Inheritance of Isocitrate Dehydrogenase, Malate Dehydrogenase, and Shikimate Dehydrogenase in Peach and Peach × Almond Hybrids

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Abstract. Eighteen isozyme systems were surveyed in the peach [Prunus persica (L.) Batsch.] plant introduction collection. Seven systems were polymorphic. Three previously unreported isocitrate dehydrogenase (IDH; EC 1.1.1.41), three malate dehydrogenase (MDH; EC 1.1.1.37) and two shikimate dehydrogenase (SDH; EC 1.1.1.25) banding patterns were detected in the clones. Isocitrate dehydrogenase was dimeric in structure, with two alleles present at a single locus. Malate dehydrogenase was dimeric in structure, with three alleles present at the fast locus, while a second locus was monomorphic. Shikimate dehydrogenase was monomeric, with one allele present in most clones, while PI 113452, PI 113650, and PI 117679 were heterozygous for a slow SDH allele. Electrophoretic evidence suggests PI 113452, PI 113650, and PI 117679 are peach × almond (P. dulcis Webb) hybrids, since they were heterozygous for alleles previously reported only in almond.

One of the main uses of isozyme analysis is to facilitate development of genetic maps through linkage studies. Isozymes have been used extensively to study gene linkage in Zea mays L. (Goodman et al., 1980b; Stuber et al., 1988) and Lycopersicon esculentum (Tanksley and Rick, 1980), and to a lesser extent in other plant species. Polymorphism is a prerequisite for use of isozymes in linkage studies. Little isozyme polymorphism has been found in peach, despite variability for numerous morphological traits. Peach is perhaps the best genetically characterized fruit tree species, with 27 simply inherited morphological traits (Hesse, 1975; Monet et al., 1985). Since peach is diploid (2n = 2x = 16), it is unusual that only two linkage groups have been reported (Hesse, 1975; Monet et al., 1985). Isozyme loci provide an opportunity to identify additional linkage groups. Previous research has identified polymorphism in six enzyme systems (Arulsekar et al., 1986b; Durham et al., 1987; Messeguer et al., 1987).

To date, most peach isozyme studies have been performed using commercial cultivars (Arulsekar et al., 1986b; Messeguer et al., 1987) or selections and seedling populations from breeding programs (Durham et al., 1987). North American peach cultivars have a narrow genetic base (Scorza et al., 1985), and it is possible that isozyme variants not present in North American cultivars exist within the species. Additional variation should be found in the USDA peach plant introduction collection because it is composed of clones from various geographical locations, including China, the center of diversity for peach (Zeven and Zhukovsk&), 1975). These clones are highly variable for bloom date, fruit size and quality, and other traits (Ackerman, 1957; Ackerman et al., 1955).

The objectives of this study were to examine 56 clones representing the USDA peach plant introduction collection to 1) determine the inheritance of any previously unreported isozyme markers found in the clones, and 2) compare polymorphism in the collection with previously documented populations.

Materials and Methods

Fifty-six peach plant introductions (Table 1) were examined. Partially to fully expanded leaves were collected from actively
Table 1. Peach plant introduction (PI) clones examined, their place of origin and respective malate dehydrogenase (MDH) phenotypes and isocitrate dehydrogenase (IDH) genotypes

