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Association of dominant loci for resistance to *Pseudomonas syringae* pv. *pisi* with linkage groups II, VI and VII of *Pisum sativum*

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Abstract Morphological characters, isoenzymes and recombinant inbred lines were employed to assign four loci for resistance to *Pseudomonas syringae* pv *pisi* to genetic linkage groups in *Pisum sativum*. A total of five morphological markers and 11 isoenzyme loci were screened in two independent F_2 *P. sativum* populations: Vinco × Hurst's Greenshaft (V×HGS) and Partridge × Early Onward (P×EO). Mapping was also carried out in two recombinant inbred populations, unrelated to the F_2 populations. Previously reported linkage between resistance genes *Ppi3* and *Ppi4* was confirmed. Linkage was also detected between resistance gene *Ppi2* and the isoenzyme locus *Aldo* (linkage group VII). The linked loci *Ppi3* and *Ppi4* were associated with *a* (linkage group II). A further resistance gene *Ppi1* was associated with linkage group VI close to the hilum colour gene *P1*. RAPD markers tested in the cross P×EO were not well targeted; however, one marker, OPA-$20_{0.71}$, showed linkage to *Ppi3*.

Keywords Resistance · Mapping · *Pisum* · Pea blight

Introduction

Pea blight is a bacterial disease of *Pisum sativum* caused by *Pseudomonas syringae* pathovar *pisi* (*Psp*) and is characterised by the appearance of water-soaked lesions on leaves, stems and pods which gradually darken and become necrotic (Sackett 1916). Taylor (1972) first identified races of *Psp* and a gene-for-gene model, involving five matching resistance/avirulence gene pairs, was postulated to explain resistant and susceptible interactions between races of *Psp* and cultivars of *P. sativum* (Taylor et al. 1989). A genetic analysis of this model indicated that up to six gene pairs were involved (Bevan et al. 1995). Considerable work has been done on the characterisation of the bacterial avirulence genes [Vivian et al. (1989) and Cournoyer et al. (1995) for specific avirulence genes from *Psp*, and Dangl (1994) and Innes (1996) for more general reviews of avirulence genes]. A further step in developing an understanding of the interaction of resistance and avirulence is the molecular characterisation of the plant resistance genes. The present work, intended as a preliminary study, aimed to locate specific resistance genes on the *Pisum* genetic linkage map.

Morphological traits, isoenzyme patterns and DNA polymorphisms were screened as potential markers. Morphological characters, which were easy to score and reasonably free of the epistatic effects of other alleles, were selected. RAPD markers were amplified from pooled F_2 DNA, enriched for sequences associated with specific resistance genes using the bulked segregant analysis approach of Michelmore et al. (1991).

Recombinant inbred (RI) populations are powerful alternative tools for linkage mapping in self-fertile plants (Haldane and Waddington 1931; Taylor 1978; Reiter et al. 1992). Each RI population is derived from a single crossing event by self-pollination and random selection of progeny until the level of homozygosity at any segregating locus approaches 100%. In practice F_8 (theoretically 99% homozygous) is the generation in which screening is started and from this point on, each successive generation can be regarded as identical for crossover events which occurred at the initial cross. The great benefit of recombinant inbred mapping is the speed by which markers can be assigned to a pre-existing map.

In this study, segregations were examined in F_2 populations from two crosses, carrying between them all the known or suspected race-specific resistance genes from *P. sativum* (*Ppi1* to *Ppi6*) and a range of morphological and isoenzyme markers. RI mapping was also conducted in two *P. sativum* populations generated at the John Innes

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Centre, which were polymorphic for the resistance genes *Ppi1* and *Ppi2*.

Material and methods

Bacterial strains

The isolates of *Psp* used in this study were as follows; 299A (race 1, ICMP[1] number 2995), 202 (race 2, ICMP number 895), 870A (race 3), 895A (race 4), 974B (race 5), 1704B (race 6, ICMP number 10222) and 2491A (race 7). The avirulence genotypes of the isolates are as follows; 299A (*Avr1, Avr3, Avr4, Avr6*[2], 202 (*Avr2*), 870A (*Avr3*), 895A (*Avr4*), 974B (*Avr2, Avr4, Avr5*[2] *Avr6*[2]), 1704B (no known avirulence genes) and 2491A (*Avr2, Avr3, Avr4*) (Taylor et al. 1989; Bevan et al. 1995). The bacterial isolates were maintained on slopes of King's medium B (KB) agar (King et al. 1954) at 4°C with monthly sub-culturing. For long-term storage, isolates grown on KB agar were suspended in a liquid medium containing nutrient broth (8 g/l) and glycerol (150 ml/l) and maintained at –80°C. For inoculation, isolates were incubated on KB agar plates for 24 h at 25°C.

