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A. Vezvaei · T. W. Hancock · L. C. Giles
G. R. Clarke · J. F. Jackson

Inheritance and linkage of isozyme loci in almond

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Abstract The segregation of seven isozyme marker genes was investigated using eight controlled crosses in almond. The cultivar 'Nonpareil' was the maternal parent in all crosses. Pollination was achieved using eight different cultivars, and a total of 3200 individual kernels were assessed. For each isozyme the goodness-of-fit test was used to test for departure from the expected frequencies assuming Mendelian inheritance. Given a higher than expected number of significant results for individual isozymes, independent segregation between pairs of isozymes was tested using the chi-square statistic on the resulting two-way contingency tables. In all crosses a highly significant association (P value < 0.001) was observed between (1) the *AAT*-1 and *IDH* isozymes loci and (2) the *LAP-1* and *PGM-2* isozymes loci, which leads to the conclusion that the respective isozyme pairs are linked.

In addition, a significant association (P value $<$ 0.001) was observed between *LAP-1* and *GPI-2* when the pollen sources were 'Fritz', 'Mission', or 'Price', but this could not be tested for the remaining five pollen sources, 'Carmel', 'Grant', 'Keane', 'Ne plus Ultra', 'Peerless', because they are homozygous at these loci. If *LAP-1* is linked with *GPI-2* and *PGM-2*, it might be expected that we should find evidence of linkage between *GPI-2* and *PGM-2*. The lack of a significant association between these two isozymes suggests that *LAP-1* is located centrally on the chromosome. These three pairs of linked loci are the first to be reported in almond.

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A. Vezvaei (✉) · G. R. Clarke · J. F. Jackson
Department of Horticulture, Viticulture and Oenology, Waite Agriculture Research Institute, University of Adelaide, Glen Osmond, South Australia 5064, Australia

T. W. Hancock · L. C. Giles
Department of Plant Science, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia 5064, Australia

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Introduction

Isozyme analysis has been used for genetic mapping through linkage studies (Torres et al. 1985). However, the application of such linkage studies are rare in woody plants for several reasons: space and other required resources, long generation times, and in many cases, technical difficulties in obtaining progeny from controlled crosses (Torres 1983). Such studies are also further complicated by the fact that woody plants are frequently heterozygous at individual loci. Recently, some species of gymnosperms and angiosperms have been investigated (Eckert et al. 1981; Lee and Ellstrand 1987; Wehling 1991; Fuong et al. 1993). A knowledge of the linkage relationships among isozyme loci would be useful for further genetic work and provide information on linkage conservation (Heemstra et al. 1991). Linkage between pairs of isozyme loci has been reported in a number of horticultural genera. In citrus two related linkage groups were found between seven isozyme loci investiaged; one of these linkage groups is between *AAT-1* and *MDH-1* (Torres et al. 1985). (In this communication we use the term AAT where others quoted refer to GOT; they refer to the same isozyme). In avocado a close, linkage has been found in *AAT-1* and *AAT-2* (Torres et al. 1986). In apple, Manganaris and Alston (1987) reported linkage between *AAT-1* and a self-incompatibility loci, and a close relationship was demonstrated between *AAT-1* and *IDH*. In blueberry, Heemstra et al. (1991) found two independent linkage groups between *GPI-2* and *LAP-1* and *PGM-2* and *6GPD-2*. In fig (*Ficus* spp.) a sex-determining gene is linked to the peroxidase gene, and *AAT* and esterase genes are linked (Valizadeh 1973). Santi and Lemoine (1990) identified two linkage groups in sweet cherry, *LAP-1* and *AAT-1* and *LAP-1* and *ME-1*. Linkage was reported in grape between *GPI-c* and *LAP-1* by Weeden et al. (1988).