| PI#      | Clone Origin                      | MDH  | IDH  |
|----------|-----------------------------------|------|------|
| 34685    | Quetta(n)                         | A    | A    |
| 36126    | Bolivian Cling                    | B    | C    |
| 43289    | Ying Tsui Tao                     | C    | C    |
| 55776    | Seed importation                  | B    | C    |
| 62602    | Pi Tao                            | A    | A    |
| 63850    | Shalil seedling                   | A    | A    |
| 65821    | Shari Thai Tao seedling           | A    | C    |
| 65974    | De Coosa(n)                       | B    | A    |
| 65977    | Gialla di Padova(n)               | B    | A    |
| 77876    | Tos China #1                      | D    | B    |
| 78544    | Terzarola Col Pizzo               | B    | C    |
| 82413    | Baladi No. 1                      | B    | A    |
| 93826    | Inkoos                            | A    | A    |
| 95501    | Seed importation                  | D    | A    |
| 101667   | Ta Tao No. 1 (= Feicheng Tao No. 1) | A    | A    |
| 101668   | Ta Tao No. 5 (= Feicheng Tao No. 5) | A    | A    |
| 101686   | Ta Tao No. 6 (= Feicheng Tao No. 6) | A    | A    |
| 101823   | Bienvenida No. 65                 | C    | A    |
| 101835   | Precoce d’Ampuis No. 56          | A    | B    |
| 102705   | No. 501                           | A    | A    |
| 104287   | Soleil d’Octobre                  | B    | B    |
| 105362   | Gaschina Novembre                 | D    | B    |
| 106062   | Killekrankenic                    | B    | C    |
| 112032   | Saharanpur No. 1                  | C    | A    |
| 112033   | Saharanpur No. 2                  | B    | B    |
| 113452   | Rogani-Gow                        | D    | C    |
| 113455   | P. persica spp. ferganesis No. 0244 | C    | C    |
| 113650   | Pollardi                          | C    | C    |
| 117679   | No. 01370                         | D    | C    |
| 119840   | No. 0195                          | E    | C    |
| 119844   | No. 0932                          | B    | C    |
| 119846   | No. 1383-T                        | C    | C    |
| 125017   | Sary Oiler(n)                     | A    | B    |
| 125025   | China Flat                        | A    | B    |
| 129674   | Angel                             | E    | A    |
| 129678   | Stanwick(n)                       | C    | C    |
| 130980   | Proskauer                         | B    | C    |
| 131034   | Spath de Hallen                   | C    | C    |
| 131075   | Violette Hative(n)                | B    | C    |
| 131209   | Pineapple(n)                      | B    | C    |
| 132007   | Rheingold                         | B    | C    |
| 133551   | Peregrine                         | C    | C    |
| 133741   | Lady Palmerston                   | D    | C    |
| 133982   | Herholdt’s Late Cling             | B    | C    |
| 133984   | Marina                            | C    | C    |
| 133987   | Noodens Herholdt’s strain         | B    | C    |
| 134150   | Bresquillo Duranzos (PI 43569) seeding | C    | C    |
| 134151   | Transvaal yellow cling (PI 87637) seeding | B    | C    |
| 134401   | PI 80089 seeding                  | A    | A    |
| 146137   | No. 142058 (PI 105057) seeding    | B    | B    |
| 151158   | Jorge (Royal George)              | A    | C    |
| 240928   | Mao Tao (PI 107838) seedling      | A    | D    |
| 442378   | Select seedling                   | C    | B    |
| 442380   | Select seedling                   | C    | B    |

A = Mdh1-3/Mdh1-3; B = Mdh1-2/Mdh1-2; C = Mdh1-1/Mdh1-1; D = Mdh1-2/Mdh1-3; E = Mdh1-2/Mdh1-1; F = Mdh1-1/Mdh1-3.

*n* = nectarine.

