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Genes determining leucine aminopeptidase and mildew resistance from the ornamental apple, 'White Angel'

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Abstract Mildew resistance in the ornamental apple 'White Angel' was found to be determined by complementary genes. The gene R_w was found to be necessary for the expression of resistance controlled by the resistance gene Pl_w. The close linkage between the isoenzyme gene, *Lap-2*, for leucine aminopeptidase and $P1_w$ was confirmed. The efficiency of *Lap-2* as a marker in screening for mildew resistance is limited, as it cannot account for susceptible plants with the $r_w r_w P1_w p1_w$ genotype. It has, however, an important role to play in combining resistance genes from different sources. The genotypes of 'White Angel' ($R_w r_w$, Pl_w pl_w, *Lap-2an*), 'Jester' ($R_w r_w$, $p1_w$ $p1_w$, *Lap-2an*) 'Katja' ($R_w r_w$, $p1_w p1_w$, *Lap-2an*) and 'Gloster 69' ($r_w r_w$, $p1_w p1_w$, *Lap-2an*) were determined. It also appeared that R_w might influence *Lap-2* activity in young seedlings.

Key words Apple · Mildew resistance · Isoenzymes · Genes *Lap-2*, $P1_w$, R_w

Introduction

While most apple (*Malus pumila* Mill.) varieties are susceptible to powdery mildew (*Podosphaera leucotricha* Ell. and Everh.), varieties carrying low levels of resistance have been identified (Brown 1959; Sarasola 1963; Misic 1966; Alston 1969; Visser et al. 1974). Such resistance, which is transmitted only to a small proportion of seedlings, appears to be polygenically determined (Brown 1959). Higher levels of stable resistance, under the control of single genes, have been identified in *M. robusta* and *M. zumi* (Knight and Alston 1968). The ornamental apple 'White Angel', which is related to *M. sargenti* and *M. sieboldii* (Simon and Weeden 1991), provides another source of high resistance, which also appears to be under monogenic control (Gallot et al. 1985). Manganaris and Alston (1992) identified four loci controlling leucine aminopeptidase (LAP) isoenzyme activity in apple, including *Lap-2*, which controls expression in the LAP II zone. They suggested that the absence of mildew resistance in *Lap-2nn* derivatives of 'White Angel' (*Lap-2an*) was the result of a linkage between *Lap-2* and a gene for mildew resistance.

The present study aims to clarify the relationship between the isoenzyme gene *Lap-2* and mildew resistance by determining the inheritance of mildew resistance from 'White Angel' and its association with alleles of *Lap-2*.

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Materials and methods

Plant material

Three progenies derived from crosses between 'White Angel' and the mildew-susceptible varieties 'Jester', 'Gloster 69' and 'Katja' were used. Mature trees provided suitable tissue for the verification of parental LAP genotypes.

Mildew screening

Potted seedlings between the four- and eight-leaf stage were inoculated in a greenhouse with a mist spray (2×10^5 spores/ml, 0.005% Agral).

Seedlings were assessed 1 month after inoculation, the first signs of sporulation being apparent after 1 week. The degree of infection on newly expanded leaves was graded according to the following scale:

0 = no sign of infection,
1 = no sporulation, but chlorotic or necrotic flecks,
2 = very slight sporulation, chlorotic or necrotic flecks,
3 = slight sporulation, chlorotic or necrotic flecks,
4 = slight sporulation, no chlorotic or necrotic flecks,
5 = severe sporulation, no chlorotic or necrotic flecks.

Plants in grades 0–2 were classified as resistant, while those in grades 3–5 were classified as susceptible.

Isoenzyme analysis

Extracts of young actively growing leaves were prepared according to Batlle and Alston (1994), and used in polyacrylamide gel electrophoresis analysis of the seedlings. Infected leaves were rinsed with distilled water and dried with a paper tissue before extraction. Gels were prepared according to Manganaris and Alston (1992) using a resolving gel (9.45% w/v concentration). Gels were run for 5 h and stained for LAP according to Arús et al. (1982) with the following modifications: 100 mg Fast Black K salt and 1 ml of L-leucine-β-naphthylamide hydrochloride solution (25 mg dissolved in 1 ml of dimethylformamide) were mixed and stirred for 3 min in 50 ml 0.2 *M* phosphate buffer (pH 6.0). Gels were stained at 30°C for 1 h, stored overnight in the staining solution, rinsed with tap water and fixed in 7% w/v acetic acid.

