Theor Appl Genet (1993) 85:609–615

THEORETICAL AND APPLIED GENETICS

Linkage mapping of *sbm-1*, a gene conferring resistance to pea seed-borne mosaic virus, using molecular markers in *Pisum sativum*

G.M. Timmerman [1], T.J. Frew [1], A.L. Miller [1], N.F. Weeden [2], and W.A. Jermyn [1]

[1] New Zealand Institute for Crop and Food Research, Private Bag 4704, Christchurch, New Zealand
[2] Department of Horticultural Science, Cornell University, Geneva, NY 14456, USA

Received July 1, 1992; Accepted September 19, 1992
Communicated by J.S. Beckmann

Summary. The location of *sbm-1* on the *Pisum sativum* genetic map was determined by linkage analysis with eight syntenic molecular markers. Analysis of the progeny of two crosses confirmed that *sbm-1* is on chromosome 6 and permitted a more detailed map of this chromosome to be constructed. The inclusion of *Fed-1* and *Prx-3* among the markers facilitated the comparison of our map with the classical genetic map of pea. The *sbm-1* gene is most closely linked to RFLP marker GS185, being separated by a distance of about 8 cM. To determine the practical value of GS185 as a marker for *sbm-1* in plant breeding programs, the GS185 hybridization pattern and virus-resistance phenotype were compared in of a collection of breeding lines and cultivars. Three GS185 hybridization patterns were discerned among the lines. A strong association was found between one of these patterns and resistance to PSbMV.

Key words: Pea – Pea seed-borne mosaic virus – Disease resistance – Genome mapping – RFLP

Introduction

Pea seed-borne mosaic virus (PSbMV) is an economically important pathogen with worldwide distribution. In peas, symptoms of PSbMV infection vary depending on host genotype and can include downward leaf curl, plant stunting, mild leaf mosaic, reduced seed size, and "tennis ball" lesions on the seed. Infection by PSbMV can cause significant yield losses (Kraft and Hampton 1980). The virus is spread mainly through planting infected pea seed and by non-persistent aphid transmission. Three strains of PSbMV have been identified, designated P-1, L and P-4 (Alconero et al. 1986). Pathotype P-1 is the most widely distributed, and economically significant, strain affecting peas. PSbMV also causes disease in lentils (*Lens culinaris*) and broadbeans (*Vicia faba*) (Alconero et al. 1986).

Previous studies have shown that some pea germplasm carries genes for resistance to PSbMV (Provvidenti and Alconero 1988a). Four recessive genes conferring resistance to the three known strains have been identified: *sbm-1* against P-1 (Gritton and Hagedorn 1975), *sbm-2* and *sbm-3* against both L and the related L-1 variant (Provvidenti and Alconero 1988b) and *sbm-4* against P-4 (Provvidenti and Alconero 1988c). These genes, and others conferring resistance to additional members of the potyvirus group that infect peas, occur in two clusters located on chromosomes 2 and 6. The chromosome-6 cluster includes the *sbm-1*, *sbm-3* and *sbm-4* genes as well as the *cyv-2* gene for resistance to clover yellow vein virus (CYVV) (Provvidenti and Muehlbauer 1990). The chromosome-2 cluster includes *sbm-2*, *cyv* for resistance to CYVV, and *mo* for resistance to bean yellow mosaic virus, as well as genes conferring resistance to other potyviruses (Provvidenti and Alconero 1988b; Provvidenti 1990). The resistance gene-cluster containing *sbm-1* has been assigned to chromosome 6 as a result of linkage studies using a number of chromosome-6 morphological and allozyme markers including *wlo* (Gritton and Hagedorn 1975), *P* and *art-1* (Skarzynska 1988), and *Prx-3* (Weeden et al. 1991). However, a rigorous placement for *sbm-1* on the linkage map was not made as a result of these studies. This was probably due to the difficulty of developing or identifying appropriate parental lines segregating for multiple chromosome-6 morphological and allozyme markers.

