Linkage Analysis of Ten Isozyme Genes in F_1 Segregating Almond Progenies

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Abstract. Ten isozyme genes were studied after analyzing the variability of eight enzyme systems— glucose phosphate isomerase (GPI), phosphoglucumutase (PGM), aspartate aminotransferase (AAT), leucine aminopeptidase (LAP), 6-phosphogluconate dehydrogenase (6PGD), isocitrate dehydrogenase (IDH), shikimate dehydrogenase (SDH), and aconitase (ACO)—in the progeny of five crosses among almond [*Prunus amygdalus* Batsch, syn. *P. dulcis* (Miller) D. A. Webb] cultivars. Six of these loci were found to be located in two linkage groups, one containing four loci (*Pgm-2*, *Gpi-2*, *Aat-2*, and *Lap-1*) and two more in the other (*Idh-2* and *Aat-1*). Genetic configurations of pairs of loci specific to segregating F_1 progeny of crosses between heterozygous parents were found in our data, for which we derived the estimate of the recombination fraction and its variance. Linkage data for the gene pairs that could be estimated in various crosses were used to obtain a joint estimation of the recombination fraction.

Isozyme genes have been the first available source of good-quality markers in fruit-tree crops (Torres, 1990). Despite the fact that only a few of them are found in most plant species, the simplicity of the technique, rapidity with which results can be obtained, and low cost of equipment and consumables have made possible their effective incorporation in fruit-tree breeding projects.

Fourteen isozyme genes have so far been identified in almond intraspecific progeny and peach x almond interspecific F_2 s (Hauage et al., 1987a; Jackson and Clarke, 1991; Mowrey et al., 1990). The level of isozyme polymorphism in almond is the highest when compared with three other *Prunus* crops: apricot, peach, and plum (Byrne, 1990). Knowledge of isozyme genetics and variability has been useful for applying these markers to cultivar fingerprinting and analyzing genetic relationships among cultivars (Cerezo et al., 1989; Hauage et al., 1987b), identifying peach x almond hybrids (Byrne and Littleton, 1988; Chaparro et al., 1987), and determining gene flow in commercial production conditions (Jackson and Clarke, 1991).

In this paper we studied the segregation of 10 isozyme loci. The genetic basis of nine of them was previously reported, and one was studied for the first time. Linkage, which was only partly analyzed in previous reports, was determined among many gene pairs, and two linkage groups were established, including six of the 10 loci examined.

We used the segregating populations that are most commonly used in tree breeding— F_1 progeny between partially heterozygous parents. When studying linkage between pairs of genes in these populations, special configurations occur that are different from the classical backcross or F_2 segregations (Ritter et al., 1990). Gene-pair configurations not studied or partially studied before were inferred from our data. For them we derived the maximum

likelihood estimator of the recombination fraction and its variance, which were later used in the analysis of the segregation data.

Materials and Methods

Plant material. Parents and progeny from five controlled crosses among almond cultivars were used for genetic analysis. Crosses were 'Ferragnes' x 'Tuono' (C1), 'Ferragnes' x 'Marcona' (C2), 'Ferraduel' x 'Cristomorto' (C3), 'Ferragnes' x 'Desmayo Langueta' (C4), and 'Primorskiy' x 'Cristomorto' (C5). These progeny, obtained as part of the almond breeding project carried out at the Institut de Recerca i Tecnologia Agroalimentàries Center of Mas Bové, were unselected with numbers ranging from 46 (C2) to 100 (C4) individuals. Isozyme phenotypes of the cultivars used as parents are shown in Table 1.

Electrophoresis. Crude extracts were prepared by crushing ≈ 1 cm² of developing leaf tissue (unexpanded or expanding leaves <4 cm long) in 100 μ l of the following extraction buffer [modified from Arulsekar and Parfitt (1986)]: 50 mM tris, 7 mM citric acid, 0.1% cysteine HCl, 0.1% ascorbic acid, 1% polyethylene glycol, 8% polyvinylpyrrolidone (PVP-40), and 1 mM β -mercaptoethanol. Extracts were taken up in 3 $\times$ 8-mm Whatman no. 3 paper wicks and inserted sequentially into a 11.5% starch (Connaught) gel. Procedures for pollen collection, extraction, and electrophoresis were as described by Cerezo et al. (1989). Pollen extracts were used only for sporophyte–gametophyte comparisons.