Growing shoots of field-grown trees. Immature leaves had the highest levels of enzyme activity, except for peroxidase and esterase enzymes, which showed greater activity in mature leaves. Mature leaves were collected =0.3 m from the shoot terminal when sampling for peroxidase and esterase staining. Samples were collected within 24 hr of electrophoresis and stored at ≈4°C.
Electrophoretic procedures used were similar to those of Stuber et al. (1988). Samples were prepared by grinding 400 mg of leaf tissue, 5 ml of extraction buffer (Arulasekar and Parfitt, 1986), and 400 mg of insoluble polyvinylpyrrolidone with a polytron (Model # PT 10 20 350D, Brinkmann Instruments, Westbury, N.Y.). Sample tubes were kept in crushed ice, except for 5 to 10 sec during grinding. The extract was absorbed onto filter paper wicks that were blotted on a paper towel, then loaded into starch gels prepared with 29.8 g Connaught starch, 15 g Electrostarch, 15 g sucrose, and 340 ml of gel buffer (Stuber et al., 1988): Histidine·citrate pH 5.0 (HIS), histidine·citrate pH 6.5 (HIS6.5), lithium-borate/Tris. citrate pH 8.3 (LBTC) (Stuber et al., 1988), and morpholine·citrate pH 6.1 (MC) (Conkle et al., 1982) gel buffers were used. The MC buffer system was modified slightly by using one-half-strength buffer in the gel and buffer tanks. Gels were run at 13.5 W constant power in a refrigerator maintained at 4 ± 1°C. Ice packs were placed on the gels for additional cooling. Wicks were removed from the gels after 10 to 15 min to improve staining resolution. HIS, HIS6.5, and LBTC gels were run for 6 hr, except when staining for peroxidase and esterase, in which case LBTC gels were run for 4 hr. MC gels were run for 7.5 hr. Following electrophoresis, gels were sliced horizontally, yielding six 2-mm-thick slices. The top slice was discarded, except when staining for leucine aminopeptidase and menadione reductase, which required 4-mm-thick slices.

Isozyme systems surveyed included acid phosphatase (ACP; EC 3.1.3.2), alcohol dehydrogenase (ADH; EC 1.1.1.1), aspartate aminotransferase (AAT; EC 2.6.1.1), catalase (CAT EC 1.11.1.6), esterase (EST; EC 3.1.1.1), glutamate dehydrogenase (GDH; EC 1.4.1.2), glucosephosphate isomerase (GPI; EC 5.3.1.9), IDH, ‘malic’ enzyme (ME; EC 1.1.1.40), MDH, phosphoglucomutase (PGM; EC 2.7.5.1), 6-phosphogluconic dehydrogenase (PGD; EC 1.1.1.44), SDH (Stuber et al., 1988), peroxidase (PER; EC 1.11.1.7), leucine amino-peptidase (LAP; EC 3.4.11.1) (Andsekar and Parfitt, 1986), aldolase (ALD; EC 4.1.2.13), glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), and menadione reductase (MNR; EC 1.6.99.2) (Conkle et al., 1982). ACP, ADH, IDH, G6PD, PGD, SDH, ME, and MNR were stained on MC gels; CAT, EST, AAT, GPI, LAP, and PER staining was performed on LBTC gels; and GDH, PGM, ALD, and MNR were stained on HIS6.5 gels. MDH staining was performed on both MC and HIS.

Migration distances were recorded to the nearest 0.5 mm. A relative migration ($R_m$) was computed from the ratio of each isozyme band to the fastest-migrating band of ‘Redhaven’ peach within an enzyme system. In systems with multiple loci, the locus with greatest anodal migration was designated as 1, slower-migrating loci were assigned progressively higher numbers. Putative alleles at each polymorphic locus were named similarly, with the fastest-migrating allele being designated 1 allele and slower migrating alleles assigned progressively higher numbers.

**Results and Discussion**

MDH, IDH, SDH, PGM, LAP, &4T, and PGM were polymorphic within the plant introductions, while ADH, ALD, GDH, GPI, ME, PER, and G6PD were monomorphic. ACP, CAT, EST, and MNR could not be characterized due to inconsistent staining, but also appeared polymorphic.

Isocitrate dehydrogenase. Three banding patterns, designated $IdhA$, $IdhB$, and $IdhC$, were observed at the main staining region for IDH (Fig. 1). Fainter-staining bands migrating both ahead and behind the main staining region were sometimes detected, but they were not characterized due to staining inconsistency. All three banding patterns were characterized by the presence of a darkly staining region and a faster-migrating, lighter-staining “shadow” region. Shadow bands were not detected on some occasions; therefore, $R_m$ calculations were based on migration of the main band. $IdhC$ was the most common banding pattern and had a $R_m$ of 1.00. The $IdhB$ banding pattern was present in only five of the clones examined. This banding pattern usually appeared as a single diffuse main band with a $R_m$ of 0.98 and a lightly staining diffuse shadow band; however, when resolution was good, three separate bands, with $R_m$s of 1.00, 0.98, and 0.95 could be distinguished in the main banding region. The $IdhA$ phenotype was present in 17 of the clones and had a $R_m$ of 0.95.