To map *sbm-1*, we conducted linkage studies using DNA markers, a class of markers which has the potential

Correspondence to: G.M. Timmerman

to provide a nearly inexhaustible supply of genetic loci. Two types of DNA markers were used: RFLPs (restriction fragment length polymorphisms) and RAPDs (randomly amplified polymorphic determinants; Williams et al. 1990). Two F_2 populations and their inbred descendants segregating for resistance to PSbMV pathotype P-1 were studied. In addition, the allozyme marker *Prx-3* has been used, enabling us to relate the RFLP markers employed in this study with classical chromosome-6 markers.

The use of DNA markers simplifies the process of mapping major genes and provides the opportunity for tagging these genes with linked genetic markers that are suitable for marker-based selection in plant breeding programs. DNA markers have recently been used both to map and to provide genetic tags for major disease resistance genes in a number of crop species, such as rice, maize, soybean, lettuce and tomato (Bentolila et al. 1991; Yu et al. 1991; Diers et al. 1992; Martin et al. 1992; Paran et al. 1992). Worldwide, considerable effort is devoted to breeding pea cultivars for resistance to PSbMV. By determining the map location of *sbm-1* with respect to chromosome-6 DNA markers, we are now in the position to develop tightly linked DNA tags.

Materials and methods

Plant material

Two crosses were made to analyze the linkage between molecular markers and the *sbm-1* gene for resistance to PSbMV. The PSbMV-resistant parents chosen for both crosses were F_5 plants from DSIR Crop Research breeding line *Pisum sativum* cv 88V1.11 (W. Jermyn, unpublished). The resistance to PSbMV incorporated into 88V1.11 originated from *P. sativum* accession PI193586. *P. sativum* inbred lines 425 (DSIR Crop Research) and Almota (Crites Moscow Growers Inc.) were used as susceptible parents. The F_1 plants were allowed to self-fertilize, giving rise to 46 F_2 plants from a cross between 88V1.11 and Almota and 88 F_2 plants from a cross between 88V1.11 and 425. The virus-resistance phenotypes of the F_2 plants in the segregating populations were inferred by testing at least four F_3 plants grown from randomly chosen seed or 5–10 F_4 plants descended from four or more different F_3 plants. Some F_2 plants set fewer than four seeds, but F_3 and F_4 descendants were still tested for the virus-resistance phenotype.

A collection of pea accessions was tested for susceptibility to PSbMV, as well as for the GS185 RFLP hybridization pattern and the *Prx-3* phenotype. Ten to fifteen plants were grown from each accession for virus susceptibility testing and DNA isolation, and five were grown for allozyme analysis.

Tests for resistance to PSbMV

Pea seed-borne mosaic virus pathotype P-1 inoculum was obtained from infected. *P. sativum* plants cv Combi. The pathotype was confirmed on a series of differential pea lines (Alconero et al. 1986). Two leaves of plants at the four-leaf stage were inoculated mechanically. All plants received a second inoculation 1 week later. Plants were classified as resistant or susceptible on the basis of Western-blot analysis. Leaf tissue was sampled from young, uninoculated leaves 14 days after the second inoculation by punching out a leaf disk using the cap of a 1.5-ml microcentrifuge tube. The sample was ground in 100 µl of 2 × Sample Buffer (4% SDS, 125 mM Tris-HCl pH 6.8, 0.14 M β-mercaptoethanol and 50% glycerol) heated at 90 °C for 10 min, iced to chill and then microcentrifuged at 13,000 rpm for 2 min to pellet particulate material. Samples of 10 µl were loaded onto a 10% SDS-polyacrylamide gel (Sambrook et al. 1989) and electrophoresed. Proteins were transferred to Hybond C-extra membrane (Amersham) using an LKB semi-dry transfer apparatus and following the procedure described by Sambrook et al. (1989). The blot was developed as described by Oberfelder (1989) using polyclonal rabbit anti-PSbMV sera, purchased from G. Mink (USDA-ARS, Prosser, Wash.), and alkaline phosphatase-conjugated goat anti-rabbit sera (Bio-Rad).