Three gel–electrode buffers were used: tris-citrate pH 7.8 (TC 7.8), histidine-citrate pH 5.7 (HC 5.7), and histidine pH 7.0 (H 7.0). TC 7.8 and H 7.0 correspond respectively to buffers F and E of Shields et al. (1983), and HC 5.7 is described in Rovira et al. (1993). TC 7.8 was used to assay for leucine aminopeptidase (LAP) and aspartate aminotransferase (AAT) enzyme activity. Glucose phosphate isomerase (GPI), phosphoglucumutase (PGM), shikimic dehydrogenase (SDH), and aconitase (ACO) isozymes were stained in H 7.0, and isocitrate dehydrogenase (IDH) and 6-phosphogluconate dehydrogenase (6PGD) were stained in HC 5.7.

Common ingredients for IDH and SDH staining solutions were 100 ml 0.1 M tris pH 8.2, 20 mg thiazolyl blue, 5 mg phenazine

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Table 1. Isozyme phenotypes of the almond cultivars used as parents.

	<i>Gpi-2</i>	<i>Lap-1</i>	<i>Aat-1</i>	<i>Aat-2</i>	<i>Pgm-1</i>	<i>Pgm-2</i>	<i>6Pgd-1</i>	<i>Sdh-1</i>	<i>Idh-2</i>	<i>Aco-1</i>
Cristomorto	a	c	ab	b	b	a	b	ab	a	b
Desmayo Langueta	a	c	ab	ab	a	ab	b	b	a	b
Ferraduel	a	bc	a	ab	b	ab	b	ab	a	b
Ferragnes	a	bc	a	ab	b	ab	b	ab	a	b
Marcona	a	c	a	b	ab	b	bc	b	a	c (n) ^z
Primorskiy	a	c	ab	b	ab	ab	b	ab	ab	b
Tuono	fa	bc	ab	b	b	ab	b	ab	ab	b

^z*Aco-1* in 'Marcona' is presumably heterozygous for a null allele.

methosulfate, and 10 mg nicotinamide adenine dinucleotide phosphate. Specific for IDH were 20 mg isocitric acid and 4 ml 10% MgCl₂, and for SDH, 20 mg shikimic acid. Procedures for the remaining enzyme stains used can be found in Rovira et al. (1993) for 6PGD, GPI, PGM, and ACO, in Vallejos (1983) for AAT, and in Arús et al. (1982) for LAP.

For GPI, PGM, LAP, 6PGD, and AAT isozymes, we have followed the terminology proposed by Arulsekar et al. (1986), for IDH that of Cerezo et al. (1989), and for SDH that of Mowrey et al. (1990).

Statistical analyses. Goodness of fit between observed and expected genotypic frequencies of individual genes and tests of independence between pairs of segregating loci were performed using the chi-square statistic. The estimation of recombination fractions of linked loci was done using the maximum likelihood methods described by Allard (1956).

Two situations specific to the segregation of crosses between heterozygous unrelated individuals were found for which the estimation equation, its variance, or both had not been determined previously. These situations and the formulae that we derived for them are described below:

- The cross between two heterozygous individuals at both loci, one in coupling phase and the other in repulsion phase ($A_1B_1/A_2B_2 \times A_1B_2/A_2B_1$). For this case we used the estimation equation obtained by Ritter et al. (1990), and the variance was found to be $V = p(1-p)(1-2p+2p^2)/2N(1-2p)^2$, where p = recombination fraction estimation and N = progeny size.
- The cross between a double heterozygote and a heterozygote for only one of the markers ($A_1B_1/A_2B_2 \times A_1B_1/A_1B_2$). In this case, the recombination frequency can be estimated as $p = f + j/f + j + e + k$, where e , f , j , and k are respectively the number of individuals of genotypes A_1B_1/A_1B_1 , A_1B_1/A_2B_1 , A_1B_2/A_1B_2 , and A_1B_2/A_2B_2 , and the variance is $V = 2p(1-p)/N$.

