	FF	FS	SS	BB
Fuerte	23/26/19	FF	FF	FS	FS	FF	FF	FS	FS	FF	MS	SS	BB
G1-66	3/19/6	FF	FF	FS	FS	FF	FF	FS	0	FF	SS	SS	BB
G3-71	3/20/5	FF	FF	FS	FS	FF	SS	SS	FF	FF	MF	SM	BB
G-6	11E/19/6	FF	FS	FF	FF	FF	FF	FF	SS	FF	FF	SM	BB
G6-72	3/20/3	FF	FS	FF	FF	FF	FF	FF	SS	FF	FF	FS?	CF?
G12	3/18/22	---	FF	FF	FF	FF?	SS?	SS	FF	FF	FM	SS	BB
G13	3/18/25	FS	FF	FF	FF	FF?	FF	SS	0	FF	SM	SS	BB
G19	3/18/24	---	FF	FF	FF	FF	SS	SS	RF	FF	FF	SS	BB
G21A	3/2/12	FF	FS	FF	FF	FF	FF	FS	SS	FF	SS	SS	BC
G21B	3/16/14	FM	FF	FS	FS	FF	FF	SS	SS	FF	SMF	SM	BB
G22	3/6/12	FF	FF	SS	SS	FF	SG	FS	FS?	FF	FM	FS	BC
Ganter	23/27/20	FF	FS	FF	FF	FF	FF	FS	SS	FF	SS	SS	CC
Guat/Brown	3/19/12	FF	FF	FS	FS	FF	FF	SS	FF	FF	FF	SS	BB
Hacienda	23/38/15	FF	FF	FF	FF	FF	FF	SS	SS	FF	SS	SS	BB
Hall	Florida	FS	FF	FS	FS	FF	FF	FS	FR	FF	---	---	---
Harms	23/26/10	FF	FF	SS	SS	FF	FS	FS	FF	FF	FS	SS	BB
Hashimoto	23/27/11	FF	FF	FS	FS	FF	FF	SS	FF	FF	FF	SS	BB
Hass	23/26/21	FF	FF	FS	FS	FF	FF	SS	FS	FF	FS	SS	BB
Haston	23/29/15	FF	FF	FS	FS	FF	SG	FS	SS	FF	SS	SS	BB
Huntalis	46/40/24	---	---	---	---	FF	FF	---	SS	FF	SS	SS	AA
Ignacio	6C/47/1	FF	FS	FF	FF	FF	FF	FS	SS	FF	SM	SS	BB
Infante	23/30/2	FF	FF	SS	SS	FF	FF	FF	RS	FF	SM	SS	BB
Irving	23/26/27	FF	FF	FS	FS	FF	FF	FF	FF	FF	MF	SS	BB
Itzamna	30/3/1	FF	FF	FF	FF	FF	FF	SS	FF	FF	FS	SS	BB