RFLP analysis

Using a modification of the Murray and Thompson (1980) procedure, DNA was extracted from young unexpanded leaves either from F_2 plants or pooled from a sufficient number of F_3 plants to reconstitute the F_2 genotype. For Southern-blot analysis, approximately 20 µg of DNA was digested with 20 units of the appropriate restriction endonuclease and electrophoresed through an 0.9% agarose, 1 × TBE gel. DNA was transferred by capillary alkaline blotting (Reed and Mann 1985) to nylon membrane (Zetaprobe, Bio-Rad). Blots were prehybridized for 2–4 h at 65 °C in a buffer containing 6 × SSC, 1% SDS, 0.01% sodium pyrophosphate, 10% dextran sulphate and 300 µg/ml of denatured, sheared herring sperm DNA. Hybridizations were carried out at 65 °C overnight in the same buffer with the addition of 50–100 ng of ^{32}P probe DNA. The membranes were washed sequentially for 30 min at 65 °C in 2 × SSC, 0.1% SDS; 1 × SSC, 0.1% SDS; and 0.1 × SSC, 0.1% SDS. Autoradiography was performed by exposing the membranes to XAR-5 film (Kodak) at −70 °C with intensifying screens.

Probe DNA

The probe DNAs used for RFLP analysis were cloned cDNAs and genomic sequences. The following probes were used: pRE80 (W. Thompson, North Carolina State University, Raleigh, N.C.); GS185 and GS341 (G. Coruzzi, Rockefeller University, New York, N.Y.); pID18 (C. Martin, John Innes Institute, Norwich, United Kingdom); rpl22 (S. Gantt, University of Minnesota, Minneapolis, Minn.); and pI49 (L. Hadwiger, Washington State University, Pullman, Wash.). Insert fragments were gel-purified and labelled with α-^{32}P dCTP using the random priming method (Feinberg and Vogelstein 1983). Unincorporated dNTPs were removed by spun-column chromatography through Sephadex G50 (Sambrook et al. 1989) before the probe was denatured and added to the hybridization buffer.

RAPD analysis

Total DNA for amplification of RAPD markers was extracted from F_2 plants using a modification of the method described by Lassner et al. (1989). Two to four leaves were placed in a small plastic bag (9 × 16 cm) with 1–1.5 ml of extraction buffer, and sap was extracted using a rolling pin or wallpaper roller. The contents were transferred to a 1.5-ml microcentrifuge tube containing 300 µl of chloroform. The procedure was then continued as described by Lassner et al. (1989).

RAPD products were amplified in 25 µl reactions containing: 20–40 ng of plant DNA, PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.01% gelatin), 100 µM of each dNTP, 0.2 µM of primer, 1U of Ampli-Taq DNA polymerase (Cetus), and was capped with 1–2 drops of parafin oil.

Products were amplified for 40 cycles (94 °C 1 min, 37 °C 1 min, 72 °C 2 min) in a Perkin Elmer Cetus DNA Thermal Cycler, with the final cycle extended so that the 72 °C incubation was 10 min long. Products were analyzed by electrophoresis on gels composed of 1% agarose, 1% Nu-Sieve and 1 × TBE (Sambrook et al. 1989). After electrophoresis gels were stained with ethidium bromide, then destained and pathographed. Primer NW04 has the sequence GTTAGGTCGT.

Allozyme analysis

Prx-3 genotypes were obtained by subjecting extracts from young roots of F_4 plants to horizontal starch-gel electrophoresis (Weeden and Marx 1987). The cathodal portion of the gel was submersed in a peroxidase assay solution as described by Weeden and Marx (1987) except that four drops of 3% H_2O_2 were used per 30 ml of assay solution. The predominant peroxidase isozyme was scored as soon as it became visible (5–10 min).

Linkage analysis

Linkage analysis was performed using the MAPMAKER program, version 2.0 (Lander et al. 1987). When scoring *sbm-1* on the 88V1.11 × 425 cross, virus-resistant progeny were coded as having a homozygous 88V1.11 genotype (coded as "A") and susceptible plants as having either a heterozygous or homozygous 425 genotype (coded as "C"). The *sbm-1* scoring was different for the 88V1.11 × Almota cross because individual F_3 or F_4 progeny were tested for PSbMV resistance permitting the genotype of F_2 plants heterozygous for *sbm-1* to be inferred. Therefore, for linkage analysis on this progeny set, F_2 plants producing only resistant progeny were scored as homozygous for the 88V1.11 genotype (coded as "A"). Progeny sets which included both resistant and susceptible segregants in the F_3 or F_4 generations were scored as heterozygous (coded as "H") and the fully susceptible progenies were scored as being due to the homozygous Almota genotype in the F_2 generation (coded as "B").