Segregation data from three crosses and two self-pollinations...
suggest that staining in the main IDH region was due to activity of a single locus possessing 2 alleles, Idh1-2 and Idh1-3 (Table 2). Allelic designations of Idh1-2 and Idh1-3 were used, since a faster-migrating Idh1 allele is present in close relatives of peach (Mowrey, unpublished data). The Idh1-2 and Idh1-3 alleles had $R_s$ values of 1.00 and 0.95, respectively. "Dimeric structure of the enzyme was indicated by the presence, of a heterodimeric band with a $R_s$ of 0.98 in heterozygous individuals. Heterozygous clones usually displayed a banding pattern that appeared to be a single, diffuse band, since average migration of the two alleles differed by only 2 mm. This diffuse band was actually three overlapping bands. Dimeric structure of the enzyme was further indicated in NC 10254, a peach x almond hybrid heterozygous for an allele with $R_s$ of 0.78. A heterodimeric band was clearly present in this clone (Fig. 1). The Idh1-2 allele was most common in the plant introductions with a frequency of 65%. Low frequency of heterozygous individuals in the plant introductions was probably due to the high rates of self-pollination that occur in peach. Variants in IDH have been reported in peach pollen (Messeguer et al., 1987), but the same alleles may not be responsible, since different loci may be active in pollen (Tanksley et al., 1981). The unexpected seedlings in the Idh1-2/Idh1-3 class in the cross PI 104488 (Idh1-2/Idh1-3) × PI 101668 (Idh1-3/Idh1-3) are probably due to accidental self-pollination or outcrossing.

Malate dehydrogenase. Six MDH banding patterns (Figs. 2 and 3) were detected in this study and identified as phenotypes A-F (Table 1). Staining of MDH was performed on both HIS and MC to distinguish bands with similar migrations on a given gel system. A total of 14 bands was detected on HIS gels (Fig. 2), while 13 bands were detected on MC (Fig. 3). Bands could be detected on each system that were not detectable on the other system. Individual bands were numbered from fastest to slowest on HIS. The same numbers were used to identify bands observed on MC. The band present on MC, but not on HIS, was identified as band 10.5, because it migrated near HIS band 10. The $R_s$ values of bands on HIS were 1.75, 1.57, 1.21, 1.19, 1.02, 1.00, 0.98, 0.81, 0.80, 0.63, 0.55, 0.40, 0.34, and 0.19 for bands 1 through 14, respectively. On MC the $R_s$ values were 1.40, 1.22, 1.12, 1.10, 1.00, 0.94, 0.91, 0.80, 0.79, 0.78, 0.71, 0.65, and 0.58 for bands 1, 2, 3, 4, 5, 6, 7, 9, 10, 10.5, 11, 13, and 14, respectively.

The MdhA phenotype consisted of four bands when observed on MC (Fig. 3), and five bands on HIS (Fig. 2). This phenotype was observed in 10 clones and has not been previously reported. The difference in band numbers between the two systems is presumed to be due to comigration of HIS bands 13 and 12 on MC.

Table 2. Genotypic ratios of goodness-of-fit for isocitrate dehydrogenase in peach crosses.

| Cross                  | Segregation classes | A: B: C | X² | Test ratio |
|------------------------|---------------------|---------|----|------------|
| PI 104488 (B) self-pollinated | 21:41:19 1:2:1 | 0.11NS | 1:2:1 | 0.11NS |
| PI 146137 (B) × PI 117679 (C) | --- 23:22:1:1 | 0.02² | --- | --- |
| PI 104488 (B) × PI 101668 (A) | 27:37:31:1:1 | 1.46² | --- | --- |
| PI 65974 (C) × PI 129674 (C) | --- 65 --- | --- | --- | --- |
| PI 55777 (C) self-pollinated | --- 74 --- | --- | --- | --- |

A = Idha, B = Idhb, C = Idhc.

'Genotypes arising from accidental self-pollination not included in x² calculations.