When more than one progeny was available for estimating the recombination fraction, we used the joint estimation method described in Allard (1956).

Results and Discussion

Inheritance. All enzymes studied produced intensely stained and well-resolved zymograms (Fig. 1). Segregation data (Table 2) agreed with the existence of eight loci—*Gpi-2*, *Lap-1*, *Aat-1*, *Aat-2*, *Pgm-1*, *Pgm-2*, *Sdh-1*, and *Idh-2*, thus confirming previous inheritance studies in inter- or intra-specific crosses involving almond or other related *Prunus* species (Byrne, 1989; Goffreda et al., 1991; Hauagge et al., 1987a; Mowrey et al., 1990). Departures from the expected values were found for *Aat-1* in C5 and for *Sdh-1* in C3, where an excess of phenotype 'a' individuals was found. In

both cases, segregations in at least three more crosses agreed with the expected frequencies, a result providing evidence for the existence of these two loci.

Two regions of activity (GPI-1 and LAP-2) presented the same band in all parents studied. An additional zone (IDH-1), anodal to IDH-2, was also observed in IDH. This zone was apparently polymorphic, but its staining intensity was too low for a reliable phenotype determination and it was not studied.

An allozyme of faster migration than those described by Hauagge et al. (1987a) for *Gpi-2* was observed in 'Tuono' by Cerezo et al. (1989). Segregation ratios for this locus in 'Ferragnes' $\times$ 'Tuono' (C1) agreed with those expected, if this allozyme is the product of a new allele (f) at *Gpi-2*.

The observed banding pattern of 6PGD was similar to that described before in almond, peach, and peach $\times$ almond hybrids or F_2 s (Byrne and Littleton, 1988; Cerezo et al., 1989; Hauagge et al., 1989). All cultivars studied had the same pattern of two bands for regions 6PGD-1 and 6PGD-2, except for 'Marcona', which had three bands instead of one at the 6PGD-1 region. The cross between 'Marcona' (presumptive heterozygote for alleles b and c) and 'Ferragnes' (presumptive homozygote for b) segregated for b and bc phenotypes (Fig. 1e), but the goodness-of-fit test was significant due to an excess of b individuals. A deviation from the expected Mendelian segregation ratio was also observed for 6PGD-1 in a peach $\times$ almond F_2 progeny studied by Mowrey et al. (1990). When we compared the leaf and pollen zymograms of 'Marcona' at the 6PGD-1 region, we found that the intermediate band was lacking in pollen, a result indicating that the enzyme has a dimeric subunit structure and the observed variation was produced by a single gene.

Aconitase isozyme variation has not been previously reported in *Prunus*. In almond, we observed two regions of activity, labeled ACO-1 and ACO-2, which had one band each (Fig. 1h), the same in all parental genotypes, except 'Marcona' (Fig. 1h, fifth lane from the left). 'Marcona' also had one band per region, but the ACO-1 band was located at a position slightly more anodal than the monomorphic ACO-2 band. The progeny 'Ferragnes' $\times$ 'Marcona' (C5) segregated at the ACO-1 region with two phenotypes: one with both 'Marcona' and 'Ferragnes' bands and the other with only the band of 'Ferragnes' (Fig. 1h). Segregation was compatible with a 1:1 ratio (Table 2). Assuming that the isozymes observed at ACO are encoded by two genes, one for each of the zones of activity, two hypotheses are consistent with these results.