Plant accessions

The pea accessions used (Table 1) were derived from single-plant selections produced in insect-free polythene structures. The recombinant inbred populations and their parental lines were obtained from the John Innes Pea Germplasm Collection. Crosses P×EO and V×HGS were made at HRI, Wellesbourne. Each F_2 family was derived from a single F_1 seed. The number of seeds in each F_2 family was small (about 30), consequently the population tested (200 plants) was made up of 20 plants from each of ten F_2 families.

Screening

Initial screens of the characters on ten plants of each accession were used to determine the uniformity of characters within an accession and variations between accessions. Morphological seed traits (Oh: basic seed-coat colour, and M: testa marbling) were recorded before sowing, and other morphological traits (A: anthocyanin production, Bt: pod tip shape, K: flower wing shape, and D: axil pigmentation) were scored as they became evident. DNA and protein (isoenzyme) extractions were made prior to bacterial inoculation.

Plants were inoculated at the most-recently fully emerged leaf node (where the stipules join the stem) according to the method of Taylor et al. (1989). Sequential inoculations in the same plant were made 7 days after the preceding inoculation, again at the most-recently emerged un-inoculated node (i.e. the next emerged node). Plants were maintained in glasshouses at 16–20°C and responses recorded 7 days after inoculation. Necrosis at the site of inoculation indicated resistance. Water-soaking spreading from the inoculation site indicated susceptibility. F_3 seed was produced from resistant plants and populations were tested to determine whether resistance(s) had been inherited in the homozygous or heterozygous state.

Two recombinant inbred populations were also screened for resistance. *Psp* race 2 was screened on five plants of each of 53 lines from the population JI281×JI399 at the F_{10} generation. Segregation of resistance to race 2 was recorded. *Psp* races 1, 2, 3 and 4 were inoculated sequentially onto five plants of each of 77 lines from the population JI15×JI399 at the F_{10} generation. Plants were scored for segregation of resistance to race 2 and for segregation

[1] ICMP – International Collection of Micro-organisms from Plants, Aukland, New Zealand

[2] Indicated genes not genetically confirmed

Table 1 Accessions of *P. sativum* used in this study, their genotypes and phenotypes

Accession	Abreviation			Morphology					Isoenzymes										
				a	*bt*	*d*	*k*	*oh*	Acp1	Acp2	Acp3	Aldo	Arg	Est2	Lap1	Pgd-p	Prx1	Prx3	Sod
Early Onward	EO	*Ppi2*		*a*	*BT*	?	*K*	?[a]	F[b]	F	S[c]	F	F	F	S	F	F	V[d]	S
Hurst's Greenshaft	HGS	*Ppi4*	*Ppi6*?	*a*	*bt*	?	*K*	?	S	S	F	F	F	S	F	S	F	F	F
Partridge	P	*Ppi3*	*Ppi4*	*A*	*BT*	*D*	*K*	*oh*	S	S	F	F	S	F	S	F	F	S	S
Vinco	V	*Ppi1*	*Ppi2*	*Ppi3*	*A*	*BT*	*D*	*K*	*oh*	S	S	F	S	F	S	F	F	S	S	F
JI15	WBH1458	*Ppi1*	*Ppi2*		A	?	D	K	?	nt[e]	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
JI281		*Ppi2*			A	Bt	D	K	Oh	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
JI399	Cennia a				?	?	D	K	?	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt

