Theor Appl Genet (1992) 83:345–352

Genetics of leucine aminopeptidase in apple

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Received May 16, 1991; Accepted May 29, 1991
Communicated by H. F. Linskens

Summary. Six zones of LAP activity were detected in apples, some of them tissue specific. Genetic studies in four of them revealed the presence of four genes *LAP-1*, *LAP-2*, *LAP-3* and *LAP-4* with 4, 5, 4 and 4 alleles respectively including two null alleles. There were no big differences in allelic frequency within cultivars, selections, rootstocks and *Malus* species. Close linkage was found between *LAP-2* and resistance to mildew derived from 'White Angel'.

Key words: *Malus pumila* Mill – Leucine aminopeptidase – Genes *LAP-1*, *LAP-2*, *LAP-3*, *LAP-4* – Mildew resistance – Linkage – Breeding

Introduction

Plant peptidases include a variety of exopeptidases that hydrolyze peptide linkages adjacent to free alpha-amino acids. They have generally been designated leucine aminopeptidases (E.C.3.4.11.1; LAP), but they usually hydrolyze a variety of other amino acid residues.

The genetic basis of LAP polymorphism has been studied in various crops. Most of them display two LAP isoenzymes, as in *Phaseolus* sp. (Wall 1968), *Lupinus* sp. (Scogin 1973), *Medicago* sp. (Quiros and Morgan 1981), cabbage (*Brassica oleracea*) (Arus and Shields 1983), beech (*Fagus sylvatica*) (Kim 1979) and almonds (*Prunus amygdalus*) (Hauagge et al. 1987). Three LAP isoenzymes have been identified in pea (*Pisum sativum*) (Weeden and Marx 1987) and four in maize (*Zea mays*) (Scandalios 1969). Twenty different amino acid-naphthylamide substrates were tested by Vodkin and Scandalios (1981), and some of them gave higher rates of hydrolysis for the four zones of aminopeptidase activity in maize. In all cases, the LAPs appeared to be monomers.

Using apple pollen extracts, Chevreau (1984) detected four bands (LAPa, LAPb, LAPc, LAPd), but genetic studies were not carried out. Extracts from leaves and cotyledons and a range of other tissues were used in the present study of the genetics of LAP polymorphism in apple.

Materials and methods

A similar electrophoretic method was followed as for glutamate oxaloacetate transaminase separation (Manganaris and Alston 1987) except that the "running" time of the gels was extended to 5 h. It was, however, essential to incoporate *Tris*-glycine buffer in the "running" gel instead of *Tris*-HCl as the latter appears to substantially reduce the activity of LAP. LAP was stained using the method of Scandalios (1969) with slight modifications. Five milliliters of L-leucine-*b*-naphthylamide HCl stock solution (250 mg dissolved in a few drops of methanol and made up to 100 ml with distilled water) and 25 mg Fast Black K salt were mixed in 50 ml 0.2 *M Tris*-maleate buffer pH 6.0. The gels were immersed in this staining solution and blue bands appeared after about 20 min.

Results

Description of the zymograms

Six zones of enzymatic activity were detected in the extracts of various tissues (Fig. 1). The first four zones were numbered in order from the most anodal, LAP-I, to the most cathodal, LAP-IV. LAP-V (located between LAP-II and LAP-III) and LAP-VI were detected at a later stage. Bands were labelled from the most anodal except for the two that only rarely occurred (LAPId, LAPIId).

The fastest zone, LAP-I, consisted of four main bands, a, b, c, d, each accompanied by a faint, more anodal secondary band. Band b was the most frequently

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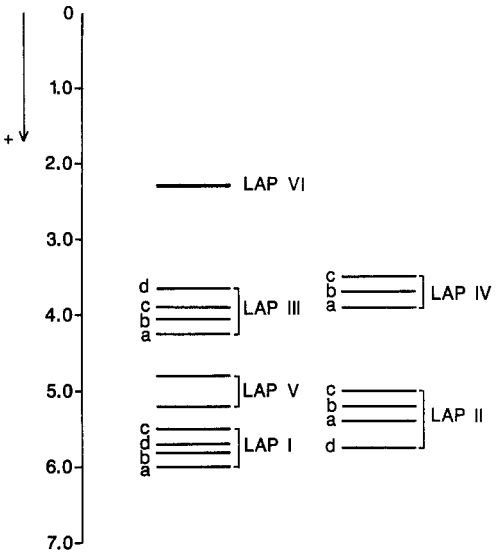