- One gene is segregating at region ACO-1, and 'Marcona' is heterozygous for an active (c) and a null (n) allele; and 'Ferragnes' homozygous for a different allele (b).
- Segregation occurs in ACO-2, with 'Marcona' heterozygous for alleles a and b and 'Ferragnes' homozygous for allele b . In this case, the pattern observed at region ACO-1 can be explained

Fig. 1. Photographs with the zymograms of the eight enzyme systems studied, with specification of the phenotypes of each isozyme gene (from left to right). Migration direction is from bottom (cathode) to top (anode). (a) PGM. *Pgm-1*: ab, b, ab, b, a, b, ab, ab, ab, and ab. *Pgm-2*: a, ab, b, ab, ab, a, ab, a, a, and a. (b) GPI. *Gpi-2*: fa, fa, fa, fa, a, fa, a, fa, a, and a. (c) AAT. *Aat-1*: a, a, a, b, a, ab, ab, a, a, and ab. *Aat-2*: b, ab, ab, b, b, b, b, b, b, and b. (d) IDH. *Idh-2*: a, a, ab, ab, a, ab, ab, ab, a, and ab. (e) 6PGD. *6Pgd-1*: a, ab, a, a, a, ab, a, ab, a, and a. (f) SDH. *Sdh-1*: a, ab, ab, ab, ab, a, a, ab, and ab. (g) LAP. *Lap-1*: c, bc, c, c, c, c, bc, bc, and c. (h) ACO. *Aco-1*: b, b, bc, b, c, bc, bc, bc, b, and bc.

considering that 'Marcona' is fixed for a null allele and 'Ferragnes' for an active allele.

Preliminarily, we have designated this presumptive gene as *Aco-1* (phenotypes in Tables 1 and 2 and Fig. 1h have been named considering hypothesis 1), but determining the correct hypothesis will require additional genetic analysis of segregating progeny.

Linkage. Twenty-six of the 45 gene pairs possible with the 10 loci studied could be examined for independence (Table 3). Significant departures from independence were observed in seven cases. In four of them (*Lap-1* – *Aat-2*, *Lap-1* – *Pgm-2*, *Pgm-2* – *Aat-2* and *Aat-1* – *Idh-2*), linkage could be studied in more than one cross, and segregations indicating linkage were consistent in all of them. A significant deviation from independence was observed in

Table 2. Single-locus segregation and chi-square analysis for 10 variable isozymes in almond.

Locus	Cross	N	Parental phenotypes	Expected ratio	Offspring phenotypes	Chi-square
<i>Gpi-2</i>	C1	61	a x fa	1:1	a(31) : fa(30)	0.02
<i>Lap-1</i>	C1	61	bc x bc	1:2:1	b(18) : bc(32) : c(11)	1.75
	C2	46	bc x c	1:1	bc(25) : c(21)	0.35
	C3	99	bc x c	1:1	bc(57) : c(42)	2.27
	C4	100	bc x c	1:1	bc(49) : c(51)	0.04
<i>Aat-1</i>	C1	61	a x ab	1:1	a(33) : ab(28)	0.41
	C3	99	a x ab	1:1	a(46) : ab(53)	0.49
	C4	100	a x ab	1:1	a(58) : ab(52)	2.56
	C5	63	ab x ab	1:2:1	a(25) : ab(23) : b(15)	7.76*
<i>Aat-2</i>	C1	61	ab x b	1:1	ab(37) : b(24)	2.77
	C2	46	ab x b	1:1	ab(26) : b(20)	0.78
	C3	99	ab x b	1:1	ab(55) : b(44)	1.22
	C4	100	ab x ab	1:2:1	a(21) : ab(54) : b(25)	0.96
<i>Pgm-1</i>	C2	46	ab x b	1:1	ab(17) : b(29)	3.13
	C5	63	ab x b	1:1	ab(38) : b(25)	2.68
<i>Pgm-2</i>	C1	61	ab x ab	1:2:1	a(9) : ab(31) : b(21)	4.74
	C2	46	ab x b	1:1	ab(20) : b(26)	0.78
	C3	99	ab x a	1:1	a(49) : ab(50)	0.01
	C4	100	ab x ab	1:2:1	a(39) : ab(52) : b(23)	0.24
	C5	63	ab x a	1:1	a(39) : ab(24)	3.57
<i>6Pgd-1</i>	C2	46	b x bc	1:1	b(32) : bc(14)	7.04*
<i>Sdh-1</i>	C1	61	ab x ab	1:2:1	a(10) : ab(33) : b(18)	2.51
	C2	46	ab x b	1:1	ab(28) : b(18)	2.17
	C3	99	ab x ab	1:2:1	a(37) : ab(46) : b(16)	9.40*
	C4	100	ab x b	1:1	ab(54) : b(46)	0.64
	C5	63	ab x ab	1:2:1	a(12) : ab(36) : b(15)	1.57
<i>Idh-2</i>	C1	61	a x ab	1:1	a(33) : ab(28)	0.41
	C5	63	ab x a	1:1	a(35) : ab(28)	0.78
<i>Aco-1</i>	C2	46	b x c(n)	1:1	bc(23) : b(n)(23)	0.00