We are now in a position to study linkage more thoroughly in almond (*Prunus dulcis* [Mill] D. A. Webb.), since in addition to the five loci identified by Hauagge et al. (1987b) (*AAT-1*, *GPI-2*, *LAP-1*, *PGM-1* and *PGM-2*), polymorphism has been demonstrated for *IDH* (Cerezo et al. 1989; Jackson and Clarke 1991) and *SKDH* (Jackson 1992).

Materials and methods

Plant materials

Healthy young leaves are suitable for isozyme analysis in almond (Jackson 1992). Leaves were sampled from each of nine cultivars ('Carmel', 'Fritz', 'Grant', 'Keane', 'Mission', 'Ne Plus Ultra', 'Nonpareil', 'Peerless' and 'Price') from a commercial orchard at Angle Vale (35 kilometres NE of Adelaide, South Australia). All samples were packed in crushed ice during transport to the laboratory at the Waite Agricultural Research Institute.

Isozyme analysis of almond leaves

The seven polymorphic isozyme loci examined were: (1) asparatate amino transferase at locus one (*AAT-1*), (2) glucose phosphate isomerase at the second locus (*GPI-2*), (3) isocitrate dehydrogenase (*IDH*), (4) leucine aminopeptidase at the first locus (*LAP-1*), (5) phosphoglucomutase at locus one (*PGM-1*), (6) phosphoglucomutase at locus two (*PGM-2*) and (7) shikimate dehydrogenase (*SKDH*). Cellogel (Chemerton, Milan, Italy) was used as the medium for electrophoresis, which was carried out as described for almond by Jackson (1992) after the general principles outlined by Richardson et al. (1986) and Granger et al. (1993).

Inheritance studies

Controlled crosses were carried out using pollen from the eight pollen sources on cv 'Nonpareil' as sole maternal source as described by Kester and Asay (1975). Two 'Nonpareil' trees were isolated in the orchard by nylon mesh cages for this purpose. For each cross 400 embryos (kernels) were analysed as described by Jackson (1992).

Results

All observed isozyme patterns for the various genotypes in the seven isozyme loci studied are represented diagrammatically in Fig. 1. Inheritance studies were crucial to the designation of null genes, which, when present in heterozygous diploid plant cells, can be scored as homozygous at a locus where it appears (e.g. *na* can be scored as *aa*). We have demonstrated that cv 'Price' has a null gene at *LAP-1* (Vezvaei et al. 1994), as do 'Fritz' and 'Mission' (Hauagge et al. 1987a, b). In addition we have shown that Peerless is *nn* at the *AAT-1* locus (Jackson and Clarke 1991).

Testing for Mendelian segregation at each enzyme locus

Mendelian inheritance of isozyme banding patterns was tested statistically with the goodness-of-fit test of expected frequencies. For each cross the expected ratio of

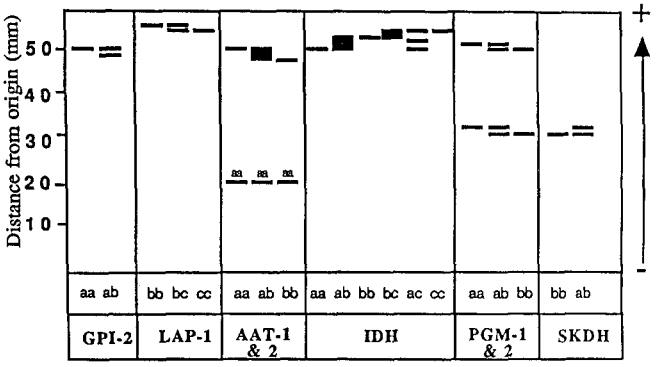

Fig. 1 Diagrammatic representation of six isozyme banding patterns

phenotypes was calculated for each isozyme locus. Table 1 shows details for the seven isozyme loci for each of the eight crosses. It should be noted that for crosses where there is no segregation occurring because both parents are homozygous, the goodness-of-fit test cannot be applied. Such cases are indicated by a "-" in Table 1 and occurred for 12 of the 56 combinations considered. Of the 44 tests, 28 supported Mendelian inheritance, but the remaining 16 tests showed significant departure from the expected ratio. This high proportion of departure needs to be carefully investigated, but we assumed that inheritance is Mendelian and proceeded to test for evidence of linkage between the seven loci.