Fig. 1. Schematic representation showing the main bands of the leucine aminopeptidase (LAP) zones. Secondary bands are not shown. The scale shows the migrational distance in cm from the origin (o)

observed amongst apples, and for this reason it was used as a reference point (Rf = 1.00) when calculating Rf values. The mean Rf values of the other LAP-I bands a, d, c were 1.03, 0.98 and 0.95, respectively. This zone showed good activity in all tissues, with flower buds showing the highest and bark the lowest activity.

Zone LAP-II consisted of four bands d, a, b, c with Rf values 0.99, 0.94, 0.90 and 0.86, respectively. Activity in this zone was weaker than in zone I. Leaves and pollen showed good activity, but it was difficult to detect these bands in other tissues. Flower buds, which generally showed low activity, showed the greatest activity for this zone at the "mouse ear" stage of development. Zones II and I overlapped, and it was not always possible to score for bands d and a in LAP-II, or bands d and c in LAP-I.

In zone LAP-III four doublets were detected. Each of them consisted of a main band and a secondary band anodal to the main band. The main bands of the four doublets a, b, c and d had Rf values 0.73, 0.70, 0.67 and 0.65, respectively. All tissues showed good activity for this zone, particularly leaves and flower buds. However, the resolution in extracts from young leaves was poor since a dark background appeared. Further difficulties arose in scoring the LAP-III bands through the presence of the secondary bands and some overlapping with the LAP-IV zone.

Zone LAP-IV showed low activity. The activity was highest in flower buds and leaves and very low in cotyledons and bark. Three doublets a, b, and c were detected with Rf values 0.67, 0.64 and 0.60, respectively. The low activity, the presence of secondary bands and the overlapping with the LAP-III zone often made it difficult to score this zone accurately.

Zone V showed activity only in the bark and roots, where it was weak. It was located between Rf 0.83 and 0.90 but when *Malus* species were examined, some extra bands were detected. However, bark and roots were used in only a few cases, and polymorphism was not recorded in this zone.

Finally, zone LAP-VI showed low activity and was detectable in a few cases where young leaves were examined. Flower buds also showed a blurred band. Two bands close to each other were detected at about Rf 0.40.

The activity of LAP decreased rapidly during the storage of the samples after preparation. Samples kept for 20 days at −15 °C showed reduced activity, losing it completely in the LAP-II, LAP-IV and LAP-VI zones. Young leaves gave the best results. Di-sodium tetraborate (5% w/v) was tried as an additive since it has been reported to inhibit phenolic complexing during grinding (Soltis et al. 1980); it resulted in increased LAP activity, but produced a viscous extract: after electrophoresis bands were sharp but insufficiently spread.

Genetic control

The segregation of bands a, b and c in the LAP-I zone from 18 progenies is presented in Table 1. Data from reciprocal crosses were pooled since no differences were found between them. These results suggest that a single gene *LAP-1* codes for the activity in this zone with three alleles *a*, *b* and *c* corresponding to bands a, b and c, respectively. Band d was detected only amongst the rootstocks and the *Malus* species. Although segregation data were not obtained for this band, it is likely that it is determined by another allele *d*.

Table 2 shows the segregation of bands a, b and d in zone LAP-II from 11 crosses. These results are in agreement with the hypothesis that a gene *LAP-2* codes for activity in this zone, with three alleles *a*, *b* and *d* determining the bands a, b and d, respectively. The results from progenies F135, F132, F71 and F41 suggest that a null allele *n* is present in one or both of the parents. Scoring in this zone was difficult when alleles *LAP-1c* and *d* were present.

Figure 2 shows segregation for *LAP-2* in progeny F132, a cross between 'Katy' (*an*) and the mildew (*Podosphaera leucotricha*) resistant var 'White Angel' (*an*); genotypes *aa* and *an* were indistinguishable. Forty seedlings from this cross were inoculated with mildew and showed clear segregation for resistance and susceptibility. These included 15 *LAP-2nn* seedlings, which were all susceptible, and 25 *LAP-2* (*aa* or *an*) seedlings of which 16 were resistant and 9 susceptible.