²Nonsignificant.

The MdhB phenotype was the most common within the plant introductions, occurring in 30 clones. This has also been reported to be the most common phenotype in commercial cultivars (Arulsekar et al., 1986b) and the Univ. of Florida peach breeding program (Durham et al., 1987).

The MdhC phenotype occurred only in PI 119846. This phenotype consisted of three bands (Figs. 2 and 3), with the middle band staining somewhat lighter than the other two. The fast band consistently stained darker than all other bands. This phenotype resembles the F/F phenotype described by Durham et al. (1987); however, there are some discrepancies. Durham et al. (1987) reported that the intermediate band in their F/F phenotype had an $R_s$ equal to the fastest-migrating band in the most common banding pattern. In this study, the $R_s$ of the intermediate band differed slightly from the $R_s$ of the fastest-migrating band of the MdhC phenotype when observed on HIS, and the two bands differed considerably when stained on MC. The MdhC phenotype observed in the plant introductions also differs from the homozygous fast phenotype described by Arulsekar et al.
consistent of seven bands. The \( MdhF \) possessed seven bands when observed on MC (Fig. 3), and eight bands when stained on HIS (Fig. 2). Differences in band number between buffer systems are thought to be due to comigration of bands 13 and 12 on MC. Overlapping of bands 5 and 7 on HIS sometimes produced what appeared to be a seven-banded phenotype. This phenotype was present only in PI 106062.

Previous investigators (Arulsekar et al., 1986b; Durham et al., 1987) have determined that the \( MdhB \) and \( MdhC \) phenotypes were produced by different alleles in the homozygous state and the \( MdhE \) phenotype is produced by these alleles in a heterozygous state. Segregation data from cross PI 146137 (\( MdhB \)) × PI 129674 (\( MdhE \)) and self-pollinations of PI 119840 (\( MdhE \)), PI 129674 (\( MdhE \)), and PI 55776 (\( MdhB \)) (Table 3) further support the hypothesis that the \( MdhB \) and \( MdhC \) phenotypes are produced in individuals homozygous for the \( Mdhl-2 \) and \( Mdhl-1 \) alleles, respectively. The \( MdhE \) phenotype is produced in individuals heterozygous for these alleles. Although PI 119846 was not used in crossing, segregation ratios observed from self-pollinations of PI 129674 and PI 119840 (both \( MdhE \)) indicate the \( MdhC \) phenotype is produced by an allele in the homozygous condition.

Self-pollination of PI 65974 (\( MdhA \)) indicates this phenotype is due to homozygosity for a previously unreported slow (\( Mdhl-3 \)) allele, since only \( MdhA \) seedlings were produced. Likewise, the \( MdhD \) phenotype is produced when individuals are heterozygous for the \( Mdhl-2 \) and \( Mdhl-3 \) alleles. The cross of PI 146137 (\( MdhB \)) × PI 101668 (\( MdhA \)) produced only \( MdhD \) seedlings, as expected when crossing two homozygous genotypes. The cross of PI 104488 (\( MdhD \)) × PI 101668 (\( MdhA \)) produced an expected segregation of 1 \( MdhA : 1 \) \( MdhD \) (Table 3). The seven \( MdhB \) seedlings are probably due to accidental self-pollinations or outcrossing.

Skewed segregation ratios were obtained in one cross and one self-pollination involving the \( MdhD \) phenotype. Self-pollination of PI 104488 (\( MdhD \)) showed a marked skewing toward the \( MdhB \) and \( MdhD \) phenotypes, while the cross of PI 146137 (\( MdhB \)) × PI 117679 (\( MdhD \)) was skewed toward the \( MdhB \) phenotype. Self-pollinations were ruled out as the cause of the skewing in PI 146137 × PI 117679, because the seed parent is male-sterile. Also, outcrossing was not a likely cause of skewing in the PI 104488 self-pollination, since flowering branches were enclosed in a fine-meshed nylon cloth during bloom to exclude bees.