Cultivars and species	UCR field location	Genotypes											
		Adh-1	*Adh-2*	*Got-1*	*Got-2*	*Lap-1*	*Lap-2*	*Mdh-1*	*Pgm-1*	*Pgm-2*	*Cpx*	*Px-1*	*Px-2*
Janboyce	23/28/20	FF	FF	FS	FS	FF	FF	SS	FF	FF	---	SS	BB
Largo	21F/3/5	FF	FF	FS	FS	FF	FF	FS	FS	FF	FS?	SM	FB
Linda	30/3/5	FF	FF	FS	FS	FF	FS	SS	FF	FF	FS	SS	BB
M5-62	3/18/28	FF	FF	FF	FF	FF	FF	FS	SS	FF	FS	SS	BB
M12-58	3/5/5	FF	FF	FS	FS	FF	FF	FS	SS	FF	SS	SS	AB
M15-58	3/6/3	FF	FS	FF	FF	FF	FF	FS	FS	FF	SS	SS	AB
M-18	3/18/21	FF	FS	FF	FF	FF	FF	SS	SS	FF	NN	SM	BB
M70-60	3/17/17	FF	FF	FS	FS	FF	FF	FF	SS	FF	MM	SS	AA[y]
M72-60	3/17/21	FF	FF	FF	FF	FF	FF	FF	SS	FF	MM	SS	AA
Marcus	Florida	FS	FF	SS	SS	FS	FF	FS	SS	FF	---	---	---
Marshelline	23/27/8	FF	FF	FS	FS	FF	FF	FF	FF	FF	FS	SS	BB
Mayo	23/27/4	FF	FS	FS	FS	FF	FF	SS	FS	FF	FS	SS	AB
McArthur	23/27/25	FF	FF	FS	FS	FF	FF	SS	FS	FF	FF	SS	BB
McGill	Florida	FS	FF	SS	SS	FS	FF	FF	FS	FF	---	---	---
Mesa	30/2/3	FF	FF	SS	SS	FF	FF	SS	FF	FF	SMF	SS	BB
Mexicola	23/26/22	FF	FF	FF	FF	FF	FF	FF	SS	FF	SM	SS	AC[y]
Nabal	23/28/9	FF	FF	FS	FS	FF	FF	SS	FF	FF	FS?	SS	BB
Nadir	Florida	SS	FF	SS	SS	SS	SG	FF	SS	FF	---	---	---
Nimlioh	23/28/4	FF	FF	SS	SS	FF	FF	SS	FF	FF	FM	SM	BB
Nordstein[x]	---	MS	FS	FS	FS	FF	FF	SS	FS?	FF	SM	SS	BB
Northrup	23/27/20	FF	FS	FF	FF	FF	FF	FS	SS	FF	SS	SS	AA
"P. americana"	3/5/10	0	0	II	FF	FF	FF	SS	FF?	FF	SM	SS	AB
P. floccosa	23/33/6	FF	FS	FS	FS	FF	FF	FM	0	FF	FS?	---	BB
P. indica	3/5/4	0	0	0	FS	FF	FF	FF	---	FF	FF	FM	BB
P. lingue	3/5/1	0	0	GG	FF	FF	FF	SS	0	0	FF	MM	BB
P. longipes	3/20/4	0	0	FF	FF	FF	FF	FF	FF?	FF	FF	SS	BB
P. nubigena	3/6/15	FM	FF	FF	FF	FF	FF	FS[w]	FF	FF	FF	SS	BB
P. packipoda	3/6/4	0	0	0	0	FF	FF	---	0	FF	FF	SM	FS?
P. schiedeana	3/2/18	FS	FF	0	SS	FF	FF	FM	MM	SS	---	---	---
P. schutchii	3/6/14	0	0	0	0	FF	FF	FF	0	FF	FF	SS	SS
PR 18	3/16/6	FF	FF	SS	SS	FF	FS	FS	SS	FF	SM	SM	BC
Pinkerton	23/28/11	FF	FF	FS	FS	FF	FF	FS	FF	FF	FS	SS	BB
Queen	30/1/1	FF	FF	SS	SS	FF	FF	SS	FF	FF	MM	SS	BB
Reed	11E/14/4	FF	FF	FS	FS	FF	FF	FS	FF	FF	FF	SS	BB
Rincon	11E/14/3	FF	FF	FS	FS	FF	FF	FF	FF	FF	MS	SS	BB
Sta. Fe. Spr.	45/78/14	FF	FS	FF	FF	FF	FF	FS	SS	FF	MS	SS	AC[y]
Semil 34	23/30/21	FF	FF	SS	FS	FS	FF	---	FS	FF	FM	SS	BB
Stewart	23/27/29	FF	FF	FS	FS	FF	FF	FS	FS/FF	FF	SS	SM	BB
Taft 142	23/28/16	FF	FF	SS	SS	FF	FF	SS	FF	FF	FS	SS	BB
Teague	23/27/1	FF	FS	FS	FS	FF	FF	FF	SS	FF	SS?	SS	SB[y]
Thille	30/1/5	FF	FF	FS	FS	FF	FF	SS	FF	FF	SS?	SS	BB
Topa Topa	23/27/23	FF	FS	FF	FF	FF	FF	FS	SS	FF	SM	SS	BB
Vaca	Florida	FS	FF	SS	SS	SG	GG	FM	FS	FF	---	---	---
Waldin	Florida	FS	FF	SS	SS	FS	FF	FF	SS	FF	---	---	---
Whitsell	23/40/2	FF	FF	FS	FS	FF	SG	SS	FS	FF	FF	SS	BB
Yama	23/27/17	FF	FF	FF	FF	FF	FF	FS	SS	FF	MS	SS	AA
Zutano	23/27/15	FF	FF	FF	FF	IT'	FS	FF	FS	FE	FS	SS	BB

[z]A dash indicates that no determination was made; a "?" indicates a doubtful determination, 0 indicates no enzymes could be detec for this system. See text for explanation of symbols.
[Y]Two-banded patterns; otherwise heterozygotes for Px-2 were three-banded.
[X]Kindly provided by Dr. Roy Young of UCR.
[W]Bands appeared to be shifted slightly toward the origin.