LAP-II band c seems likely to be determined by another allele, *c*, however no crosses were made which involved this band.

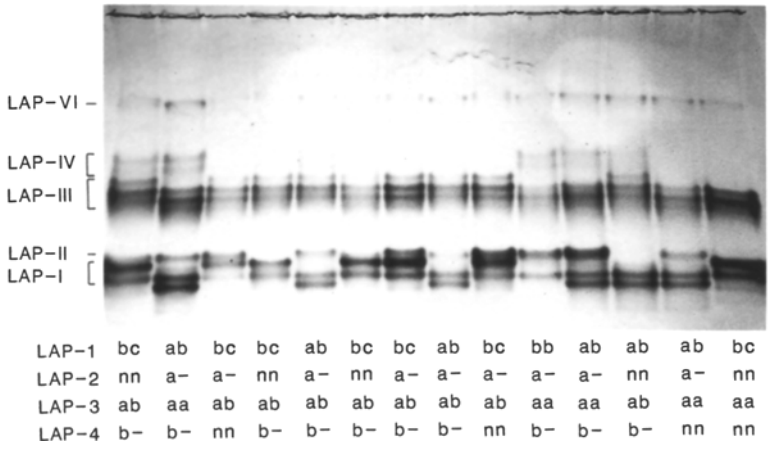

Fig. 2. Leucine aminopeptidase zymograms from leaf extracts from progeny F132 ('Katy' × 'White Angel'). Segregation was observed for *LAP-1* (*bb* × *ac*), *LAP-2* (*an* × *an*), *LAP-3* (*aa* × *ab*) *LAP-4* (*bn* × *bn*) and for LAP-VI bands

Table 1. Segregation for *LAP-1*

Family		Parental genotypes	Progeny genotypes	Expected ratio	χ^2	*P*
F24	('Golden Delicious' × 'Golden Hornet')	bc × ac	2ab:9ac:9bc:6cc	1:1:1:1	5.07	0.17
F9,10[a]	('Golden Delicious' × A463–70)	bc × bb	27bb:20bc	1:1	1.04	0.31
F32	('Fiesta' × 'Spartan')	bb × bc	7bb:6bc	1:1	0.08	0.78
F36,49[a]	('Northern Spy' × 'Winter Majetin')	bb × bc	43bb:49bc	1:1	0.39	0.53
F46	('Discovery' × 'Red Jade')	bc × bb	13bb:8bc	1:1	1.19	0.27
F72	(604 × A853/1)	bc × bb	14bb:3bc	1:1	7.11	0.01*
F93	('Jonathan' × A849/7)	bb × bc	23bb:11bc	1:1	4.23	0.04*
F115	('Kent' × 'Fiesta')	bc × bb	6bb:3bc	1:1	1.00	0.32
F633	('S. Seedless' × 'G. Carpenter') (normal)	bb × bc	32bb:28bc	1:1	0.27	0.60
F633	('S. Seedless' × 'G. Carpenter') (pale green lethal)	bb × bc	9bb:12bc	1:1	0.43	0.51
F33	('Gala' × 'Elstar')	bc × bb	12bb:12bc	1:1	0.00	1.00
F34	('Idared' × 'Spartan')	bb × bc	23bb:23bc	1:1	0.00	1.00
F37	('Granny Smith' × 'Kent')	bb × bc	18bb:27bc	1:1	1.80	0.18
F23	('Vista Bella' × 'Katy')	bc × bb	12bb:9bc	1:1	0.42	0.51
F27	('Delprim' × 'Katy')	bc × bb	19bb:14bc	1:1	0.76	0.38
F25	('Gloster 69' × 'Golden Hornet')	bb × ac	14ab:11bc	1:1	0.36	0.55
F30	('Idared' × 'Golden Hornet')	bb × ac	9ab:5bc	1:1	1.14	0.28
F132	('Katy' × 'White Angel')	bb × ac	12ab:16bc	1:1	1.25	0.26
F140	('Glengyle Red' × 3762)	bc × cc	15bc:21cc	1:1	1.00	0.32