*Significant at $P \leq 0.05$.

Table 3. Pairs of genes analyzed in five almond progenies.

	<i>Lap-1</i>	<i>Aat-1</i>	<i>Aat-2</i>	<i>Pgm-1</i>	<i>Pgm-2</i>	<i>6Pgd-1</i>	<i>Sdh-1</i>	<i>Idh-2</i>	<i>Aco-1</i>
<i>Gpi-2</i>	C1	C1	---	---	C1*	---	C1	C1	---
<i>Lap-1</i>		C1	C1*,C2*,C3,C4	---	C1*,C2*,C3*,C4*	---	C1,C2,C3,C4	C1	---
<i>Aat-1</i>			C4	C5	C1,C4,C5	---	C1,C3,C5	C1*C5*	---
<i>Aat-2</i>				---	C1*,C2*,C3*,C4*	---	C1,C2,C3,C4	—	—
<i>Pgm-1</i>					C5	C2	C5	C5	C2
<i>Pgm-2</i>					---	C1,C2*,C3,C4	C1	C5	---
<i>6Pgd-1</i>							---	---	C2
<i>Sdh-1</i>								C1,C5	---
<i>Idh-2</i>									---

*Chi-square independence test, $P \leq 0.05$

C2 for *Sdh-1* – *Pgm-2*, but this linkage was not confirmed in three other crosses, a result suggesting that linkage is loose or that the positive result is an artifact. Finally, two more pairs of loci that could only be studied in one segregation showed tight linkage (*Gpi-2* – *Lap-1* and *Gpi-2* – *Pgm-2*).

All of the possible two-gene configurations expected in F_1 progeny of heterozygous unrelated parents were recovered in the 16 situations in which linkage could be estimated (Table 4). Half of them correspond to well-known cases of F_2 (one) or backcross (seven) segregations. The configurations found in the remaining eight progeny were typical of segregating F_1 s: seven were crosses that had one of the parents heterozygous for both loci and the other heterozygous for only one of them, and the last one corresponded

to a cross between two double heterozygotes, one in coupling and the other in repulsion phase.

The homogeneity of the recombination estimates for a given gene pair obtained from different crosses was tested with a chi-square statistic (Allard, 1956). Estimations of recombination were homogeneous for *Pgm-2* – *Aat-2* ($\chi^2 = 0.98$, nonsignificant), *Pgm-2* – *Lap-1* ($\chi^2 = 3.85$, nonsignificant), and *Idh-2* – *Aat-1* ($\chi^2 = 0.07$, nonsignificant), but not in *Lap-1* – *Aat-2* ($\chi^2 = 8.89$, $0.05 > P > 0.01$), where the difference between the recombination estimate of cross ‘Ferragnes’ x ‘Desmayo Largueta’ and the rest was responsible for this result. Interestingly, the recombination proportion of *Pgm-2* – *Aat-2* and *Pgm-2* – *Lap-1* was also larger in this cross than in the others, a result suggesting differences in recom-

Table 4. Two locus segregations in the pairs of genes with deviations from independent assortment.