[a] Genotype unknown due to *a* background

[b] Fast isoform

[c] Slow isoform

[d] Isoenzyme pattern variable within a species

[e] Not tested

of resistance to race 1 in the absence of resistance to races 3 and 4. This latter segregation could then be attributed to the segregation of resistance gene *Ppi1*. The inoculations of isolates 870A (race 3) and 895A (race 4) were included as controls; however, neither parental accession showed resistance to either isolate (Table 1). For analysis of isoenzyme patterns, two leaflets (approximately 0.1 g of tissue) were extracted according to the method of Tanksley and Orton (1983). Extractions for peroxidase activity (expressed most strongly in root tissue and sensitive to 2-mercaptoethanol) were made from 10-mm sections of root tip in extraction buffer without 2-mercaptoethanol. Samples were run on a horizontal 11% (w/v) hydrolysed potato starch gel with a discontinuous buffer system, using an appropriate buffer (Tanksley and Orton 1983) at 300 V for 30 min at 4°C. After electrophoresis the gel was sectioned horizontally, each section being stained for a different enzyme activity according to the protocols of Tanksley and Orton (1983). Staining was stopped when bands were clearly visible. Multiple alleles were ordered according to electrophoretic mobility, with allele-1 representing further migration than allele-2 etc. Where organellar localisation of alleles was known, the lowercase letters c (cytosolic), p (plastid) or m (mitochondrial) represented this.

For analysis of RAPD banding patterns, DNA was extracted from 0.1 g of tissue per plant in 220 mM Tris/HCl pH 8.0, 800 mM NaCl, 140 mM sorbitol, 35 mM N-lauryl sarcosine, 22 mM EDTA (Na_2), and 20 mM cetyltrimethyammonium bromide. The DNA solution was extracted twice with 1:1 phenol: (23:1 chloroform : isoamyl alcohol) and once with chloroform, and the DNA precipitated. DNA was washed in twice in 70% (v/v) ethanol at 0°C and once in 100% ethanol at 0°C, dried under vacuum and re-suspended in sterile nuclease-free water. The concentration of the DNA solution was calculated from the absorbance at 260 nm against a sterile distilled water standard and adjusted to 500 ng/µl.

PCR-amplification was carried out in 25-µl reactions containing, 2-µl of 5-µM primer DNA and 1 ng of template DNA, and 2.1 mM $MgCl_2$. An amplification regime of 2 min 30 s @ 94°C, 1 min @ 36°C, 2 min @ 72°C (1 cycle), 20 s @ 94°C, 1 min @ 36°C and 2 min @ 72°C (40 cycles) in a Hybaid Omnigene thermal cycler was employed. Amplified DNA was visualised in a 2% (w/v) TAE agarose gel.

Bulked segregant analysis

F_2 individuals were identified as homozygous dominant or recessive for particular alleles on the basis of F_3 data. Pooled DNA samples representing the homozygous dominant and recessive alleles of each resistance locus were constructed from 1 µg of DNA from each of ten plants. Polymorphisms were generated by amplifying bulk DNA pools in pairs (representing resistance or susceptibility to each race of *Psp*) using a range of decameric primers (OPA-01 to OPA-20, Operon Technologies). Only bands appearing in triplicate-amplifications of one bulk and absent in the appropriate amplifications of the complementary bulk were accepted as genuine polymorphisms. Polymorphic bands were sized by comparison with a λ/*Eco*RI+*Hin*dIII ladder and scored in duplicate DNA samples from each F_2 individual.

Statistical analysis

The segregation of characters in each cross was examined using χ^2 analysis. The homogeneity of the data for each character was evaluated according to the method of Fisher (1954). In cases where the data were significantly heterogeneous, data from any family which indicated statistically significant deviation from the expected segregation ratio were eliminated from the analysis. Linkages between characters were detected using χ^2 analysis and the degree estimated using the maximum-likelihood method of Fisher (1954). The data were also analysed using the linkage-analysis programme Mapmaker/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992a, b).

Results

Uniformity of characters and marker selection in parental accessions

Resistance to races 2, 3 and 4 of *Psp*, morphological characters *oh* (basic seed coat colour), *k* (reduced flower wings), *d* (axil pigmentation), *bt* (sharp pod tip shape) and *a* (anthocyanin production), and 18 of 34 isoenzyme loci were uniform within the *Pisum* accessions tested. Ten of the isoenzyme loci and the morphological character *k* showed no variation between plant accessions.

Expression of *a* is epistatic over *d* and *oh* (Blixt et al. 1978), thus segregation in these characters can only be distinguished against an *A/–* genetic background. Where axil pigmentation could be distinguished, only the dominant phenotype (D) was observed. In *A/–* plants only brown seed-coat colour (*oh*) was detected. Since cultivars EO and HGS both exhibited the phenotype corresponding to an *a/a* genotype, however, the possibilities of *d* and *oh* segregating in crosses with these cultivars could not be excluded.