* $P<0.05$

[a] Pooled data from reciprocal crosses

Table 2. Segregation for *LAP-2*

Family		Parental genotypes	Progeny genotypes	Expected ratio	χ^2	*P*
F5,6[a]	('Idared' × T31-12)	ab × aa	27aa:27ab	1:1	0.00	1.00
F11,21[a]	('Idared' × 'Fiesta')	ab × aa	9aa:17ab	1:1	2.46	0.12
F30	('Idared' × 'Golden Hornet')	ab × aa	7aa:6ab	1:1	0.08	0.78
F34	('Idared' × 'Spartan')	ab × aa	16aa:15ab	1:1	0.03	0.86
F93	('Jonathan' × A849-7)	ab × aa	15aa:15ab	1:1	0.00	1.00
F135	('Idared' × A679-12)	ab × an	28ab:38(aa+an)[b]:19bn	1:2:1	2.92	0.23
F71	(A140-7 × A172-2)	an × an	12(aa+an)[b]:5nn	3:1	0.18	0.67
F132	('Katy' × 'White Angel')	an × an	37(aa+an)[b]:18nn	3:1	1.75	0.20
F12,17[a]	(T30-24 × 'Cox')	ab × ad	15(aa+ad)[b]:9(ab:bd)[b]	1:1	1.50	0.22
F3	('Cox' × A463-70)	ad × dd	5ad:6dd	1:1	0.09	0.77
F41	('Cox' × 'Baskatong')	ad × nn	27an:34dn	1:1	0.80	0.40

[a] Data from reciprocal crosses were pooled

[b] Genotypes indistinguishable either because of the null allele or because of poor resolution

Table 3. Segregation for *LAP-3*

Family		Parental genotypes	Progeny genotypes	Expected ratio	χ^2	*P*
F32	('Fiesta' × 'Spartan')	ab × aa	8aa : 6ab	1 : 1	0.11	0.74
F93	('Jonathan' × A849-7)	bb × ac	12ab : 12bc	1 : 1	0.00	1.00
F132	('Katy' × 'White Angel')	aa × ab	15aa : 25ab	1 : 1	2.50	0.12
F18	('Cox' × 'Fiesta')	ab × ab	2aa : 4ab : 5bb	1 : 2 : 1	2.27	0.15
F12	('Cox' × T30-24)	ab × ab	4aa : 6ab : 4bb	1 : 2 : 1	0.28	0.82

Table 4. Segregation for *LAP-4*

Family		Parental genotypes	Progeny genotypes	Expected ratio	χ^2	*P*
F32	('Fiesta' × 'Spartan')	ab × aa	8aa : 5ab	1 : 1	0.69	0.40
F101	(A723-6 × 'Jester')	ab × aa	9aa : 7ab	1 : 1	0.25	0.62
F5, 6[a]	('Idared' × T31-12)	an × ab	26 (aa + an)[b] : 30 (ab + bn)[b]	1 : 1	0.28	0.59
F36, 49[a]	('N. Spy' × 'Winter Majetin')	bn × cn	46 (bn + nn)[b] : 43 (bc + cn)[b]	1 : 1	0.10	0.75
F48	('Jonathan' × 'Idared')	ab × an	11 (aa + an)[b] : 10 (ab + bn)[b]	1 : 1	0.05	0.83
F29	('Katy' × 'Delprim')	bn × bn	8 (bb + bn)[b] : 6nn	3 : 1	2.38	0.13
F132	('Katy' × 'White Angel')	bn × bn	28 (bb + bn)[b] : 12nn	3 : 1	0.53	0.46
F135	('Idared' × A679-12)	an × bn	21an : 40 (ab + bn)[b] : 24nn	1 : 2 : 1	0.50	0.78

[a] Pooled data from reciprocal crosses
[b] Genotypes indistinguishable either because of the *n* allele or because of poor resolution

The segregation of LAP-III bands from five progenies (Table 3) suggests that a gene, *LAP-3*, codes for the activity in that zone with three alleles *a*, *b* and *c* corresponding to bands a, b and c, respectively. Segregation data were not available for the d band, but it may be supposed that it is determined by another allele *d*.

The results in Table 4 suggest the presence of a single gene *LAP-4* with three alleles *a*, *b* and *c* determining the bands a, b and c, respectively, and a null allele in the LAP-IV zone.