Testing for linkage between isozyme loci

If two loci are unlinked, then the segregation pattern at one locus should be independent (or unrelated) to the segregation pattern at the other locus. Given the data recorded, the potential exists to test the $7 \times (7-1)/2 = 21$ pairwise combinations of the seven isozyme loci for each of the eight crosses investigated. However, as segregation did not occur at all loci in all crosses, it was not possible to perform a test in all instances. The observed phenotypes were tabulated for each pair of isozyme loci for each cross. Given the high proportion of departure from the expected ratios when individual isozyme loci were tested, it seemed unwise to test the two-way contingency tables against their corresponding expected ratios with the goodness-of-fit test. Instead, for each pair of isozyme loci the null hypothesis that the two isozyme loci independently segregate was tested by carrying out a chi-square test of independence on the appropriate contingency table.

The results of the chi-square test for independence are summarised in Table 2. Table 3 shows the observed and expected frequencies for each significant association. For every cross a highly significant association (P value < 0.001) was observed between the following pairs (1) *AAT-1* and *IDH* and (2) *LAP-1* and *PGM-2*. Additionally, a highly significant association (P value < 0.001) found between *LAP-1* and *GPI-2* when the pollen

Table 1 Summary of observed frequencies, expected ratios and goodness-of-fit test for each of the eight crosses by seven isozyme loci