Segregation of Characters in the F_2 of crosses P×EO and V×HGS

Chi-square analysis of the segregation data is presented in Table 2. Resistance to races 2 and 3 of *Psp* segregated according to the expected 3:1 ratio in both crosses. Resistance to race 4 segregated according to the expected 3:1 ratio in the cross V×HGS, but showed a statistically significant deviation from the expected ratio in P×EO. Morphological markers *a* and *oh* (the latter distinguished only in *A/–* plants) segregated in both crosses whilst *bt* segregated only in V×HGS. Segregation of all three characters was in agreement with a 3:1 ratio. No segregation of *d* was observed in plants with pigmented flowers. The isoenzyme markers *Acp*1, *Acp*2, *Acp*3, *Aldo*, *Lap*1, *Prx*1, *Sod*, *Pgd*-p and *Prx*3 segregated in agreement with the expected 1:2:1 ratio (homozygous fast:heterozygous:homozygous slow). *Arg* and *Est*2 in V×HGS showed statistically significant deviation from the expected ratio.

Seven of the twenty primers used for RAPD amplifications in P×EO, amplified polymorphisms. Three polymorphisms were identified in bulks representing resistance and susceptibility to race 2 of *Psp*, and six in bulks representing resistance and susceptibility to race 3. Of these, only one polymorphism in each bulk pair was associated with the resistant bulk. A single polymorphism, associated with the susceptible bulk, was detected in the paired bulks representing resistance and susceptibility to race 4.

Linkage analyses

The linkage analyses are presented in Table 3. Analysis of data from the cross P×EO indicated linkage between *Ppi3* and *Ppi4*, between *Ppi2* and *Aldo* and possibly be-

Table 2 Segregation of resistance, morphological characters and isoenzymes in the crosses P×EO and V×HGS

P×EO

Resistance to	R:S[a]	Expected (3:1)	χ^2	*df*	*p*
Race 2	131 : 54	138.75 : 46.25	1.731	1	0.100–0.500
Race 3	118 : 54	129.00 : 43.00	3.752	1	0.050–0.100
Race 4	109 : 53	121.50 : 40.50	5.144	1	0.020–0.050
Isoenzyme	**S:H:F[b]**	**Expected (1:2:1)**	**χ^2**	**df**	***p***
Acp-1	43 : 101 : 47	45.25 : 90.50 : 45.25	2.834	2	0.200–0.500
Acp-2	45 : 94 : 47	46.50 : 93.00 : 46.50	0.064	2	0.950–0.980
Acp-3	33 : 99 : 55	46.75 : 93.50 : 46.75	5.823	2	0.050–0.100
Aldo	44 : 91 : 52	46.75 : 93.50 : 46.75	0.818	2	0.500–0.800
Est-2	35 : 104 : 39	44.50 : 89.00 : 44.50	5.236	2	0.050–0.100
Lap-1	41 : 104 : 42	46.75 : 93.50 : 46.75	2.362	2	0.200–0.500
Prx-1	33 : 67 : 37	34.25 : 68.50 : 34.25	0.299	2	0.800–0.900
Sod	34 : 77 : 37	37.00 : 74.00 : 37.00	0.365	2	0.800–0.900
Morphological Characters	**D : r[c]**	**Expected (3 : 1)**	**χ^2**	***df***	***p***
A	98 : 46	108.00 : 36.00	3.703	1	0.050–0.100
OH (in *A*/–)	74 : 20	70.50 : 23.50	0.695	1	0.200–0.500

V×HGS

Resistance to	R : S	Expected (3 : 1)	χ^2	*df*	*p*
Race 2	116 : 42	118.50 : 39.50	0.211	1	0.500–0.800
Race 3	159 : 38	147.75 : 49.25	3.426	1	0.050–0.100
Race 4	98 : 33	98.25 : 32.75	0.003	1	0.950–0.980
Isoenzyme	**S : H : F**	**Expected (1 : 2 : 1)**	**χ^2**	***df***	***p***
Est-2	27 : 83 : 25	33.75 : 67.50 : 33.75	7.187	2	0.020–0.050
Lap-1	34 : 102 : 39	43.75 : 87.50 : 43.75	5.019	2	0.050–0.100
Pgd-p	48 : 73 : 36	39.25 : 78.50 : 39.25	2.605	2	0.200–0.500
Prx-3	25 : 51 : 25	26.25 : 52.50 : 26.25	0.390	2	0.800–0.900
Sod	31 : 82 : 38	37.75 : 75.50 : 37.75	1.769	2	0.200–0.500
Morphological characters	**D : r**	**Expected (3 : 1)**	**χ^2**	***df***	***p***
A	149 : 41	142.50 : 47.50	1.186	1	0.200–0.500
OH (in *A*/–)	89 : 33	91.50 : 30.50	0.273	1	0.500–0.800
BT	119 : 53	29.00 : 43.00	3.101	1	0.050–0.100