Resolution was sufficient for scoring the LAP-VI zone in only one progeny, F132 (Fig. 2). This progeny also segregated for *LAP-1*, *LAP-2*, *LAP-3* and *LAP-4*, and the corresponding genotypes are shown. One seedling (position 10 from the left) appeared to show only one *LAP-1* allele, *b*; neither of the alleles from its mildew resistant parent 'White Angel' (*LAP-1ac*) was represented. This seedling did not show inconsistency for any of the 13 other isoenzymes examined and appeared resistant to mildew. It seems probable, therefore, that it was an aneuploid.

Distribution of LAP classes in Malus

Tables 5, 6 and 7 present the genotypes for leucine aminopeptidase in 66 cultivars, 37 rootstocks and 38 *Malus* species and species derivatives, respectively.

Two alleles (*b*, *c*) for *LAP-1* were found in the cultivars giving three different genotypes (*bb*, *bc*, *cc*) (Fig. 3). A further allele (*d*) was found in the apple rootstocks and an additional allele (*a*) amongst the *Malus* species, giving nine different genotypes for *LAP-1*. Allele *b* predominated with the highest frequency in the rootstocks (85%) and the lowest in the *Malus* species (48.5%) (Table 8). Triploid cvs 'Blenheim Orange', 'Holstein' and 'Jonagold', showed the b band more intensively than the c band, and for this reason genotype *bbc* was assigned to them. Similarly, as the b band in *M. fusca* was more intense than band c, the genotype *bbc* was assigned to that species. In rootstocks M.4, M.14 and M.21 the genotype *bc* was determined but the b band appeared weak, even when other zones showed good activity. It is probable that these rootstocks have genotype *cc*, and the weak b band corresponds to the secondary band of the main band c. No differences were found between M.9 and M.9 EMLA or between M.13 and its tetraploid chimera.

It was not always possible to record LAP-2 genotypes due to overlapping with the LAP-I zone and the occurrence of null alleles. When only one band was present in the LAP-II zone, the assigned genotype was incomplete. In this case complete genotypes could only be determined through progeny tests. Genotype *nn* was assigned to varieties which repeatedly did not show activity in this zone. Although the data are incomplete, it appears that alleles

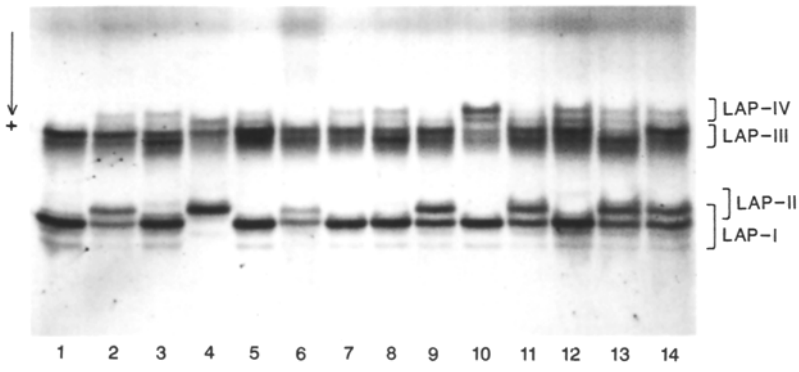

Fig. 3. Leucine aminopeptidase zymograms from flower bud extracts of apple cultivars. *1* 'Idared', *2* 'Greensleeves', *3* 'James Grieve', *4* 'Early Victoria', *5* 'Jonathan', *6* 'Discovery', *7* 'Bramley's Seedling', *8* 'Jupiter', *9* 'Starkrimson', *10* 'Gloster 69', *11* 'Gala', *12* 'Ashmead's Kernel', *13* 'Kent', *14* 'Golden Delicious'

Table 5. Leucine aminopeptidase (*LAP-1*, *LAP-2*, *LAP-3*, *LAP-4*) genotypes of apple cultivars