Results and Discussion

Consistent with earlier nomenclature, genes which specify the slower or slowest anodally migrating isozyme set will be designated by the number *1*, and the genes which code for successively faster migrating sets will be called 2 (Fig. 1). The allele which specifies the slowest anodally migrating isozyme within a set is generally called S; M and F stand for Medium and Fast. The R (for Retarded) allele of Pgm-1 specifies a polypeptide which migrates slower than that of S, and G codes for newly detected isozymes which migrate faster than those of the already established F. The names A, B and C, like M, are given to alleles whose products migrate between those of S and F.

Thus, allelic designations correspond to the slow to fast order of migration of their isozymes: R, S, M, A, B, C, F, G.

NEW ALLELES IN EARLIER STUDIED SYSTEMS. While the genetic control of mesocarp ADH, GOT, LAP and PGM was reported earlier (13), the broader survey of cultivars and other Persea spp. in the present study revealed some new alleles for these systems (Table 1).

Adh-1 alleles known from the previous survey included *S, M* and *F*. No new alleles were detected. *S* of *Adh-1* was found. with one exception, only in Florida cultivars which are largely or entirely of West Indian derivation (1, 7). The exception was in G13, a collection from Guatemala of unknown ancestry and racial composition grown at Riverside. The *M* allele had previously been found in *P. nubigena* L. O. Williams, a small-fruited relative of *P. americana* collected in Guatemala, but the *M* allele also occurs in CR4, G21B and 'Nordstein' (an Israel cultivar).

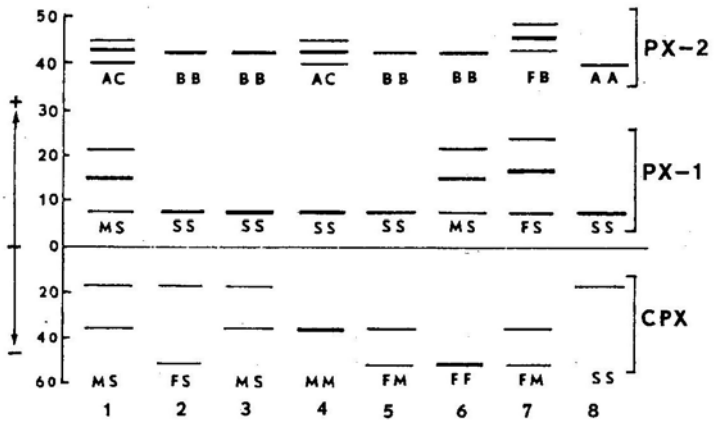

Fig. 1. Schematic peroxidase zymograms of avocado cultivars. Rp values (×100) along the left, isozyme designations along the right; 0 = origin, + = anode, - = cathode. Genotypes indicated along the bottom of each set. Channels are numbered along the bottom. Channel 1, 'Duke'; 2, 'Pinkerton'; 3, 'Fuerte'; 4, 'Duke 7'; 5, Irving': 6, G-6; 7, 'Coock'; 8, 'Huntalis'.

No new alleles for *Adh-2* were found. 'Duke' is *F/S*, whereas it was previously reported as *S/S* (13). The earlier 'Duke' sample was found, through examination of pedigree, to be a self-pollinated 'Duke' seedling (12).

In addition to *S* and *F*, new alleles of *Got-1* were *M* (found as the hornozygote *M/M*) in a *P. americana* sample and *G* (as *G/G*) in *P. indica* (L.) Spreng., both grown at Riverside. *Persea indica* is native to the Canary and Azores Islands.

Genotypes of *Got-1* and *Got-2* were always the same (Table 1), hence they were assumed to be linked. The first indication of apparent crossing over was found in 'Semil 34' which is *S/S* for *Got-1* but *F/S* for *Got-2*.