Cultivar Non-Parell	*AAT-1* *ab*	*GPI-2* *aa*	*IDH* *ab*	*LAP-1* *bc*	*PGM-1* *ab*	*PGM-2* *ab*	*SKDH* *bb*
Carmel	*ab*	*aa*	*ab*	*nc*	*ab*	*bb*	*bb*
Expected	*aa*:*ab*:*bb* = 1:2:1	*aa* = 1	*aa*:*ab*:*bb* = 1:2:1	*bb*:*bc*:*cc* + *nc* = 1:1:2	*aa*:*ab*:*bb* = 1:2:1	*ab*:*bb*: = 1:1	*bb* = 1
Observed	135:187:78	400	89:207:104	94:92:214	89:217:94	188:212	400
Goodness-of-fit	17.13 on 2 *df****	–	1.65 on 2 *df* ns	1.98 on 2 *df* ns	3.03 on 2 *df* ns	1.44 on 1 *df* ns	–
Fritz	*ns*	*ab*	*ab*	*nb*	*ab*	*bb*	*bb*
Expected	*aa* + *na*:*ab*:*bb* = 2:1:1	*aa*:*ab* = 1:1	*aa*:*ab*:*bb* = 1:2:1	*bb* + *nb*:*bc*:*cc* = 2:1:1	*aa*:*ab*:*bb* = 1:2:1	*ab*:*bb*: = 1:1	*bb* = 1
Observed	219:102:79	174:226	86:209:105	171:111:118	71:196:133	194:206	400
Goodness-of-fit	6.54 on 2 *df**	6.77 on 1 *df***	2.70 on 2 *df* ns	8.65 on 2 *df**	19.30 on 2 *df****	0.36 on 1 *df* ns	–
Grant	*aa*	*aa*	*bc*	*bb*	*ab*	*ab*	*bb*
Expected	*aa*:*ab* = 1:1	*aa* = 1	*bb*:*ab*:*acbc* = 1:1:1:1	*bb*:*bc* = 1:1	*aa*:*ab*:*bb* = 1:2:1	*ab*:*ab*:*bb* = 1:2:1	*bb* = 1
Observed	212:188	400	101:119:82:98	199:201	50:203:147	116:208:76	400
Goodness-of-fit	1.44 on 1 *df* ns	–	6.09 on 3 *df* ns	0.01 on 1 *df* ns	49.99 on 2 *df****	9.03 on 2 *df**	–
Keane	*ab*	*aa*	*ac*	*cc*	*ab*	*bb*	*ab*
Expected	*aa*:*ab*:*bb* = 1:2:1	*aa* = 1	*aa*:*ab*:*ac*:*bc* = 1:1:1:1	*bc*:*cc* = 1:1	*aa*:*ab*:*bb* = 1:2:1	*ab*:*bb*: = 1:1	*ab*:*bb* = 1:1
Observed	98:207:95	400	123:102:94:81	171:229	69:191:140	195:205	193:207
Goodness-of-fit	0.45 on 2 *df**ns	–	9.19 on 3 *df**	8.44 on 1 *df***	45.42 on 2 *df****	0.25 on 1 *df* ns	0.49 on 1 *df* ns
Mission	*ab*	*ab*	*aa*	*nc*	*aa*	*bb*	*bb*
Expected	*aa*:*ab*:*bb* = 1:2:1	*aa*:*ab* = 1:1	*aa*:*ab* = 1:1	*bb*:*bc*:*cc* + *nc* = 1:1:2	*aa*:*ab* = 1:1	*ab*:*bb*: = 1:1	*bb* = 1
Observed	104:216:80	199:201	199:201	102:82:216	152:248	178:222	400
Goodness-of-fit	5.7 on 2 *df* ns	0.01 on 1 *df* ns	0.01 on 1 *df* ns	4.47 on 2 *df* ns	23.26 on 1 *df****	4.84 on 1 *df**	–
Ne Plus Ultra	*ab*	*aa*	*ac*	*bc*	*bb*	*ab*	*bb*
Expected	*aa*:*ab*:*bb* = 1:2:1	*aa* = 1	*aa*:*ab*:*acbc* = 1:1:1:1	*bb*:*bc*:*cc* = 1:2:1	*ab*:*bb* = 1:1	*aa*:*ab*:*bb* = 1:2:1	*bb* = 1
Observed	125:198:77	400	115:97:79:109	129:141:130	202:198	99:194:107	400
Goodness-of-fit	11.55 on 2 *df***	–	7.77 on 3 *df* ns	35.33 on 2 *df****	0.04 on1 *df* ns	0.67 on 1 *df* ns	–
Peerless	*nn*	*aa*	*bb*	*bb*	*ab*	*ab*	*bb*
Expected	*an*:*bn* = 1:1	*aa* = 1	*aa*:*bb*: = 1:1	*bb*:*bc* = 1:1	*aa*:*ab*:*bb* = 1:2:1	*aa*:*ab*:*bb* = 1:2:1	*bb* = 1
Observed	198:202	400	218:182	190:210	85:228:87	94:193:113	400
Goodness-of-fit	0.04 on 1 *df* ns	–	3.24 on 1 *df* ns	1.00 on 1 *df* ns	7.89 2 *df**	2.24 on 2 *df* ns	–
Price	*aa*	*ab*	*ab*	*nc*	*aa*	*bb*	*bb*
Expected	*aa*:*ab*: = 1:1	*aa*:*ab* = 1:1	*aa*:*ab*:*bb* = 1:2:1	*bb*:*bc*:*cc* + *nc* = 1:1:2	*aa*:*ab* = 1:1	*ab*:*bb*: = 1:1	*bb* = 1
Observed	225:175	198:202	99:205:96	109:92:198	186:214	202:198	400
Goodness-of-fit	6.26 on 1 *df**	0.04 on 1 *df* ns	0.29 on 2 *df* ns	1.3 on 2 *df* ns	1.96 on 1 *df* ns	0.04 on 1 *df* ns	–

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns, non-significant

Table 2 Summary of all possible chi-square tests of association between isozyme loci [–two-way table cannot be formed (at least one homozygous locus)]