Cultivar	*LAP-1*	*LAP-2*[a]	*LAP-3*	*LAP-4*[a]	Cultivar	*LAP-1*	*LAP-2*[a]	*LAP-3*	*LAP-4*[a]
'Akane'	bb	d–	bb	ab	'Jester'	bb	an	bb	aa
'Andre Briollay'	bb	a–	ab	ab	'Jonagold'	bbc	–	bbb	–
'Ashmead's Kernel'	bb	c–	bb	ab	'Jonathan'	bb	ab	bb	ab
'Beauty of Bath'	bc	–	bb	a–	'Jupiter'	bbb	ad–	ab–	b–
'Blenheim Orange'	bbc	–	bbb	ab–	'Katy'	bb	an	aa	bn
'Bountiful'	bb	aa	aa	ab	'Kent'	bc	–	aa	b–
'Bramley's Seedling'	bbb	c–	bbb	bb–	'King of the Pippin's'	bc	–	–	–
'Cloden'	bbb	nnn	bbb	ab–	'Lane's Prince Albert'	bb	nn	bb	a–
'Court Pendu Plat'	bb	nn	bc	–	'Laxton's Superb'	bc	–	bb	ab
'Cox'	bb	ad	ab	bb	'Leonie de Sonnaville'	bb	ad	ab	ab
'Delcorf'	bc	–	bb	ab	'Lodi'	bc	–	ab	b–
'Delprim'	bc	–	ab	bn	'Lord Derby'	bb	–	–	–
'Discovery'	bc	–	ab	a–	'Louiton'	cc	–	ab	ab
'Early Victoria'	cc	–	bb	aa	'McIntosh Starkspur'	bb	aa	cd	–
'Edward VII'	bb	ac	bc	–	'McIntosh Wijcik'	bb	aa	cd	–
'Elstar'	bb	ab	bb	b–	'Marie Joseph'	bb	d–	ab	ab
'Falstaff'	bc	–	ab	ab	'Miller's Seedling'	bc	–	–	–
'Fiesta'	bb	aa	ab	ab	'Newton Wonder'	bb	–	–	–
'Gala'	bc	c–	ab	ab	'Northern Spy'	bb	an	bb	bn
'George Carpenter'	bc	a–	ab	ab	'Reinette Clochard'	bb	ad	bb	ab
'Gloster 69'	bb	nn	ab	bb	'Reinette de France'	bbb	a–	abc	–
'Golden Delicious'	bc	–	bb	ab	'Reverend W. Wilks'	bc	–	–	–
'Golden Noble'	bc	–	–	–	'Ribston Pippin'	bbb	a–	bbb	a–
'Granny Smith'	bb	c–	bb	–	'Rome Beauty'	bc	–	bb	ab
'Gravenstein'	bc	–	–	–	'Spartan'	bc	aa	aa	aa
'Greensleeves'	bc	–	bb	ab	'Spencer's Seedless'	bb	ad	ab	b–
'Grenadier'	cc	–	bc	–	'Starkrimson'	bc	–	bb	ab
'Holstein'	bbc	–	–	–	'Suntan'	bbb	a–	ab–	a–
'Howgate Wonder'	bb	–	–	–	'Vista Bella'	bc	–	bc	–
'Idared'	bb	ab	bb	an	'Wagener'	bb	a–	bb	–
'Indo'	bb	nn	bb	a–	'White Transparent'	bc	–	bb	a–
'Ingrid Marie'	bb	ab	ab	ab	'Winter Majetin'	bc	an	bb	cn
'James Grieve'	bb	ad	ab	b–	'Worcester'	bb	ad	ab	a–

[a] Some genotypes not specified either due to null alleles or due to poor resolution

a and *n* predominated for *LAP-2*. Rootstock M.24 showed a band that was faster than the normal band a; possibly it is determined by a different allele.

Four alleles (*a*, *b*, *c*, *d*) for *LAP-3* gave seven different genotypes including one with three different alleles, 'Reinette de France'. Allele *b* predominated (65–68.5%) in all groups (Table 8). No large differences were found between the frequency of the *LAP-3* alleles. Rootstocks M.15 and M.16 showed a band which moved faster than the normal band a.

It was not always possible to record the *LAP-4* alleles because of the appearance of secondary bands of nearly the same intensity at the same positions that overlapped with the LAP-III zone and the occurrence of null alleles. Genotypes were assigned for those with two distinct bands and for the parents of the examined crosses.