The \( Mdhl-1 \) allele is found in commercial germplasm at low frequencies, possibly due to some type of selection pressure against the allele (Arulsekar et al., 1986b). Although no significant skewing of segregation ratios was noted in crosses and self-pollinations involving the \( Mdhl-1 \) allele, it is possible the \( Mdhl-3 \) and \( Mdhl-1 \) alleles have reduced viability in certain genetic backgrounds. This would explain the high frequency of the \( Mdhl-2 \) allele in all populations examined so far and the skewed segregation ratios obtained in the aforementioned cross and self-pollination.

The cross PI 104488 (\( MdhD \)) × PI 129674 (\( MdhE \)) gave a segregation ratio that was not significantly different from the expected ratio of 1 \( MdhB : 1 \) \( MdhD : 1 \) \( MdhE : 1 \) \( MdhF \), indicating the observed variation in the population was due to polymorphism at one locus. An open-pollinated seedling population from PI 106062 (\( MdhF \)) also was examined in this study. PI 106062 is male-sterile and blooms too early for dependable seed production under the usual climatic conditions of Raleigh, N. C.; consequently, it was not used in controlled crosses. How-
ever, 1987 climatic conditions were such that heavy fruit set was obtained on PI 106062. PI 106062 bloomed with PI 129674 (MdhE) and in 3 weeks before most male-fertile clones in the planting. Therefore, most seed produced on PI 106062 was due to crossing with PI 129674. A segregation of 6 MdhA : 23 MdhC : 16 MdhD : 16 MdhI-1/MdhI-3 was obtained from the open-pollinated population. This ratio was not significantly different from the expected ratio of 1:1:1:1 (χ² = 3.94).

The six seedlings in the MdhA class probably were due to outcrossing with PI 113452 (MdhD) or PI 117679 (MdhD), which overlapped in bloom slightly with PI 106062. Even though this population was not from a controlled cross, it further supports the hypothesis that the variation was due to polymorphism at a single locus.

Although segregation data indicated MDH variability is due to polymorphism at a single locus, banding patterns obtained were quite complex (Figs. 2 and 3). Observation of phenotypes (Durham et al., 1987) have determined that MDH is a dimeric enzyme. Due to cellular compartmentalization, mitochondrial MDH does not produce heterodimeric bands with other forms of MDH (Goodman et al., 1980a). Therefore, band 13 may be excluded from consideration when determining production of heterodimeric bands between nonmitochondrial MDHs.

Segregation ratios observed in this study support the hypothesis that MDH is controlled by two loci in peach, as previously proposed (Arulasekar et al., 1986b; Durham et al., 1987). The Mdh2 locus appears to be monomorphic and responsible for production of the mitochondrial band, while Mdh1 is polymorphic and responsible for the variation observed in peach. From the MdhA, MdhB, and MdhC phenotypes, it can be deduced that the alleles produce two proteins in the homozgous state that can interact to produce heterodimeric and homodimeric bands. In the MdhA phenotype, bands 14 and 11 were probably homodimeric, while bands 6 and 10 were homodimeric and 9 was heterodimeric in the MdhB phenotype. Since band 1 consistently stained more darkly than other bands, it is probably produced by comigration of more than a single band. We propose that band 1 is actually produced by comigration of two monomeric bands and a heterodimeric band. The origin of band 7 is not known; however, it does not seem to interact with other proteins to produce heterodimeric bands and was peculiar to the MdhA, MdhC, and MdhF phenotypes. Band 7 did not appear to be present in any phenotype produced by the MdhI-2 allele in the homozygous or heterozygous state. Band 8 in the MdhD phenotype was a heterodimeric band produced between bands 11 and 6, from the MdhI-3 and MdhI-2 alleles, respectively. The heterodimeric band produced by interaction of the proteins responsible for bands 6 and 14 probably was obscured by comigration with bands 10 and 11. Likewise, the heterodimeric band produced by interaction of the proteins producing bands 10 and 11 is also obscured due to comigration. Band 10.5 on MC was probably a heterodimeric band produced by the interaction of the proteins producing bands 11 and 6. In the MdhE phenotype, bands 2 and 3 are heterodimeric bands. Band 2 was produced by interaction of the proteins producing bands 1 and 6, while band 3 was produced by the interaction of the proteins producing bands 14 and 6. In the MdhF phenotype, bands 4 and 5 are heterodimeric bands produced by the interaction of the proteins producing bands 11 and 1 and 14 and 1, respectively.