Results

Segregation for mildew resistance

The three progenies derived from 'White Angel' (Table 1) showed a clear distinction between the response of resistant (grades 0–2) and susceptible (grades 3–5) seedlings, as was also observed in 'White Angel' derivatives by Gallot et al. (1985), Lespinasse (1989) and Manganaris (1989). The segregations in two progenies agreed well with a 3 resistant: 5 susceptible ratio, E294 ('Jester' × 'White Angel', χ^2=1.84) and E296 ('Katja' × 'White Angel', χ^2=0.44). E295 ('Gloster 69' × 'White Angel') gave close agreement to a 1 resistant:3 susceptible distribution (χ^2=2.61). These segregations may be explained on the basis of complementary gene action, with 'White Angel' carrying a dominant allele Pl_w for mildew resistance, as suggested by Manganaris (1989), together with a complementary dominant allele, R_w. 'Jester', 'Katja' and 'White Angel' were heterozygous for R_w, and 'Gloster 69' (*rw*, *rw*) homozygous for the recessive allele.

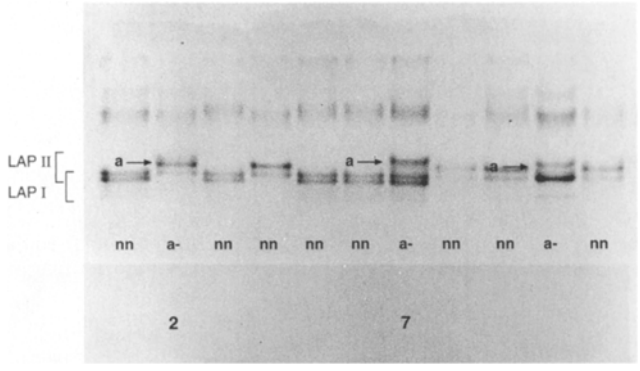

Fig. 1 Leucine aminopeptidase zymograms from leaf extracts of E296 'Katja' (*Lap-2an*) × 'White Angel' (*Lap-2an*). Samples 2 and 7 were mildew resistant; all the others were susceptible

LAP isoenzyme analysis

The pattern of bands and their distribution were consistent with that described by Manganaris and Alston (1992) Fig. 1. Clear resolution was obtained in zones LAP-I and LAP II, although sometimes it was difficult to distinguish bands in the latter zone due to low activity. The modified staining recipe developed from Arús et al. (1982) gave improved resolution. Zones I and II showed the highest activity when young leaves were used; activity decreased during storage of samples at –20°C.

The *Lap-2* genotypes recorded for 'White Angel', 'Jester' and 'Katja' agreed with those observed by Manganaris and Alston (1992), but that of 'Gloster 69' was found to be *an*, thus differing from the genotype (*nn*) previously assigned.

Mildew infection of leaf tissue did not affect the observation of LAP-II phenotypes by electrophoresis.

Segregation for *Lap-2*

All three progenies analysed were *an* × *an* for *Lap-2* (Table 2); genotypes *aa* and *an* were indistinguishable. Progeny E294 ('Jester' × 'White Angel', χ^2=0.62) fitted the expected 3 *a–* (*aa* + *an*):1 *nn* ratio but progenies E295 ('Gloster 69' × 'White Angel', χ^2=90.94) and E296 ('Katja' × 'White Angel', χ^2=14.51) did not. In both of these progenies there was an excess of *Lap-2nn* seedlings; as would result if preferential fertilization of *Lap-2n* egg cells by pollen carrying the *n* allele was occurring in 'Katja' and 'Gloster 69' or that *Lap-2nn* zygotes had the highest chance of survival in those varieties.

Joint mildew resistance and *Lap-2* segregations

The cosegregations for mildew resistance and *Lap-2* in the three progenies are shown in Table 2. Of the 104 *Lap-2nn* seedlings 102 were susceptible to mildew and only 2 were resistant, while of the 95 *Lap-2a* (*aa* or *an*) seedlings 39 were susceptible and 56 resistant. These results agreed with

Table 1 Segregation for mildew resistance in progenies derived from 'White Angel'

Cross	Parentage	Number of seedlings	Mildew grade						Mildew reaction[a]		Parental genotypes	Expected ratio	χ^2 (1 *df*)	*P*
			0	1	2	3	4	5	R	S				
E294	'Jester' × 'White Angel'	40	1	7	3	4	4	21	11	29	$R_w r_w pl_w pl_w \times R_w r_w Pl_w pl_w$	3:5	1.84	0.18
E295	'Gloster 69' × 'White Angel'	80	0	5	9	3	3	60	14	66	$r_w r_w pl_w pl_w \times R_w r_w Pl_w pl_w$	1:3	2.61	0.11
E296	'Katja' × 'White Angel'	80	1	25	7	12	12	23	33	47	$R_w r_w pl_w pl_w \times R_w r_w Pl_w pl_w$	3:5	0.44	0.51

[a] R = resistant (grades 0–2), S = susceptible (grades 3–5)

Table 2 Segregations for *Lap-2* and cosegregations for *Lap-2* and mildew resistance in progenies derived from 'White Angel'