Table 6. Leucine and aminopeptidase (*LAP-1*, *LAP-2*, *LAP-3*, *LAP-4*) genotypes of apple rootstocks

Rootstock	*LAP-1*	*LAP-2*	*LAP-3*	*LAP-4*	Rootstock	*LAP-1*	*LAP-2*	*LAP-3*	*LAP-4*
M.1	bb	a–	ab	b–	M.19	bb	b–	ab	ab
M.2	bb	a–	ab	ab	M.20	bb	a–	bb	b–
M.3	bb	a–	bb	bc	M.21	*bc*[a]	–	bc	–
M.4	*bc*[a]	a–	ab	a–	M.23	bc	b–	ab	b–
M.5	bb	a–	ab	b–	M.24	bb	a–[c]	ab	bc
M.6	bd	a–	ab	b–	M.25	bb	d–	ab	b–
M.7	bb	a–	ab	ab	M.26	bb	b–	ab	a–
M.8	bb	b–	bb	b–	M.27	bb	a–	bb	b–
M.9	bb	a–	ab	b–	MM.101	bb	a–	ab	b–
M.10	bd	b–	bb	b–	MM.102	bb	a–	bb	b–
M.11	cd	–	bc	b–	MM.104	bb	da	ab	b–
M.12	bc	b–	ab	b–	MM.105	bb	da	ab	b–
M.13	bb	ab	bb	ab	MM.106	bb	a–	ab	b–
M.13 chimera	bb	ab	bb	ab	MM.109	bb	da	bb	b–
M.14	*bc*[a]	–	ab	nn	MM.110	bb	a–	bb	b–
M.15	bd	a–	a–[b]	ab	MM.111	bb	a–	ab	b–
M.16	*bc*[a]	b–	a–[b]	ab	MM.112	bb	d–	bb	bc
M.17	bb	a–	ab	b–	MM.115	bb	d–	bb	b–
M.18	bb	a–	bb	a–					

[a] Italicized alleles show more intensive bands (Possible cc genotype)
[b] Allelic band LAP-IIIa faster than the normal
[c] Allelic band LAP-IIa faster than the normal

Discussion

Six zones of LAP activity were detected, and four genes were found with 17 alleles including two null alleles. Apart from null alleles all *LAP* alleles were expressed by well-defined independent bands – hybrid bands did not occur – thus supporting a monomeric basis for the enzymes coded by the *LAP* genes. The occurrence of null LAP alleles has been reported in beech (Kim 1979), almonds (Hauagge et al. 1987) and alfalfa (Quiros and Morgan 1981).

In progeny F633 ('Spencer's Seedless' × 'George Carpenter') plants showing pale green lethal symptoms (Klein et al. 1961) were compared with normal plants, and no isoenzymic differences were observed. In progeny F132 ('Katy' *LAP-1bb* × 'White Angel' *LAP-1ac*) one seedling (Fig. 2), showed only a LAP-I b band. No inconsistencies were noticed for 13 other isoenzymes in this seedling. In addition the seedling was resistant to mildew, which was presumably derived from 'White Angel'. It is possible that chromosomal modifications were involved. The chromosome region including the gene *LAP-1* may have been involved in an interchromosomal translocation in the male parent leading during microspore development to pollen hypoploid for the *LAP-1* region. Fusion of such pollen cells with normal egg cells could then result in zygotes haploid for *LAP-1*, which would be recorded as *LAP-1bb*.

Where two different bands were present for LAP-I the genotype of triploids could be identified on the basis of differing degrees of band intensity. Such a distinction was not possible with triploids where only one LAP-I band is represented, and thus this position cannot provide satisfactory confirmation of the proposed triploid nature of 'Ashmead's Kernel' so clearly demonstrated through alleles of glutamate oxaloacetate transaminase (GOT) and peroxidase (PRX) genes (Manganaris and Alston 1989).

Gallot et al. 1985 suggested a monogenic basis for the mildew resistance carried by 'White Angel'. The absence of mildew resistance among *LAP-2nn* derivatives of 'White Angel' (*LAP-2an*) may be a result of linkage between *LAP-2* and a gene for resistance. Further crosses for linkage tests and mildew resistance studies are needed to define more clearly the inheritance of resistance and its relationship with *LAP-2*. Some such crosses should include as parents derivatives of 'White Angel' resistant to mildew, which also carry two active alleles for LAP-2, since the presence of null alleles substantially reduces the efficiency of linkage estimations.