Variation has now been found for *Lap-1* so that the common allele can be designated *F/F.* The *S* allele occurs in some of the Florida cultivars, in 'Semil 34' and in 'Cellon's Hawaii'. The majority of California cultivars are Mexican × Guatemalan hybrids and the *S* allele is lacking in these, thus we may tentatively infer that the *S* allele of *Lap-1*, like *S* of *Adh-1*, originated in the West Indian race.

F and *S* alleles of *Lap-2* were recognized previously, but evidence of an allele designated *G* which codes for a faster migrating isozyme was found. It occurs in a group of cultivars, 'Clifton', G22, 'Haston', 'Nadir', 'Vaca', 'Yama', and 'Whitsell', which for

the most part have no known immediate relationship to each other. 'Haston' is a supposed 'Hass' × 'Clifton' F_1 so that the presence of *Lap-2G* in 'Haston' is attributable to 'Clifton'. 'Vaca' and 'Nadir' are Florida cultivars. The origin of 'Yama' is unknown, and 'Whitsell' is thought to be an F_1 of 'Hass' × 'Rincon', neither of which has G. Thus the reputed parentage of 'Whitsell' is open to question.

A new allele, *R* (for *Retarded*) of *Pgm-1* was found in G19, 'Hall' and 'Infante'. There is no known racial or genetic relation between these three cultivars.

Pgm-2 was monomorphic in all cultivars of *P. americana* examined. However, one specimen of wild *P. schiedeana* Nees collected in Mexico had a single isozyme in the *Pgm-2* region which migrated more slowly than the usual; the *Pgm-2* genotype of *P. schiedeana* could therefore be designated as *S/S* and all other *P. americana* genotypes become *F/F*.

MESOCARP AND LEAF ZYMOGRAMS COMPARED. LAP, PGM, and MDH leaf and fruit zymograms of the same cultivar were identical in pattern, relative intensity and positions of the isozymes. It appears that the same genes in leaves and fruit specify these 3 enzyme systems. Fruit peroxidase has not yet been clearly resolved, but mesocarp tissue yielded a zymogram entirely different from that of the leaf.

NEW SYSTEMS; LEAF PX, LEAF AND MESOCARP MDH. Considerable experimentation was required to obtain satisfactory resolution of certain leaf enzyme systems because of the presence of phenolics and/or their derivatives in avocado leaves (5).

Interpretable leaf PX zymograms consisted of isozymes in 3 regions of the gel, 1 cathodal set and 2 anodal sets as illustrated in Fig. 1. The cathodal set resolved clearly in leaves 10-14 da after harvest stored at 2-3C. They appeared to be isozyrnes which formed as the leaf senesced, or perhaps they became accessible by our extraction methods as membranes and/or cell walls deteriorated.

The PX isozyme set of the cathodal gel section is designated CPX, and for a given cultivar, there were 1 or 2 isozyme bands (Fig. 1) suggesting that the isozyrnes are monomers. If I band was present, it was in 1 of 3 different positions leading to the inference that the gene which specifies these isozymes, *Cpx,* has 3 alleles, *S*, *M* and *F*.

A segregation analysis for *Cpx* was carried out with a population of 200 seedlings of 'Pinkerton' which has 2 bands and is *F/S*. There were 56 *S/S*, 97 *F/S* and 47 *F/F*. Data are not significantly different from the expected 1:2:1 ratio (χ^2=0.59, p > 0.5). 'Hass' is also *F/S* for *Cpx* and of 30 F_1's from selfing, there were 10 *S/S*: 12 *F/S*:8 *F/F*; the data are not significantly different from 1:2:1 (χ^2=1.46, p > 0.3). The 'Duke' zymogram consisted of 2 bands in the *Cpx* region, one S and one M. A population of 10 'Duke' selfed seedlings was examined and the observed results (5:4:2) did not deviate significantly from the expected (χ^2=2.8, p > 0.2). These and other crossing data suggest that *Cpx* has 3 alleles, *S*, *M* and *F*, which specify monomeric cathodal isozymes.