Cross	Parentage	Number of seedlings	Parental genotypes	Progeny genotypes[a]	Expected ratio	χ^2 [b] (1 *df*)	*P*	Mildew reaction[c] and *Lap-2* genotypes[a]	
								R	S
E294	'Jester' × 'White Angel'	39	*an* × *an*	27*a*–:12*nn*	3:1	0.62	0.43	11*a*–:0*nn*	16*a*–:12*nn*
E295	'Gloster 69' × 'White Angel'	80	*an* × *an*	23*a*–:57*nn*	3:1	90.4	<0.001	12*a*–:2*nn*	11*a*–:55*nn*
E296	'Katja' × 'White Angel'	80	*an* × *an*	45*a*–:35*nn*	3:1	14.51	<0.001	33*a*–:0*nn*	12*a*–:35*nn*

[a] *Lap-2 a*– includes both *aa* and *an* genotypes
[b] Calculated using a continuity correction
[c] R = resistant, S = susceptible

those of Manganaris and Alston (1992) who studied a 'Katja' × 'White Angel' progeny of 40 seedlings. In that progeny there were 15 *Lap-2nn* seedlings, none of which were resistant to mildew. The cosegregation of mildew resistance with the alleles of *Lap-2* agrees with a three-gene hypothesis based on close linkage between *Lap-2* and a gene for mildew resistance, Pl_w previously found in 'White Angel' by Manganaris and Alston (1992), and the action of an independent complementary gene R_w.

Discussion

These results, giving fair agreement, for mildew, with either 1:3 or 3:5 resistant:susceptible ratios, suggest that the mildew resistance of 'White Angel' is determined by two loci. The results of Gallot et al. (1985) and Manganaris (1989) also support an hypothesis that more than one resistance gene is involved. However, in contrast to their results Lespinasse (1989) found clear 1:1 segregations in progenies derived from 'White Angel'.

The close linkage between *Lap-2* and resistance to mildew determined by Pl_w (Manganaris and Alston 1992) was also observed in this work. A dominant allele of the complementary gene R_w was found to be necessary for the expression of mildew resistance determined by Pl_w. The genotype of 'White Angel' is then $R_w r_w$, Pl_w pl_w, *Lap-2an*; that of 'Jester' and 'Katja', $R_w r_w$, pl_w pl_w, *Lap-2an*; and that of 'Gloster 69', $r_w r_w$, pl_w pl_w, *Lap-2an*.

The excess of *Lap-2nn* genotypes could be because this type of zygote has higher viability or because *Lap-2a* is linked to lethal genes, but in view of its association with mildew resistance this seems unlikely. An alternative explanation is that in the juvenile phase of seedlings R_w controls activity in the LAP-II zone, in addition to mildew resistance, so that in its absence, in the recessive state ($r_w r_w$), LAP-II activity cannot be expressed. Thus, in this case the putative association of LAP II with mildew resistance cannot be ruled out, particularly in view of suggestions that LAP may be a major component in plant defence mechanisms (Pautot et al. 1993). However, since in Family E295 two seedlings without LAPII activity (*Lap-2nn*) were found to be resistant to mildew, it appears that *Lap-2* activity is not necessary for the full expression of resistance, as suggested by Manganaris and Alston (1992) in an alternative explanation to linkage. In these seedlings R_w must be present to facilitate the expression of mildew resistance, and in that situation the full expression of a *Lap-2* allele would be expected were it present.

A recombination fraction for *Lap-2* and Pl_w was calculated using the method of maximum likelihood after assuming that they are linked but that R_w segregates independently of both. This resulted in a recombination fraction of 0.026 with a 95% likelihood based on confidence interval functions of (0.004, 0.080). Another approach was also followed; the recombination fraction was estimated only amongst those plants carrying *Lap-2nn* because of the distorted segregations of *Lap-2* in progenies E295 and E296. This resulted in the similar value of 0.031 with 95% confidence intervals of (0.005, 0.094).

The efficiency of *Lap-2* as a marker for early mildew screening is limited, partly due to mildew resistance being based on two complementary genes. Screening for *Lap-2* activity in progenies derived from 'White Angel' would reduce the susceptible component of the population, but it could not eliminate all susceptible plants, in particular those with *Lap-2a* and $r_w r_w$. There is a role for *Lap-2* in selection towards combining Pl_w with Pl_1 or Pl_2 from *M. robusta* and *M. zumi*, respectively (Knight and Alston 1968). If parents with Pl_1 or Pl_2 accompanied by *Lap-2nn* are chosen, and all mildew-resistant derivatives from crosses between them and mildew resistance derivatives of 'White Angel' are then screened for *Lap-2*, the proportion of plants carrying two mildew resistance genes (Pl_1 and Pl_w or Pl_2 and Pl_w) would be increased by discarding the *Lap-2nn* derivatives.

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