Since all 15 seedlings without activity for *LAP-2* (*nn*) in family F132 were found to be susceptible to mildew, an alternative hypothesis to linkage must be considered at this stage. This is that *LAP-2* is a component of mildew resistance in 'White Angel', and its activity is necessary for the full expression of resistance (complementary gene action). This hypothesis could best be tested through crosses between 'White Angel' and susceptible parents homozygous for the *LAP-2n* allele.

Allele *LAP-1a* was found only in *Malus* species, hybrids and derivatives. This allele was found in 11 accessions spread over the genus and was not associated with

geographical origin. Five accessions of *M. baccata* origin carried the *LAP-1a* allele. Since *M. baccata* is thought to be a progenitor of cultivated apple, it then seems likely that the *LAP-1a* allele was discarded during the evolution of the apple. Of the rootstocks only M.6, M.10, M.11 and M.15 carry the *LAP-1d* allele, but they do not show any morphological similarities, either between themselves (Tydeman 1955) or with *M. hupehensis*, the only species carrying this allele. There were no big differences in allelic frequency within cultivars, species or rootstocks. Similar cases of contrast between the high levels of morphological polymorphisms and low polymorphisms in genes coding isoenzymes has been reported in Indian amaranths (Jain et al. 1980).

Table 7. Leucine aminopeptidase (*LAP-1*, *LAP-2*, *LAP-3*, *LAP-4*) genotypes of *Malus* species, hybrids and derivatives

Malus sp.	*LAP-1*	*LAP-2*	*LAP-3*	*LAP-4*
M. baccata flexilis	aa	ac	ab	a–
M. baccata 'Gracilis'	cc	–	bb	b–
M. baccata jackii	bb	a–	bb	a–
M. × *arnoldiana*	ab	a–	bb	nn
M. × *hartwigii*	bb	b–	bb	b–
M. × *robusta* 'Erecta'	bb	ac	ab	a–
M. × *robusta* o.p. (3760)	aa	bc	bb	ab
M. × *robusta* o.p. (3762)	cc	–	bb	ab
M. × *zumi* o.p. (3753)	ab	a–	bb	–
M. 'Golden Hornet'	ac	aa	cc	–
M. brevipes	bb	a–	bb	b–
M. coronaria 'Charlottae'	ab	a–	ab	a–
M. × *platycarpa*	ab	nn	ab	nn
M. floribunda J.	ab	a–	ab	a–
M. × *scheideckeri* 'Hillieri'	bb	a–	ab	–
M. florentina	cc	b–	bc	–
M. fusca	bbc	b–	ab	nn
M. glaucescens	aa	a–	aa	a–
M. hupehensis theifera	dd	–	bc	–
M. hupehensis robusta o.p. (3759)	dd	a–	ab	ab
M. hupehensis derivatives (A878-5, 9, 10, 11, 25)	bd	a–	–	–
M. prunifolia loceinea	ac	–	bc	–
M. × *purpurea* 'Aldenhamensis'	bb	a–	ab	–
M. sargentii	bc	–	bb	–
M. sieboldii	bc	b–	aa	–
M. × *atrosanguinea*	bb	b–	bc	–
M. × *moerlandsii*	bb	ab	bb	a–
M. 'White Angel'	ac	an	ab	bn
M. × *soulardii*	bb	d–	ab	nn
M. toringoides	bb	–	bc	–
M. trilobata	cc	–	bb	–
M. yunnanensis	cc[a]	bc	ab	–
M. 'Baskatong'	bb	nn	bb	–
M. 'Red Jade'	bb	b–	bb	a

[a] Faint c band

Acknowledgements. We are grateful to the Ministry of Agriculture Fisheries and Food for supporting this work and to the Greek Scholarship Foundation for further financial support to the senior author.

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Table 8. Frequencies of *LAP-1* and *LAP-3* alleles in apple cultivars, rootstocks and *Malus* species

	LAP-1				*LAP-3*			
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*
Cultivars	0.0	0.740	0.260	0.0	0.240	0.680	0.060	0.020
Rootstocks	0.0	0.850	0.095	0.055	0.285	0.685	0.030	0.0
Malus sp.	0.185	0.485	0.185	0.145	0.240	0.650	0.110	0.0

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