In addition to *sbm-1*, the F_2 genotypes for a number of the other genetic markers were inferred by examining pooled F_3 or F_4 progeny. There were some F_2 plants which produced three of less progeny, however, resulting in an unacceptably high probability of misscoring heterozygotes as homozygotes. For genotype-scoring on these individuals, the ambiguous codes "C" or "D" were used, indicating that the genotype was "not homozygous 88V1.11" or "not homozygous 425 or Almota", respectively. The markers and number of progeny that required this handling of the data were: pRE80 (four plants); *sbm-1* (three plants); GS185 (one plant); *Prx-3* (seven plants); pI49 (seven plants); and pID18 (one plant).

Chi-squared analysis of segregation of *sbm-1* was performed using the Linkage-1 program (Suiter et al. 1983). For chi-squared analysis, the virus-resistant progeny were scored as being homozygous for the 88V1.11 genotype (AA) and susceptible progeny were scored as being either heterozygous or homozygous for the susceptible parental genotype (B_).

Results

Segregation of resistance to PSbMV pathotype P-1

Analysis of resistance among the F_3 and F_4 progeny from the cross between 88V1.11 and 425 suggested that the F_2 was composed of 61 susceptible and 25 resistant plants. Similarly, results on the F_3 and F_4 progeny of the cross between 88V1.11 and Almota indicated that the plants tested from this F_2 population consisted of 28 susceptible and ten resistant plants. These ratios are close to the 3:1 ratio expected for Mendelian segregation of homozygous dominant and recessive alleles, and this was supported by chi-squared analysis of the data. The chi-squared value for the 88V1.11 × 425 progeny was 0.759 ($P=0.38$) and for the 88V1.11 × Almota progeny was 0.035 ($P=0.85$). Examination of four F_3 progeny from each F_2 would theoretically cause us to misscore a heterozygous (susceptible) F_2 plant as resistant at a frequency of only $(1/4)^4 = 1/256$. Some F_2 lines from both crosses were omitted from the analysis, due to the small numbers of F_3 progeny (< three seeds).

Marker analysis

Six markers (pRE80, GS185, pI49, rpl22, GS341a and *Prx-3*) previously mapped to chromosome 6 (Weeden and Wolko 1990; Wolko and Weeden 1990; Gantt et al. 1991; Polans et al. 1991) were segregating in the 88V1.11 × 425 progeny. RFLP markers pRE80 and GS185 are probes for the loci *Fed-1* and *Gs-p*, respectively. In addition, two previously unmapped markers, RFLP marker pID18 and RAPD marker $NW04_{950}$, were segregating in this progeny and showed linkage to chromosome-6 markers. All of these markers except rpl22 and GS341a also segregated in the 88V1.11 × Almota progeny. These markers gave F_2 segregation ratios that did not differ significantly from the ratios expected for Mendelian markers.

Joint segregation analysis of *sbm-1* with the marker loci revealed clear linkage between *sbm-1* and markers on chromosome 6 (Table 1). Homogeneity tests were performed and indicated that the results from the two progenies could be combined. The approximate position of *sbm-1* relative to chromosome-6 markers is given in Fig. 1. RFLP marker GS185 shows closest linkage to *sbm-1* at a genetic distance of about 7.7 cM. The distances presented in Fig. 1 are the result of multipoint analysis and therefore differ slightly from the estimates presented in Table 1 which were made by pairwise comparisons. The locus order presented in Fig. 1 is the most favored one. Brackets are used to indicate regions where the favored locus order for a pair of markers is less than 1000-times more likely than the next best order (i.e., $\Delta LOD < 3.0$). For the closely linked marker pair $NWO4_{950}$ and pI49 there were two F_2 plants with recombination between these loci, permitting their order to be determined.