Fresh leaves of a given cultivar produced either I or 3 PX isozymes in the set which migrated most slowly toward the anode. The single isozyme bands occurred in 1 of 3 positions. These observations suggested that the isozymes have a 2 subunit structure and are specified by *Px-1* having 3 alleles, *S*, *M* and *F*. There were very few among all of the cultivars examined with the 3-banded pattern for which there were also selfed seedlings available for analysis. 'Duke', however, is *S/M* for *Px-1*, and of 15 selfs, 4

were *S/S*, 8 were *M/S* and 3 were *M/M*. Again, the difference between the theoretical 1:2:1 ratio and the observed numbers was nonsignificant (χ^2=0.2, p > 0.9). 'Pinkerton' trees and those of other cultivars nearby that could have been pollen sources for the 200 seedlings examined were all *S/S* for *Px-1*; hence, all of the progeny were expected to be *S/S* and all were. Thus, the hypothesized genetic control of the *Px-1* isozymes was supported by the obtainable segregation data.

Isozymes of *Px-2*, just anodal from those of *Px-1*, were unusual in that some cultivars produced 1 band, others 2 and yet others 3. One band would suggest homozygosity, 2, heterozygosity for a monomeric system, and 3, heterozygosity for a dimeric system. The question of a quaternary structure must remain open but the genetic analysis could proceed. The single bands occurred in 1 of 5 different positions suggesting the controlling gene, *Px-2*, has alleles which have been named *S*, *A*, *B*, *C*, and *F*, in order of the migration of their products from slow to fast. Again, material to study segregation ratios was scarce, but the same 200 'Pinkerton' seedlings were all identical to the parents and are of presumed genotype *B/B*. 'Duke' produced a three-banded pattern; 1 at the A, 1 at the C, and 1 at an intermediate position. In the same 'Duke' F_1 population as examined for *Px-1*, there was a 3:9:3 segregation ratio to compare with the expected 1:2:1. The observed ratio did not deviate significantly from the expected (χ^2=0.6, p > 0.7). 'Duke' has been assigned the genotype *A/C* and its isozymes were used as a standard.

Data from the F_1 'Duke' PX's, although the sample size is small (N = 15), suggested that *Px-1* and *Px-2* may be linked because of the more frequent association of certain combined genotypes and the reduced frequency of others. The expected F_2 dihybrid ratio of phenotypes with codominance and independent assortment would be 1:2:1:2:4:2:1:2:1; 1*SS*:2*SM*:1*MM* for *Px-1* × 1*AA*:2*AC*:1*CC* for *Px-2*. The corresponding observed ratio was 2*SS*/*AA*:2*SS*/*AC**: 0*SS*/*CC***: 1*SM*/*AA**: 7*SM*/*AC*: 0*SM*/*CC**: 0*MM*/*AA***: 0*MM*/*AC**: 3*MM*/*CC*. The single asterisked genotypes would require a crossover in only one parent, while those with double asterisks would necessitate a crossover in both parents. The χ^2 value for independent assortment is 12.5 (p < 0.2); assuming linkage, however, the χ^2 value is 1.3 (p > 0.7). More data should be gathered to test linkage because of the possible relationship between peroxidases and disease resistance (3, 8, 14, 15). Indeed, cultivars that have demonstrated resistance to avocado root rot (*Phytophthora cinnamomi* Rands) were commonly *M/S* for *Px-1*. If *Px-1* and *Px-2* were linked, 'Duke' progeny could be scored for crossovers and tested for resistance or be used as parents in resistance breeding programs (2, 17, 18). The data did not suggest linkage between *Cpx* and *Px-1* or *Px-2*, but the limited number of plants available precluded adequate testing.

'Teague' is a hybrid of 'Fuerte' × 'Duke' as judged from morphology and documented by isozymes (12, 13). The expected 'Teague' genotype/phenotype for *Px-2* would be *A/B* or *B/C* with either 2 or 3 bands. However, 'Teague' produced an isozyme for *Px-2* at the B position and a slow moving band not found in either parent nor in any other cultivar sampled. The isozyme has tentatively been ascribed to an allele, *S*, but may well be an artifact of unknown origin.

MDH has been widely studied in a variety of organisms. Two forms exist in animals, 1 in the cytosol and 1 in the mitochondria (6, 16). Plants have an additional MDH in their microbodies. Most variation in isozyme patterns has been observed in the mitochondrial

form which is specified by nuclear genes and is dimeric in all organisms studied (4). Recently, the first microbody MDH polymorphism has been reported in *Opuntia* (9) and the 2 isozymes of the heterozygotes suggested this MDH was monomeric.