		AAT-1	*GPI-2*	*IDH*	*LAP-1*	*PGM-1*	*PGM-2*
GPI-2	Carmel	–					
	Fritz	ns					
	Grant	–					
	Keane	–					
	Ne Plus	–					
	Mission	ns					
	Peerless	–					
	Price	ns					
IDH	Carmel	***	–				
	Fritz	***	ns				
	Grant	***	–				
	Keane	***	–				
	Ne Plus Ultra	***	–				
	Mission	***	ns				
	Peerless	***	–				
	Price	***	ns				
LAP-1	Carmel	ns	–	ns			
	Fritz	ns	***	ns			
	Grant	ns	–	ns			
	Keane	ns	–	ns			
	Ne Plus Ultra	ns	–	ns			
	Mission	ns	***	ns			
	Peerless	ns	–	ns			
	Price	ns	***	ns			
PGM-1	Carmel	ns	–	*	ns		
	Fritz	ns	ns	ns	ns		
	Grant	ns	–	ns	ns		
	Keane	ns	–	ns	ns		
	Ne Plus Ultra	ns	–	ns	ns		
	Mission	ns	ns	ns	ns		
	Peerless	ns	–	ns	ns		
	Price	ns	ns	ns	ns		
PGM-2	Carmel	ns	–	ns	***	ns	
	Fritz	ns	ns	ns	***	ns	
	Grant	ns	–	ns	***	ns	
	Keane	ns	–	ns	***	ns	
	Ne Plus Ultra	ns	–	ns	***	ns	
	Mission	ns	ns	ns	***	ns	
	Peerless	ns	–	ns	***	ns	
	Price	ns	ns	ns	***	ns	
SKDH	Carmel	–	–	–	–	–	–
	Fritz	–	–	–	–	–	
	Grant	–	–	–	–	–	–
	Keane	ns	–	ns	ns	ns	ns
	Ne Plus Ultra	–	–	–	–	–	–
	Mission	–	–	–	–	–	–
	Peerless	–	–	–	–	–	–
	Price	–	–	–	–	–	–

* Association significant at $P < 0.05$; *** association significant at $P < 0.001$; ns, association non-significant

source was 'Fritz', 'Mission' or 'Price', but could not be tested for the remaining five pollen sources, as there was no segregation. An isolated association of *PGM-1* and *IDH* was found for the cross involving 'Carmel' (P value < 0.05), but in the absence of supporting evidence for the other parents, this appears to be a chance result and not proof of linkage. Given the large number of pairwise combinations tested (100), it is not surprising that this seemingly anomalous result was found.

Identifying parental type when isozyme loci are linked

By considering the genotype of the parent and the observed frequency of the phenotypes in the embryo, we were able to identify the pairing of the alternative alleles at the linked isozyme loci. For example, consider the 'Nonpareil' × 'Ne Plus Ultra' cross for isozyme loci *LAP-1* and *PGM-2*. Consider both parents are *bc* for *Lap-1* and *a'b'* for *PGM-2*. The two way contingency

Table 3 Summary of observed (obs) and expected (exp) frequencies for 'Nonpareil' crossed with different pollen sources

With eight different pollen sources: AAT-1 & IDH									
Pollen source	*AAT-1*	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
		IDH							
Carmel		*aa*		*ab*		*bb*			
	aa	10	30.04	61	69.53	64	35.44		
	ab	35	41.61	117	96.31	35	49.09		
	bb	44	17.35	28	40.17	6	20.48		
Fritz		*aa*		*ab*		*bb*			
	aa + *an*	28	47.08	104	114.43	87	57.49		
	ab	49	21.93	45	53.29	8	26.77		
	bb	9	16.99	60	41.28	10	20.74		
Grant		*ab*		*bb*		*ac*		*bc*	
	aa	41	63.07	78	53.53	22	43.46	71	51.94
	ab	78	55.93	23	47.47	60	38.54	27	46.06
Keane		*aa*		*ab*		*ac*		*bc*	
	aa	10	30.14	20	25.14	16	22.30	52	20.33
	ab	53	63.35	66	53.05	60	46.87	27	42.74
	bb	60	29.52	17	24.72	15	21.84	4	19.92
Mission		*aa*		*ab*					
	aa	32	51.24	71	51.76				
	ab	112	107.96	105	109.04				
	bb	55	39.80	25	40.20				
Ne Plus Ultra		*aa*		*ab*		*ac*		*bc*	
	aa	21	35.94	18	30.31	20	24.69	66	34.06
	ab	45	37.21	66	48.26	49	39.30	39	54.23
	bb	49	21.85	13	18.43	10	15.01	4	20.71
Peerless		*ab*		*bb*					
	an	53	107.91	145	90.09				
	bn	165	110.09	37	91.91				
Price		*aa*		*ab*		*bb*			
	aa	27	55.69	116	114.75	82	54.56		
	ab	72	43.31	88	89.25	15	42.44		