Survey of P. sativum accessions

To estimate the potential value of GS185 and *Prx-3* as markers for *sbm-1* in pea breeding programs, a group of *P. sativum* accessions were screened for their susceptibility to PSbMV pathotype P-1, as well as for GS185 RFLP

Table 1. Joint segregation analyses involving chromosome-6 loci

Markers	No. of F_2 progeny in each phenotypic class[a]									n	Pairwise LOD	Pairwise distance in CentiMorgans (Kosambi function)
	11,11	12,11	11,12	12,12	11,22	12,22	22,11	22,12	22,22			
sbm-1[b], RE80	30		45		10		1	17	12	115	3.69	31.9
sbm-1[b], GS185	31		52		4		1	5	28	121	19.99	7.4
sbm-1[b], *Prx-3*	23		36		2		1	8	19	89	10.27	12.8
sbm-1[b], $NWO4_{950}$[b]		80			8		15		16	119	4.96	15.4
sbm-1[b], pI49	26		52		6		1	14	16	115	6.64	17.0
RE80, GS185	16	15	14	34	2	19	1	10	11	122	4.44	35.6
GS185, *Prx-3*	21	4	5	36	0	0	0	4	22	92	24.8	6.8
$NWO4_{950}$[b], GS185	34		57		11		0	4	20	126	10.9	13.4
GS185, pI49	23	6	10	48	0	5	1	12	19	124	17.7	14.8
$NWO4_{950}$[b], *Prx-3*	24		43		6		0	2	15	90	10.17	8.4
Prx-3, pI49	16	4	9	37	0	3	1	6	13	89	12.42	14.8
$NWO4_{950}$[b], pI49	30		67		1		0	1	19	118	19.99	2.4
pI49, pID18	10	10	14	48	5	10	1	13	9	120	2.68	35.4
pI49, rpI22	5	5	9	34	3	6	1	7	8	78	2.19	32.0
pID18, rpI22	8	3	4	43	0	4	0	1	13	76	14.7	8.4
	22,22	12,22	22,12	12,12	22,11	12,11	11,22	11,12	11,11			
GS341a[c], pID18	9		25		1		0	5	3	43	2.18	16.9
GS341a[c], rpI22	11		22		0		0	3	5	41	4.40	7.3

[a] Phenotypic designations: 1, Almota or 425; 2, 88V1.11

[b] 11 and 12 cannot be distinguished because of dominance for markers *sbm-1* (susceptible) and $NWO4_{950}$

[c] 12 and 22 cannot be distinguished because of a similar hybridization pattern for marker GS341a

Table 2. Survey of *P. sativum* accessions for PSbMV pathotype P-1 susceptibility, GS185 hybridization pattern and *Prx-3* allotype

Plant line	PSbMV (P-1)[a] susceptibility	GS185[b] pattern	PRX-3 phenotype[c]	Source[d]
88V1.11	R	A	S	1
OSU442-15	R	A	S	2
OSU445-66	R	A	F	2
WISC 7105	R	A	S	3
VF-74-410-2	R	A	S	4
B636-320	R	C	S	5
X78006	R	C	F	6
X78124	R	A/B	F	6
X78125	R	A	S	6
PI193586	R	A	S	7
PI269774	R	A	F	7
PI269818	R	A	C	7
PI347464	R	A	C	7
PI347492	R	A	C	7
PI193835	R	C	S	7
PI193835	S	B	D	8
PI193836	S	B	D	7
PI314795	S	B	F	7
PI347329	S	C	S	7
PI347422	S	C	nd	7
PI347528	S	B	F	7
425	S	B	nd	1
Almota	S	B	nd	9
Rovar	S	B	nd	10
Primo	S	B	F	10
Trounce	S	B	nd	1
Whero	S	B/C	nd	1
Bohatyr	S	B/C	nd	11
Huka	S	B/C	nd	1
Apex	S	C	nd	1
Summit	S	C	nd	1
Pania	S	C	nd	1