The variable avocado fruit and leaf MDH zymograms were identical; however, 1 unresolvable, but apparently invariable, mesocarp isozyme set was absent in leaves. Preliminary sucrose density gradient experiments suggested that the variable MDH enzymes analyzed here were mitochondrial and the set missing in leaves could be those of the microbodies or the cytoplasm. The basic MDH zymograms are illustrated in Fig. 2 where they are labelled as the patterns A, B, C and D. Only a few cultivars had patterns other than these, and the most common one was D. Pattern E (not shown) was basically like D but the 2 lower bands were shifted toward the anode.

The zymograms of several progeny of known parents and a population of 'Bacon' selfed F_1's provided evidence concerning the genetic control of bands 1, 3 and 4 in Fig. 2. The lower three isozymes of the D pattern are thought to be specified by *Mdh-1*. We believe *Mdh-1* produces dimers whereby the homozygous *S/S* genotype yields the lower isozyme of the A pattern. The heterozygote, *F/S*, yields 3 isozymes as expected but the faster migrating isozyme is at the same position as the upper isozyme of the A pattern or the lower isozyme of the B pattern (band 4). Band 4 was always present, thus it is probably the product of a second gene, *Mdh-2*. Under this interpretation, the genotype of pattern A is *S/S* for *Mdh-1* and *C/C* (for *Constant*) for *Mdh-2*. Pattern D is *F/S* for *Mdh-1* and again *C/C* for *Mdh-2*; pattern B is *F/F* for *Mdh-1* and *C/C* for *Mdh-2*. Some of the segregation data in support of this interpretation are given in Table 2. 'Bacon', with pattern D, would be *1F*/*1S*, *2C*/*2C* and its progeny from selfing should segregate as 1*SS*: 2*FS*: 1*FF* or as patterns 1A:2D:1B. Band 4 would always be present because of *2C*/*2C*. Of 65 progeny, the observed ratio was 14:38:13 which is a nonsignificant deviation from the expected (χ^2=1.89, p > 0.3).

The above interpretation of the genetic control of MDH carries certain implications which pertain to band 4: band 4 in pattern A consists only of 2C-2C dimers (or 2C monomers) and band 4 in patterns B and D consists of at least 2 isozyme types, 2C-2C (or 2C monomers), but also 1F-1F dimers. If this hypothesis is correct and includes the assumption that the *Mdh-1* products are dimers, then the separate A and B patterns should be combinable by dissociation-recombination of the polypeptide subunits into pattern D. That is, S subunits of band 1 of pattern A should dimerize with F subunits of band 4 of pattern B to yield the F-S dimer of band 3 which is not possessed by either "parent." A dissociation-recombination experiment gave zymogram results as predicted and consistent with the hypothesized subunit composition and genetic control of isozyme bands 1, 3, and 4.

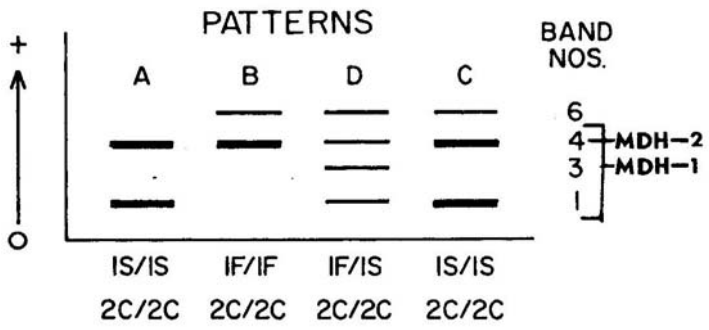

Fig. 2. Schematic illustration of variable avocado fruit and leaf MDH zymograms; letter designations above, assumed genotypes below, band numbers at right. *Mdh-1* bands are 1, 3 and 4. *Mdh-2* band 4 is constant at the same position as the *Mdh-1* F-F homodimer isozyme. Band 1 is the *Mdh-1* S-S homodimer isozyme, band 3 the *Mdh-1* F-S heterodimer.