Gene	Cross		Genotypic classes										
pair	Cross	genotype ^z	e	f	g	h + i	j	k	l	m	n	p	SE
<i>Pgm-2</i> – <i>Aat-2</i>	C1	i x k			0	20	9	11		17	4	0.10	0.06
	C2	i x n				2		18		24	2	0.09	0.04
	C3	i x j			7	48	42	2				0.09	0.03
	C4	i x h	3	18	19	14	3	20	0	21	2	0.13	0.02
<i>Pgm-2</i> – <i>Lap-1</i>	C1	i x i	0	4	1	24	8	3	14	7	0	0.11 ^y	0.02 ^y
	C2	i x n				3		17		22	4	0.15	0.05
	C3	i x j			9	48	40	2				0.11	0.03
	C4	i x k			6	25	19	27		18	5	0.23	0.06
<i>Lap-1</i> – <i>Aat-2</i>	C1	h x k			18	18	0	14		1	10	0.03	0.03
	C2	h x n				24		2		1	19	0.07	0.04
	C3	h x n				54		1		3	41	0.04	0.02
	C4	h x m		18		26		5	3	28	20	0.17	0.05
<i>Lap-1</i> – <i>Gpi-2</i>	C1	k x i			2	17	16	15		11	0	0.07	0.05
	C1	k x h			9	19	0	12		2	19	0.07	0.05
<i>Idh-2</i> – <i>Aat-1</i>	C1	e x i	10	23	23	5						0.25	0.06
	C5	h x g	19	6	13	10	3	12				0.23	0.07
												0.24 ^y	0.04 ^y

^zGenotypic classes: e = A₁B₁/A₁B₁; f = A₁B₁/A₂B₁; g = A₁B₁/A₁B₂; h = A₁B₁/A₂B₂; i = A₁B₂/A₂B₁; j = A₁B₂/A₁B₂; k = A₁B₂/A₂B₂; l = A₂B₁/A₂B₁; m = A₂B₁/A₂B₂; n = A₂B₂/A₂B₂.

^yValue of the joint estimation of p and its SE using the data of all individual progeny.

bination rates for this chromosome segment associated with specific crosses. Given that 'Ferragnes' has been used as the female parent in two more crosses yielding similar *P* values, our results suggest that 'Desmayo Langueta' has a higher recombination rate in general or when used as pollen parent than 'Ferragnes' as a pistillate parent. Differences in recombination behavior associated with genotypes and sex have been described previously (de Vicente and Tanksley, 1991; Stadler, 1926).

Two linkage groups were detected (Fig. 2), one with four isozyme genes (*Pgm-2*, *Gpi-2*, *Aat-2*, and *Lap-1*) and the other with two (*Idh-2* and *Aat-1*). In the former group, the position of *Gpi-2* and *Aat-2* could be determined with respect to *Pgm-2* and *Lap-1*, but not between each other. Using the information obtained from the progeny, it was possible to determine the phase of the parents for the loci included in these two linkage groups (Table 5). The isozyme phenotypes of the parents of 'Ferragnes' x 'Ferraduel' (selected from the progeny of 'Ai' x 'Cristomorto') were reported by Cerezo et al., 1989. Both cultivars have an identical phenotype for all isozymes studied in this paper, and the linkage phase of *Pgm-2* – *Aat-2* – *Lap-1* group, for which they were in heterozygosis, was also the same, agreeing with what was expected from the phenotypes of their parents.

Our results on linked and unlinked isozyme loci agree in general with those obtained previously on pairwise linkage analyses performed in almond or other related *Prunus* species. Preliminary evidence on linkage between *Lap-1* and *Pgm-2* was provided by Hauagge et al. (1987a). Santi and Lemoine (1990) found linkage between LAP and AAT loci, probably coincident with the pair *Lap-1* – *Aat-2* studied here with a similar (0.09) estimate of linkage intensity. On the other hand, Hauagge et al. (1987a) reported independent assortment between *Gpi-2* and *Pgm-2*, and Goffreda et al. (1991) found *Idh* (presumably *Idh-2*) and *Aat-1* to behave independently, whereas our results indicated linkage in both cases.