[a] R:S, ratio of resistant to susceptible plants
[b] S:H:F, ratio of plants expressing slow (S), heterozygous (H) or fast (F) isoforms of an enzyme
[c] D:r, ratio of plants expressing dominant and recessive alleles of morphological characters: underlined *p* values indicate statistically significant deviation from the expected ratio

tween RAPD marker OPA-$20_{0.71}$ and *Ppi3*. A distant linkage between *Ppi4* and *a* was also indicated. Analysis of the data from the cross V×HGS indicated possible linkage between *Ppi2* and *Ppi4*, and between *Ppi3* and *Ppi4*. Multipoint analysis performed by the Mapmaker programme on the data from the cross P×EO associated *Ppi3* and *Ppi4* with each other, with the RAPD polymorphism from the race-3 resistant bulk (OPA-$20_{0.71}$), and with *a*. The programme also associated *Ppi3* and *Ppi4* with each other on the basis of the V×HGS data. Both analyses were carried out at a LOD score cut-off limit of 3.00.

Recombinant inbred (RI) mapping

A set of 77 RI lines from the cross JI15×JI399, segregating for 348 mapped loci, were screened for the segregation of *Ppi1* and *Ppi2*. A second set of 53 RI lines from the cross JI281×JI399, segregating for 421 mapped markers, were screened for the segregation of *Ppi2* only. Linkage analyses (Table 3) placed *Ppi1* on linkage group VI between *GS-cp* (the gene for the plastid form of Glutamine synthetase) and *P1* (the black hilum locus). *Ppi2* was mapped to linkage group VII near the locus for the ribosomal DNA gene *Rrn2* (Fig. 1).

Discussion

The majority of the alleles tested for in the F_2 populations segregated in agreement with the expected single-gene segregation ratios (3:1, or 1:2:1 in the case of isoenzymes). Three exceptions to these segregations were *Ppi4* in the cross P×EO and *Arg* and *Est2* in the cross

Table 3 Linkages analyses

P×EO	Loci		χ^2	*df*	*p*	LOD
	Ppi3	*Ppi4*	43.621	3	0.262	4.04
	Ppi2	*Aldo*	21.613	5	0.262	8.41
	Ppi3	$OPA20_{0.71}$	30.628	3	0.284	2.00
	Ppi4	*a*	19.605	3	0.464	8.87
V×HGS	Loci		χ^2	*df*	*p*	LOD
	Ppi3	*Ppi4*	12.259	3	0.210	2.80
	Ppi2	*Ppi4*	20.171	3	0.220	0.268

Multipoint MAPMAKER analyses

P×EO		LOD
	Ppi3–[10.6 cM]–*Ppi4*–[28.9 cM]–*a*	3.00[a]
V×HGS		
	Ppi4–[27.3 cM]–*Ppi3*	3.00[a]

Recombinant inbred population JI15×JI399

Loci		χ^2	*df*	*p*	LOD
Ppi1	*P1*	28.201	3	0.232	4.77
Ppi1	*cDNA 289/2*	8.700	3	0.228	2.36
Ppi1	*5S/3*	12.469	3	0.268	3.24
Ppi1	*5S/3a*	14.419	3	0.268	3.24
Ppi1	*GScp*	13.384	3	0.268	3.24
Ppi1	*5A/3–*	15.582	3	0.275	3.74
Ppi2	*cDNA 136*	60.121	3	0.016	17.03
Ppi2	*5B/13+*	55.367	3	0.067	14.33

[a] Minimum LOD score limit set in the analysis parameters for the Mapmaker package