Table 2. MDH-1 phenotypes and assumed genotypes for avocado parents and progeny.

Cross	Phenotype pattern	Hypothetical genotype for *Mdh-1*
Hass ×	A	S/S
Rincon =	B	F/F
Pinkerton	D	F/S
Duke ×	D	F/S
Fuerte =	D	F/S
Teague	D	F/S
Edranol ×	A	S/S
Anaheim =	B	F/F
151-2	D	F/S
Nabal ×	C	S/S
Anaheim =	B	F/F
Reed	D	F/S
Bacon ×	D	F/S
Bacon =	D	F/S
14 F_1	A	S/S
38 F_1	D	F/S
13 F_1	B	F/F

We are not yet prepared to address the problem of band 6 present in the B, C and D patterns. The band could be an artifact, a product of a third locus possibly producing monomers and having null or silent alleles or it could be a case of dimers incapable of certain cross dimerizations. We cannot presently distinguish between these various possibilities. If, however, band 6 were the fast moving allozyme of a third isozyme migrating to the band 4 position, and if 'Bacon' were heterozygous *F/S* for this hypothetical third gene, then its progeny should segregate as 3:1 band 6 vs. no band 6. There were 51 of 65 'Bacon' selfed seedling progeny with band 6 (patterns B and D) and 14 without (pattern A). This ratio is very close to 3:1 (χ^2=0.41, $p > 0.5$). These data, however, would fit other interpretations and the lack of certain possible recombinant patterns among the 65 seedlings (e.g., band 4 alone and a pattern consisting of bands 1, 4 and 6) indicates that linkage would also have to be invoked. Further, the interpretation assumes that the third gene's isozymes are monomeric and/or may not dimerize in certain combinations.

Mdh-1 apparently specifies a dimeric system as shown by progeny tests and the dissociation-recombination experiment and has 3 alleles, *F*, the rare *M*, and *S*. *Mdh-2* is apparently monomorphic and specifies an isozyme of unknown quaternary structure which has the same electrophoretic properties as that of the subunit F of *Mdh-1*. The nature of band 6 and its allozymes remains an enigma.

Conclusions

Newly found alleles are reported for *Got-1*, *Lap-1*, *Lap-2*, *Pgm-1*, and *Pgm-2*. It is apparent from Table I that some alleles are prevalent and others are rare. The data also

clearly suggest that there is a high or low frequency of certain alleles in relation to the racial origin of the cultivars. Thus, there is now the possibility that isozyme data can be used to examine systematic and evolutionary problems.

A comparison of leaf and mesocarp isozymes of LAP, MDH, and PGM indicated that the same genes were operating in these tissues. ADH was not detected in leaves and GOT has not been resolved in leaves. Leaf and fruit PX produce different zymograms.

Segregation ratio data for PX and MDH suggest that these enzymes are specified by at least 3 genes and 1 gene, respectively. All of the genes and alleles found to date in avocados and allied taxa are summarized in Table 3. The 6 enzyme systems studied are specified by 12 genes with 37 alleles. Undoubtedly, new polymorphisms will be found among West Indian cultivars which are presently isozymically poorly known.

Several of the uses of isozyme markers noted earlier for the avocado could be applied to other crops as well. In addition, they could be used to characterize clones isozymically, especially in patent applications, to assess evolutionary relationships and in citrus and mango to distinguish asexual nucellar from recombinant zygotic seedlings in breeding programs. Other uses for this tool will undoubtedly be found depending on the problems peculiar to a particular crop.

Table 3. Genes, alleles, and tissues analyzed in cultivated *P. americana* and *Persea spp*.

Gene	Alleles	Tissue[z]
Adh-1	*S, M, F*	F
Adh-2	*S, F*	F
Got-1	*S, M, F, G*	F
Got-2	*S, F*	F
Lap-I	*S, F, G*	F, L
Lap-2	*S, F, G*	F, L
Mdh-1	*S, M, F*	F, L
Pgm-I	*R, S, M, F*	F, L
Pgm-2	*S, F*	F, L
Cpx	*S, M, F*	L
Px-1	*S, M, F*	L
Px-2	*S, A, B, C, F*	L
Adh-1	*S, M, F*	F
Adh-2	*S, F*	F

[z]F = fruit, L = leaf

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