The MdhI-3, MdhI-2, and MdhI-1 alleles were present in the plant introductions at frequencies of 0.25, 0.67, and 0.08, respectively. As observed in IDH, heterozygous genotypes were present at a much lower frequency than homozygous genotypes. The genotypes of each clone are given in Table 1. It is interesting that we found no report of MdhI-3 in North American cultivars and breeding programs, since it was the second most frequent allele in the plant introductions and the most frequent allele in clones originating directly from China. This may be due to the narrow germplasm base of the North American cultivars and breeding programs, or perhaps to reduced viability of the genotype in certain genetic backgrounds under North American climatic conditions.

Shikimate dehydrogenase. Two SDH banding patterns were observed in the plant introductions (Fig. 4). All of the plant introductions displayed a single band, except for PI 117679, PI 113452, and PI 113650, which possessed a slower-migrating band in addition to the band present in the rest of the plant introductions. Faint bands were sometimes detected migrating ahead and behind the main region of activity, but they were not consistent enough to characterize. The single-banded phenotype was referred to as SdhA, and the double-banded phenotype as SdhB. The Rv values of the two bands were 1.00 and 0.89.

| Cross                  | A  | B  | C  | D  | E  | F  | Test ratio | χ²   |
|------------------------|----|----|----|----|----|----|------------|------|
| PI 104488 (D) x PI 129674 (E) | ---| 13 | ---| 10 | 24 | 13 | 1:1:1:1:1 | 5.58NS |
| PI 104488 (D) self-pollinated | 11 | 23 | ---| 56 | ---| ---| 1:2:1:1 | 8.05** |
| PI 129674 (E) self-pollinated | ---| 21 | 24 | ---| 42 | ---| 1:1:2:1 | 0.29NS |
| PI 119840 (E) self-pollinated | ---| 8  | 12 | ---| 24 | ---| 1:1:1:1 | 1.08NS |
| PI 104488 (D) x PI 101668 (A) | 31 | 7  | ---| 30 | ---| ---| 1:1 | 0.02NS |
| PI 146137 (B) x PI 129674 (E) | ---| 70 | ---| 57 | ---| ---| 1:1 | 1.41NS |
| PI 146137 (B) x PI 117679 (D) | ---| 32 | ---| 15 | ---| ---| 1:1 | 5.33*  |
| PI 65974 (A) x PI 117679 (D) | 16 | ---| ---| 21 | ---| ---| 1:1 | 0.64NS |
| PI 65974 (A) x PI 129674 (E) | 1  | ---| ---| 32 | ---| 32 | 1:1 | 0.00NS |
| PI 55776 (B) self-pollinated | 48 | ---| ---| ---| ---| ---|      |      |
| PI 146137 (B) x PI 101668 (A) | ---| 74 | ---| ---| ---| ---|      |      |

* A = MdhA, B = MdhB, C = MdhC, D = MdhD, E = MdhE, F = MdhF.

**Genotypes arising from accidental self-pollination not included in χ² calculations.

NS, ** Nonsignificant or significant at P = 0.05 or 0.01, respectively.
Two crosses [PI 146137 (SdhA) × PI 117679 (SdhB) and PI 65974 (SdhA) × PI 117679 (Sdbh)] were made to determine inheritance of SDH, but fruit set on these crosses was poor. Although fewer seedlings were obtained in these crosses, the observed ratio of 23 SdhA : 21 SdhB obtained in the cross of PI 146137 × PI 117679 was close to a 1:1 ratio ($x^2 = 0.09$ NS), indicating that SDH is monomorphic and encoded by one locus. Allelic designations of Sdh-2 and Sdh-3 were given because a faster-migrating Sdh allele is present in species closely related to peach and almond (Mowrey, unpublished data). The cross of PI 65974 × PI 117679 gave a considerably different ratio of 21 SdhA : 7 SdhB ($x^2 = 10.05\ast\ast$). The cause of this discrepancy is not known.