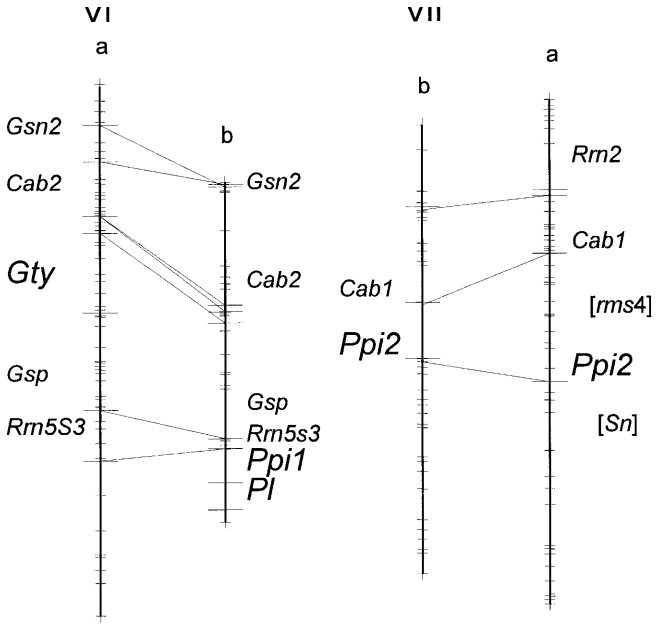

Fig. 1 Linkage mapping of *Ppi1* and *Ppi2*. Maps of pea linkage groups VI and VII, derived from the recombinant inbred populations JI281×JI399 (*a*) and JI15×JI399 (*b*) are shown. Markers in common between these two populations are connected by *thin diagonal lines*. These maps are essentially as described by Ellis et al. (1998) and Lacou et al. (1998), but with some additional data. Markers which can be taken as general reference markers are indicated by *name*. The classical markers *Gty* (gritty testa) and *P1* (black hilum) are shown, as are the approximate positions of *rms4* and *Sn* (Lacou et al. 1998)

V×HGS. In these latter cases no significant heterogeneity was detected by χ^2 analysis and no data set for any family showed significant deviation from the expected ratio. These two results were assumed to be due to the accumulation of small deviations which were not individually statistically significant. The data for *Ppi4* in P×EO did show significant heterogeneity and a single family showed significant disagreement with the expected 3:1 ratio. Elimination of the data from this family, however, still resulted in a statistically significant excess of susceptible plants.

Bevan et al. (1995) had previously reported this phenomenon for *Ppi4* in crosses involving cv Partridge and suggested that this was due to incomplete expression of the resistance in certain genetic backgrounds. This background effect may have been due to the segregation of a gene or genes that effected the expression of *Ppi4*-mediated resistance. Examples of similar phenomena reported in the literature include the tomato gene *Rcr3*, reportedly required in addition to the resistance gene *Cf-2* for resistance to downy mildew (Dixon et al. 2000), and the *Ror* genes which are reported to modify the effect of the *Mlo* locus for resistance to powdery mildew in barley (Freialdenhoven et al. 1996).

The observation of a higher than expected number of susceptible individuals in F_2 populations from crosses involving cv Partridge could be explained in two ways. Firstly, a second, dominant gene also required for resistance to *Psp* race 4 in addition to *Ppi4*; or secondly, that *Ppi4* was a weakly expressed gene, the expression of which was altered by modifying genes. In the first case, the observed data differed significantly from the expected ratio for co-dominant control of resistance (9:7); however, the data could be explained by linkage between *Ppi4* and the other component of resistance in cv Partridge. In the case of a weakly expressed dominant gene, the assumption was made that the modifying genes enhanced the expression of resistance in heterozygous plants. Thus with both *Ppi4* and the modifying gene present in cv Partridge, resistance to *Psp* race 4 would be uniform within the cultivar, as was observed. In crosses where both parents carried the modifying genes, the segregation of resistance to *Psp* race 4 would have been 3:1 (e.g. in V×HGS); whereas if the modifying genes were also segregating then there would be a segregation among the plants heterozygous for *Ppi4*, with plants where the modifier was expressed showing resistance and plants where the modifier was not expressed showing an intermediate phenotype. Mis-classification of the intermediate phenotype as susceptible would allow for the observed excess of susceptible individuals. If the modifying gene was segregating independently of *Ppi4*, the ratio of resistant to susceptible plants would be in the order of 10:6 (close to the observed segregation ratio for the cross P×EO).