With eight different pollen sources: *LAP-1* & *PGM-2*							
Pollen source	*LAP-1*	Obs	Exp	Obs	Exp	Obs	Exp
		PGM-2					
Carmel		*ab*		*bb*			
	bb	80	44.18	14	49.82		
	bc	72	43.24	20	48.76		
	cc + *cn*	36	100.58	178	113.42		
Fritz		*ab*		*bb*			
	bb + *bn*	136	82.94	35	88.06		
	bc	25	53.83	86	57.17		
	cc	33	57.23	85	60.77		
Grant		*aa*		*ab*		*bb*	
	bb	97	57.71	83	102.98	19	38.31
	bc	19	58.29	124	104.02	58	38.69
Keane		*ab*		*bb*			
	bc	123	83.36	54	100.26		
	cc	67	111.64	144	97.74		
Mission		*ab*		*bb*			
	bb	87	56.61	15	45.39		
	bc	70	45.51	12	36.49		
	cc + *cn*	65	119.88	151	96.12		
Ne Plus Ultra		*aa*		*ab*		*bb*	
	bb	74	31.94	46	62.56	9	34.51
	bc	12	34.65	93	67.90	35	37.45
	cc	13	32.42	55	63.53	63	35.04
Peerless		*aa*		*ab*		*bb*	
	bb	69	44.65	90	91.20	31	54.15
	bc	25	49.35	102	100.8	83	59.85
Price		*ab*		*bb*			
	bb	86	54.77	23	54.23		
	bc	68	46.23	24	45.77		
	cc + *cn*	47	100.00	152	99.00		

Table 3 (Continued)

With three different pollen sources: *LAP-1* & *GPI-2*					
Pollen source	*LAP-1*	Obs *GPI-2*	Exp	Obs	Exp
Fritz		*aa*		*ab*	
	bb + *bn*	82	96.61	89	74.38
	bc	101	62.72	10	48.28
	cc	43	66.67	75	51.33
Mission		*aa*		*ab*	
	bb	16	50.49	86	51.51
	bc	65	40.59	17	41.41
	cc + *cn*	117	106.92	99	109.08
Price		*aa*		*ab*	
	bb	11	53.96	98	55.04
	bc	77	45.54	15	46.46
	cc + *cn*	110	98.50	89	100.49

Table 4 Deduced parental type showing linkage of alleles for *AAT-1* and *IDH*, *LAP-1* and *PGM-2* and *LAP-1* and *GPI-2* (see Table 3)

Cultivar	Isozyme-linked groups		
	AAT-1 & IDH	*LAP-1 & PGM-2*	*LAP-1 & GPI-2*
Nonpareil	$\frac{a\ b'}{b\ a'}$	$\frac{b\ a'}{c\ b'}$	$\frac{b\ a'}{c\ a'}$
Carmel	$\frac{a\ b'}{b\ a'}$	$\frac{c\ b'}{n\ b'}$	
Fritz	$\frac{a\ a'}{n\ b'}$	$\frac{b\ b'}{n\ b'}$	$\frac{b\ a'}{n\ b'}$
Grant	$\frac{a\ b'}{a\ c'}$	$\frac{b\ a'}{b\ b'}$	
Keane	$\frac{a\ c'}{b\ a'}$	$\frac{c\ b'}{c\ b'}$	
Mission	$\frac{a\ a'}{b\ a'}$	$\frac{c\ b'}{n\ b'}$	$\frac{c\ a'}{n\ b'}$
Ne Plus Ultra	$\frac{a\ c'}{b\ a'}$	$\frac{b\ a'}{c\ b'}$	
Peerless	$\frac{n\ b'}{n\ b'}$	$\frac{b\ a'}{b\ b'}$	
Price	$\frac{a\ a'}{a\ b'}$	$\frac{c\ b'}{n\ b'}$	$\frac{c\ a'}{n\ b'}$