[a] R, resistant; S, susceptible
[b] GS185 patterns: A, 11.2- and 4.7-kb fragments; B, 5.3- and 2.8-kb fragments; C, 6.2- and 2.8-kb fragments
[c] PRX-3 phenotypes: S, slow; F, fast; C and D, see Results for description; nd, not done
[d] Sources of pea lines: 1, DSIR Crop Research, Christchurch, New Zealand; 2, Baggett and Hampton 1977; 3, Hagedorn and Gritton 1971; 4, Kraft and Giles 1978; 5, G. Marx Cornell University, Geneva NY; 6, Muehlbauer 1983; 7, USDA-PGRU, Pullmann Wash.; 8, USDA-PGRU, maintained in the DSIR Crop Research collection; 9, Crites Moscow Growers Inc., Moscow Idaho; 10, Cebeco, Lelystad, The Netherlands; 11, OSEVA, Prague, Czechoslovakia

pattern and *PRX-3* phenotype (Table 2). The lines tested were known PSbMV-resistant lines, selected on the basis of data published by Provvidenti and Alconero (1988a), as well as PSbMV-susceptible lines. Our scoring of susceptibility to PSbMV pathotype P-1 was similar to that of Provvidenti and Alconero (1988a), except that PI347464 was resistant. This disparity may reflect heterogeneity in PI347464.

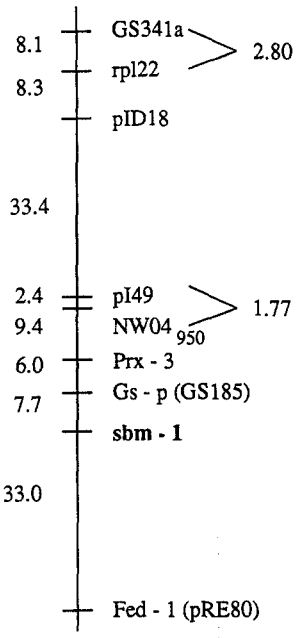

Fig. 1. Linkage map of chromosome 6 of pea showing the location of the *sbm-1* gene. Map distances, written on the left, are in centiMorgans. Pairs of linked genes that were not unambigously ordered (i.e., the ΔLOD for an alternative order is <3.0) are indicated by *brackets*. The numbers to the right of the brackets are the differences between the LOD scores for the order presented and the next most likely order. The loci *Gs-p* and *Fed-1* were examined using RFLP probes GS185 and pRE80, respectively

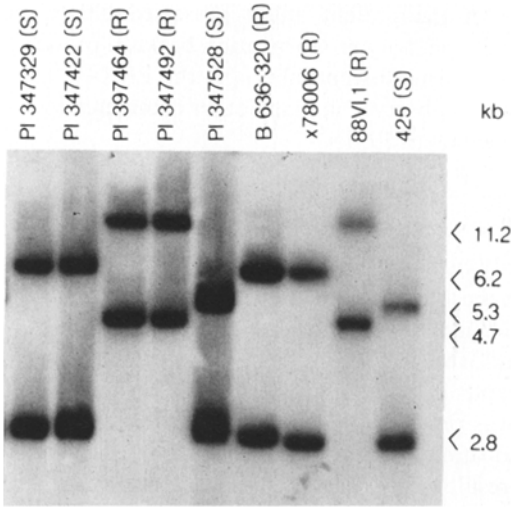

Fig. 2. GS185 hybridization patterns for resistant and susceptible *P. sativum* accessions. Total plant DNA was digested with *Eco*RV and Southern blots prepared, permitting the GS185 hybridization patterns to be discerned in the accessions listed in Table 2. The DNA samples loaded are indicated at the top of the figure. The latters in *brackets* indicate whether the ten plants tested were susceptible (*S*) or resistant (*R*) to infection by PSbMV pathotype P-1. The sizes in kilobase pairs of the labeled fragments are indicated

*Eco*RV-digested total plant DNA generated three GS185 hybridization patterns on Southern blots (Fig. 2). The "A" pattern (fragments of 11.2 kb and 4.7 kb) was most prevalent among the resistant lines and was never found among the susceptible lines examined. The "B" pattern (fragments of 5.3 kb and 2.8 kb) was most prevalent among the susceptible lines and was only detected for the resistant accession X78124, which showed both the "A" and "B" patterns. The "C" pattern (fragments of 6.2 kb and 2.8 kb) occurred among both resistant and susceptible accessions. The samples tested from accession PI193835 showed differences in susceptibility to PSbMV and also in GS185 hybridization pattern, depending on the source of the seed. Heterogeneity in this seed lot has been noted previously (Provvidenti and Alconero 1988a).