PI 117679, PI 113452, and PI 113650 were also heterozygous at both LAP loci and both PGD loci. PI 113650 was also heterozygous at the Pgm2 and Aat2 loci. Peach is monomorphic at these loci, while almond possesses contrasting alleles (Arulsekar et al., 1986a; Chaparro et al., 1987; Hauagge et al., 1987). Comparison with almond clones of known genotype indicated PI 117679, PI 113452, and PI 113650 were heterozygous for almond alleles at Lap1, Lap2, Pgdl, and Pgdl2. PI 113650 was also heterozygous for the slow Pgm2 and Aat2 alleles present in almond. These clones have phenotypes resembling peach × almond hybrids and are probably of interspecific origin. This relationship suggests that the Sdh1-3 allele is of almond origin. The probable hybrid origin of PI 117679 and PI 113452 might be responsible for the poor fruit set obtained when these clones were used as pollen parents. This origin may also be the reason for the skewing toward the Sdh1-2/Sdh1-2 genotype observed in the cross of PI 65974 × PI 117679. Backcrossing interspecific hybrids of Lycopersicon sometimes results in anomalous segregation ratios at isozyme loci (Vallejos and Tanksley, 1983). This phenomenon was also observed in segregation ratios of both PGD loci in the cross PI 146137 × PI 117679. PGD is inherited in a Mendelian manner in F1 peach × almond hybrids (Chaparro et al., 1987), but ratios of 30 Pgdl-2/Pgd1-2 : 17 (Pgdl-1/Pgd1-1 ($x^2 = 4.28\ast$) and 14 Pgd2-1/Pgd2-2 : 33 Pgd2-1/Pgd2-1 ($x^2 = 6.58\ast$) were observed at the PGD loci. In each of these cases, the peach genotype was the most common in the seedling population. Accidental self-pollination does not appear to be the cause of this phenomenon, because the Mdh1 segregation ratio in cross PI 65974 × PI 117679 was as expected, and PI 146137 is male-sterile.

The cross PI 146137 × PI 117679 was examined for possible linkages, because the pollen parent was heterozygous at six loci and the female parent was homozygous at these loci. Pgdl1, Pgdl2, and Mdh1 could not be included in the test due to abnormal segregation ratios. The Lap1 and Lap2 loci appear to be linked, (5 Lap1-1/Lap1-1 Lap2-2/Lap2-3 : 16 Lap1-1/Lap1-1 Lap2-3/Lap2-3 : 17 Lap1-1/Lap1-2 Lap2-2/Lap2-3 : 3 Lap1-1/ Lap1-2 Lap2-3/Lap2-3, with $x^2 = 14.33\ast\ast$) as reported by Hauagge et al (1987). No other linkages were detected.

This study identified new genetic markers at two loci (Idh1 and Mdh1) in peach. The Mdh1-3 allele will not be as useful for linkage studies as the Idh1 alleles, since it is allelic to previously reported MDH variants. Previous workers have suggested lack of isozyme variability in peach is due to the narrow genetic base of North American cultivars and breeding programs. Findings of this study indicate little isozyme variation is present in the species as a whole, since clones originating in 16 countries, including 12 clones from the center of diversity of peach, were examined.

Considerable isozyme polymorphism is present in almond (Arulsekar et al., 1986a; Chaparro et al., 1987; Hauagge et al., 1987), P. davidiana, P. kansuensis, and P. mira (B. D. M., unpublished data) that could be used, through interspecific hybridization, for genetic mapping in peach. Skewed segregation ratios observed when peach × almond hybrids were backcrossed to peach indicate care must be taken when using this approach to avoid biased results. One possible way to avoid skewing of segregation ratios would be to use as many peach clones and interspecific hybrids as feasible, because skewing may be more pronounced in certain genetic backgrounds. Male-sterile parents should also be used when possible, to avoid accidental self-pollination.

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