Bevan et al. (1995) noted that some plant-responses to inoculation with *Psp* race 4 were difficult to score due to both water-soaking and necrosis developing at the same inoculation site. This observation would be consistent with the weak expression of resistance in heterozygous individuals in the absence of modifying genes. This observation favoured the latter scenario as an explanation in the case of *Ppi4*.

The multipoint linkage analyses of the V×HGS data calculated by the Mapmaker programme at a minimum LOD limit of 3.00 (Table 3) associated *Ppi2* with *Aldo* and provided evidence to place *Ppi2* on linkage group VII (Weeden et al. 1993). Analysis of the segregation of resistances in RI lines from two populations, (JI15×JI399 and JI281×JI399) also mapped *Ppi2* to linkage group VII, near to *Rrn*2. This reinforced the observed linkage between *Ppi2* and *Aldo* in P×EO, mapping *Ppi2* to linkage group VII.

The suggested loose association between *Ppi2* and *Ppi4* was discounted as evidence for linkage on the basis of a LOD of 2.68, and this was supported by the mapping (in two RI populations) of *Ppi2* to linkage group VII near *Rrn2*, coupled with the detection of linkage between *Ppi4* and *a* (group II) in the P×EO F_2 population. With the data for the cross P×EO the Mapmaker programme associated *Ppi3* and *Ppi4* (linked) with the anthocyanin locus *a* (linkage group II). The data for the two crosses were consistent indicating a genetic distance of between 26.2 and 27.5 cM between *Ppi3* and *Ppi4*, although one analysis (the Mapmaker analysis of the P×EO data) produced a distance of 10.6 cM between the two loci.

The fortuitous segregation of *Ppi1* in the parental lines of the RI mapping population derived from the cross JI15×JI399 (selected for segregation of *Ppi2*), permitted *Ppi1* to be mapped to a locus on linkage group VI near to *P1* (black hilum) (Fig. 1) with some degree of confidence. The segregation ratio for resistance to *Psp* race 1 in the population, however, indicated the possibility of multiple loci conferring resistance to *Psp* race 1 (isolate 299A) One component of such resistance may be located at a locus on linkage group IV. It has been noted, however, that other markers in this region, from this particular population, have a tendency to show biased segregation in the mapping population JI15×JI399 (N. Ellis personal communication).

The targeting of pea resistance genes with RAPD polymorphisms was not effective in this study. Two factors may account for this. Firstly, a number of the DNA samples selected for inclusion in the bulk pools failed to amplify individually with certain of the primers; secondly, some mis-classification of susceptible plants may have occurred where confirmation of the F_2 genotype was not possible (i.e. due to death of the plant before the production of F_3 seed). Both situations would have resulted in a reduction in specificity in the bulked DNA pools. Additionally, the number of primers tested (20) was, with hindsight, insufficient to produce a large enough population of unambiguous polymorphisms to work with. Despite these problems, a possible linkage of RAPD marker OPA-$20_{0.71}$ to *Ppi3* in coupling phase was detected, although the LOD score calculated for this predicted linkage was 2.00 (not generally accepted as sufficiently stringent for confirmation of linkage). The level of recombination detected (p=0.284) meant, however, that, even were the linkage genuine, the polymorphic locus would be too distant to be of use as a selectable marker for *Ppi3*.

This study was undertaken to provide a starting point for the molecular investigation of resistance to bacterial blight in *P. sativum* by assigning the resistance genes to previously defined linkage groups. In this respect, the conclusions drawn from the analyses of the four populations (P×EO, V×HGS, JI15×JI399 and JI281×JI399) were generally consistent. *Ppi1* (on the basis of RI data from one population only) mapped to linkage group VI, close to *P1*. *Ppi2* mapped to linkage group VII, near to *Rrn*2. The predicted linkage between *Ppi3* and *Ppi4* was also confirmed and the evidence indicated that these genes were probably located on linkage group I, with a possible linkage to *a*.

Further mapping studies using molecular markers known to be associated with linkage groups II, VI and VII in the JI populations would help to more-accurately locate *Ppi*1 and *Ppi*2, confirm the locations of *Ppi3* and *Ppi4*, and help elucidate the number of loci which may be involved in resistance to race 1 of *Psp*. Should further markers be required for more fine-scale mapping, AFLP or micro-satellite techniques, combined with multiple rounds of bulked segregant analysis, should prove the most efficient method for generating sufficient numbers of linked polymorphisms.

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