The *PRX-3* phenotype was also examined (Table 2). Four patterns were discerned among the breeding lines surveyed. In addition to the "slow" and "fast" alleles, two other *Prx-3* patterns, designated as "C" and "D", were observed among the lines tested. The "C" pattern was similar to the "fast" phenotype except that it lacked a secondary peroxidase band (PRX-4) which is present and monomorphic in most varieties and in all progeny from the two crosses. Instead, the "C" pattern contained another secondary band (PRX-5) characteristically associated with the "slow" phenotype. The "D" pattern was similar to the "slow" phenotype except that it lacked the PRX-5 band. Of the resistant lines tested, 11 of 15 displayed either the "slow" or "C" PRX-3 phenotype, whereas most of the susceptible lines tested for PRX-3 phenotype in the present study possessed either the "fast" or "D" phenotype. Correlation between pea seedborne mosaic virus susceptibility and the PRX-3 "fast" phenotype was observed in many other commercial varieties (Weeden et al. 1991).

Discussion

In this study we used RFLP, RAPD, and allozyme markers to map the location of the *sbm-1* gene on chromosome 6 of pea (Fig. 1). For three of these markers, *Prx-3* (Wolko and Weeden 1990), *Fed-1* (Polans et al. 1991) and rpl22 (Gantt et al. 1991), linkage to chromosome-6 markers was previously demonstrated, permitting us to infer the location of *sbm-1* on the classical genetic map of pea. Our results place *sbm-1* between *Prx-3* and *Fed-1* at distances of approximately 14 cM and 33 cM, respectively. Polans et al. (1991) demonstrated that *Fed-1* was separated from *Pl* (black hilum) by approximately ten recombination units and Wolko and Weeden (1990) estimated that *Prx-3* and *Pl* are separated by approximately 20 recombination units.

Molecular markers for *sbm-1* would be very valuable to plant breeders interested in developing cultivars resistant to PSbMV. Linked markers would facilitate identification of putative resistant progeny as early as the F_2 generation, without infecting these plants with PSbMV, which can cause a severe decrease in seed set in susceptible plants and result in seed-borne transmission of the virus into subsequent generations. Identification of plants heterozygous for this recessive trait could also be very valuable. Since *sbm-1* is a recessive gene, heterozygotes cannot be distinguished from homozygous susceptibles by virus-susceptibility testing, and potentially valuable heterozygous plants could be identified only by progeny-testing subsequent generations. In particular, the use of linked molecular markers for this recessive gene will accelerate a backcross breeding program because heterozygotes are identifiable. Finally, since *sbm-1* appears to be part of a cluster of virus-resistance genes (Provvidenti and Muehlbauer 1990), linked markers will also serve as markers for genes for resistance to PSbMV pathotypes L and P-4 and to clover yellow vein virus. Having mapped the location of *sbm-1* we are in a position to identify closely linked molecular markers. A potentially powerful strategy for identifiying linked RAPD markers is bulked segregant analysis (Michelmore et al. 1991).

Of the genetic markers examined in our study, the RFLP marker GS185 shows the closest linkage to *sbm-1*. Although *sbm-1* and GS185 are separated by a genetic distance of about 8 cM, the survey of PSbMV-resistant and susceptible lines supports the notion that GS185 may be a valuable marker for *sbm-1* (Table 2). The coupling between the "A" allele of GS185 and *sbm-1* is well maintained throughout much of the available resistant breeding material.

The PRX-3 allele may be less useful for screening new germplasm for the presence of the *sbm-1* gene since there are a number of exceptions to the fast = susceptible, slow = resistant correlation (Table 2). In breeding programs, however, PRX-3 may be a valuable initial screening tool because it is relatively easy to score. RFLP analysis using the GS185 probe would obviously be more predictive of an *sbm-1* genotype but may be unacceptable to many programs because RFLPs are technically more demanding and generally use radiolabels.

Acknowledgements. We thank Adrian Russell and Dave Goulden for helpful discussions and Tony Conner and Richard Pickering for critical reading of the manuscript. This work was funded in part by NRICGP/USDA grant #91-37300-6417 to N.F.W.