table, showing observed frequency and expected frequency for these crosses is given in Table 3. The placement of the alleles on the chromosomes could be either of the following two alternatives for both 'Nonpareil' and 'Ne Plus Ultra': $\frac{b\ a'}{c\ b'}$ or $\frac{b\ b'}{c\ a'}$ for both parents. By considering the expected frequency of parental and recombinant types and comparing these to the observed numbers in the contingency table, we were able to conclude that the most likely linkage arrangement for both 'Nonpareil' and 'Ne Plus Ultra' is $\frac{b\ a'}{c\ b'}$.

Thus, the *b* allele of the *LAP-1* loci is linked with the *a'* allele of the *PGM-2* loci and similarly, *c* is linked with *b'* for both 'Nonpareil' and 'Ne Plus Ultra'. In Table 4 the relationship among the alleles for the linked loci is indicated for all eight crosses considered.

Discussion

In this, the first linkage study of almond with eight crosses tested for seven isozyme loci, a significant association (P value < 0.001) is found between *AAT-1* and *IDH* and also between *LAP-1* and *PGM-2*, which leads us to conclude that these isozyme pairs are linked. Additionally a highly significant association is found between *LAP-1* and *GPI-2*. However, as *LAP-1* appears linked to two isozyme loci, evidence of linkage might be expected between *PGM-2* and *GPI-2*. The lack of a significant association between these two isozymes sug-

gests that *LAP-1* is located more centrally on the chromosome with the other two isozymes at opposite ends of the same chromosome.

Two of these linked isozyme loci are also found to be linked in other studies. Linkage between *LAP-1* and *PGM-2* is tentatively suggested in almond by Hauagge et al. (1987a, b), and linkage is indicated between *AAT-1* and *IDH* in apple where both in turn appear linked to the self-incompatibility gene (Manganaris and Alston 1987). It can be suggested that as the mechainism of self-incompatibility is gametophytic in both related genera, it could be that the linkage group *AAT-1* and *IDH* is itself linked to self-incompatibility in almond; this idea can be investigated in the future. In blueberry, Heemstra et al. (1991) found a similar linkage group between *LAP-1* and *GPI-2*. Moran and Bell (1983) reported the linkage of *LAP-1* and *GPI-2* in Eucalyptus. In *Asparagus officinalis* L., Maestri et al. (1991) found linkage between *IDH-2* and *AAT-2* and *CP-1*. The conservation of linked loci among different species can have important implications for the evolution of the various species (Weeden and Wendel 1990). It must be recognised that for the results presented here, where there is significant departure from the expected ratio using a goodness-of-fit test as in Table 1, it could be misleading to estimate linkage distance.

An isolated association of *PGM-1* and *IDH* was found for the cross involving 'Carmel' (P value < 0.05), but we concluded that this is a chance result and not proof of linkage. Finally, we consider there is significant evidence in almond of linkage between two pairs of loci, namely (1) *AAT-1* and *IDH* and (2) *LAP-1* and *PGM-2*. Additionally, *LAP-1* and *GPI-2* were observed to be linked when the pollen source was heterozygous for *GPI-2*. However, as *PGM-2* and *GPI-2* are not significantly associated, we propose that *LAP-1* is located in a more central position on the chromosome with *PGM-2* and *GPI-2* at opposite ends.

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