Additional linkages have been detected by other researchers: *Lap-1* and *Lap-2* were found to be tightly linked (*P* = 0.03 – 0.05) in interspecific peach x almond progeny by Hauagge et al. (1987a);

Fig. 2. Schematic linkage map of almond. Position of *Aat-2* and *Gpi-2* is correct with respect to *Pgm-2* and *Lap-1*, but tentative between each other.

Table 5. Genotype of the almond cultivars used as parents, with specification of their phase for the two linkage groups detected.

	<i>Pgm-2</i>	<i>Gpi-2</i>	<i>Aat-2</i>	<i>Lap-1</i>	<i>Idh-2</i>	<i>Aat-1</i>
Cristimorto	<i>a</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>a</i>
	<i>a</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>
Desmayo Langueta	<i>a</i>	<i>a</i>	<i>a</i>	<i>c</i>	<i>a</i>	<i>a</i>
	<i>b</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>
Ferragnes	<i>a</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>a</i>
	<i>b</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>
Ferraduel	<i>a</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>a</i>
	<i>b</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>
Marcona	<i>b</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>a</i>
	<i>b</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>a</i>
Primorskiy	<i>a</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>a</i>
	<i>b</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>b</i>	<i>b</i>
Tuono	<i>a</i>	<i>f</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>
	<i>b</i>	<i>a</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>a</i>

this result was later confirmed by Mowrey et al. (1990). Tight linkage ($P = 0.05$) was also found between a LAP gene (presumably *Lap-1*) and a malic enzyme encoding gene (*Me-1*) by Santi and Lemoine (1990) in cherry. Goffreda et al. (1991) detected linkage between *Aat-1* and *6Pgd-2* ($P = 0.36$) in crosses between peach and related wild species. If a high level of synteny exists among the chromosomes of almond, peach, and cherry, many of the isozyme genes of these species will be located in two linkage groups, one containing six tightly linked loci (*Pgm-2*, *Gpi-2*, *Aat-2*, *Lap-1*, *Lap-2*, and *Me-1*) and another with three loci (*Idh-2*, *Aat-1*, and *6Pgd-2*).

Polymorphic isozymes not studied here were reported in almond (Arulsekhar et al., 1986; Cerezo et al., 1989; Jackson and Clarke, 1991) and other species of *Prunus* (Byrne, 1989; Durham et al., 1987; Monet and Gibault, 1991; Santi and Lemoine, 1991). Thus, more isozyme loci can be identified and studied for linkage in addition to those reported here. The development of isozymes and other sources of markers like restriction fragment length polymorphisms (Eldredge et al., 1992) and polymerase chain reaction-derived polymorphisms are likely to lead to the construction of high-density maps in *Prunus* in the near future. Benefits expected from the application of these maps are especially important in *Prunus*, where marker-assisted selection may result in enormous savings of time because of the long inter-generation period of these crops.

Literature Cited

- Allard, R. W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. *Hilgardia* 24:235–278.
- Arulsekhar, S. and D.E. Parfitt. 1986. Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio, and fig. *HortScience* 21:928–933.
- Arulsekhar S., D.E. Parfitt, and D.E. Kester. 1986. Comparison of isozyme variability in peach and almond cultivars. *J. Hered.* 77:272–274.
- Arús, P., S.D. Tanksley, T.J. Orton, and R.A. Jones. 1982. Electrophoretic variability as a tool for determining seed purity and for breeding hybrid varieties in *Brassica oleracea*. *Euphytica* 31:417–428.
- Byrne, D.H. 1989. Inheritance of five isozyme loci in apricot. *HortScience* 24:1015–1016.
- Byrne, D.H. 1990. Isozyme variability in four diploid stone fruits compared with other woody perennial plants. *J. Hered.* 81:68–71.
- Byrne D.H. and T.G. Littleton. 1988. Verification of the parentage of presumed peach x almond hybrids by isozyme analyses. *Fruit Var. J.* 42:130–134.
- Cerezo, M., R. Socias i Company, and P. Arús. 1989. Identification of almond cultivars by pollen isoenzymes. *J. Amer. Soc. Hort. Sci.* 114:164–169.
- Chaparro, J.X., R.E. Durham, G.A. Moore, and W.B. Sherman. 1987. Use of isozyme techniques to identify peach x ‘Nonpareil’ almond hybrids. *HortScience* 22:300–302.
- de Vicente, M.C. and S.D. Tanksley. 1991. Genome-wide reduction in recombination of backcross progeny derived from male versus female gametes in an interspecific cross of tomato. *Theoretical Applied Genet.* 83:173–178.
- Durham, R.E., G.A. Moore, and W.B. Sherman. 1987. Isozyme banding patterns and their usefulness as genetic markers in peach. *J. Amer. Soc. Hort. Sci.* 112:1013–1018.
- Eldredge, L., R. Ballard, W.V. Baird, A. Abbott, P. Morgens, A. Callahan, R. Scorza, and R. Monet. 1992. Application of RFLP analysis to genetic linkage mapping in peaches. *HortScience* 27:160–163.
- Goffreda, J.C., J.M. Armstrong Nick, S.A. Mehlenbacher, and N. Vorsa. 1991. Inheritance of isozymes in peach x *Prunus kansuensis* and peach x *Prunus davidiana* hybrids. *Euphytica* 54:161–168.
- Hauagge, R., D.E. Kester, and R.A. Asay. 1987a. Isozyme variation among California almond cultivars: I. Inheritance. *J. Amer. Soc. Hort. Sci.* 112:687–693.
- Hauagge R., D.E. Kester, S. Arulsekhar, and D.E. Parfitt. 1987b. Isozyme variation among California almond cultivars: II. Cultivar characterization and origins. *J. Amer. Soc. Hort. Sci.* 112:693–698.
- Jackson, J.E. and G.R. Clarke. 1991. Gene flow in an almond orchard. *Theoretical Applied Genet.* 82:169–173.
- Monet, R. and B. Gibault. 1991. Polymorphisme de l’a-amylase chez le pêcher. *Etude genetique. Agronomie* 11:353–358.
- Mowrey B.D., D.J. Werner, and D.H. Byrne. 1990. Inheritance of isocitrate dehydrogenase, malate dehydrogenase, and shikimate dehydrogenase in peach and peach x almond hybrids. *J. Amer. Soc. Hort. Sci.* 115:312–319.
- Ritter E., G. Gebhardt, and F. Salamini. 1990. Estimation of recombination frequencies and construction of RFLP linkage maps in plants from crosses between heterozygous parents. *Genetics* 125:645–654.
- Rovira, M., N. Aletà, E. Germain, and P. Arús. 1993. Inheritance and linkage relationships of ten isozyme genes in hazelnut. *Theoretical Applied Genet.* 86:322–328.
- Santi, F. and M. Lemoine. 1991. Genetic markers for *Prunus avium* L.: Inheritance and linkage of isozyme loci. *Ann. Sci. For.* 47:131–139.
- Shields C.R., T.J. Orton, and C.W. Stuber. 1983. An outline of general resource needs and procedures for the electrophoretic separation of active enzymes from plant tissue, p. 443–468. In: S.D. Tanksley and T.J. Orton (eds.). *Isozymes in plant genetics and breeding. part A*. Elsevier, Amsterdam.
- Stadler, L.J. 1926. The variability of crossing over in maize. *Genetics* 11:1–27.
- Torres, A.M. 1990. Isozyme analysis of tree fruits, p. 192–205. In: D.E. Soltis and P.S. Soltis (eds.). *Isozymes in plant biology*. Chapman and Hall, London.
- Vallejos, C.E. 1983. Enzyme activity staining, p. 469–516. In: S.D. Tanksley and T.J. Orton (eds.). *Isozymes in plant genetics and breeding. part A*. Elsevier, Amsterdam.