References

Alconero R, Provvidenti R, Gonsalves D (1986) Three pea seedborne mosaic virus pathotypes from pea and lentil germ plasm. Plant Dis 70:783–786

Baggett JR, Hampton RO (1977) Oregon B422-15 and B445-66 pea seedborne mosaic virus-resistant breeding lines. Hort Science 12:506

Bentolila S, Guitton C, Bouvet N, Nykaza S, Freyssinet G (1991) Identification of an RFLP marker tightly linked to the *Ht1* gene in maize. Theor Appl Genet 82:393–398

Diers BW, Mansur L, Imsande J, Shoemaker RC (1992) Mapping *Phytophthora* resistance loci in soybean with restriction fragment length polymorphism markers. Crop Sci 32:377–383

Feinberg A, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Gantt JS, Baldauf SL, Calie PJ, Weeden NF, Palmer JD (1991) Transfer of rpl22 to the nucleus greatly preceded its loss from the chloroplast and involved the gain of an intron. EMBO J 10:3073–3078

Gritton ET, Hagedorn DJ (1975) Linkage of the genes *sbm* and *wlo* in peas. Crop Sci 15:447–448

Hagedorn DJ, Gritton ET (1971) Registration of Wisconsin 7105 and 7106 pea germplasm. Crop Sci 11:945–946

Kraft JM, Giles RA (1978) Registration of VR74-410-2 and VR1492-1 pea germplasm. Crop Sci 18:1099

Kraft JM, Hampton RO (1980) Crop losses from pea seedborne mosaic virus in six processing pea cultivars. Plant Dis 64:922–924

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lassner MW, Peterson P, Yoder JI (1989) Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. Plant Mol Biol Rep 7:116–128

Martin GB, Williams JGK, Tanksley SD (1992) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Muehlbauer FJ (1983) Eight germplasm lines of pea resistant to pea seedborne mosaic virus. Crop Sci 23:1019

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4326

Oberfelder R (1989) Immunoblotting: comparison of detection methods. BRL Focus 11:1–5

Paran I, Kesseli R, Michelmore R (1992) Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome 34:1021–1027

Polans NO, Folta KM, Elliot RC, Thompson WF (1991) Inheritance and linkage mapping of *ferredoxin-1* in pea. J Hered 82:259–261

Provvidenti R (1990) Inheritance of resistance to pea mosaic virus in *Pisum sativum*. J Hered 81:143–145

Provvidenti R, Alconero R (1988a) Sources of resistance to pathotypes of pea seed-borne mosaic virus in the US plant introductions of *Pisum sativum*. *Pisum* Newslett 20:30–31

Provvidenti R, Alconero R (1988b) Inheritance of resistance to a lentil strain of pea seed-borne mosaic virus in *Pisum sativum*. J Hered 79:45–47

Provvidenti R, Alconero R (1988c) Inheritance of resistance to a third pathotype of pea seed-borne mosaic virus in *Pisum sativum*. J Hered 79:76–77

Provvidenti R, Muehlbauer FJ (1990) Evidence of a cluster of linked genes for resistance to pea seedborne mosaic virus and clover yellow vein virus on chromosome 6. *Pisum* Newslett 22:43–45

Reed KC, Mann DA (1985) Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res 13:7207–7221

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Cold Spring Harbor, New York

Skarzynska A (1988) Supplemental mapping data for chromosome 6. *Pisum* Newslett 20:34–36

Suiter KA, Wendel JF, Case JS (1983) LINKAGE-1: a PASCAL computer program for the detection and analysis of genetic linkage. J Hered 74:203–204

Weeden NF, Marx G (1987) Further genetic analysis and linkage relationships of isozyme loci in the pea. J Hered 78:153–159

Weeden NF, Wolko B (1990) Linkage map for the garden pea *Pisum sativum* based on molecular markers. In: Genetic maps, 5th ed (O'Brien SJ, ed). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Weeden NF, Provvidenti R, Wolko B (1991) *Prx-3* is linked to *sbm*, the gene conferring resistance to pea seedborne mosaic virus. *Pisum* Genet 23:42–43

Williams JGK, Kubelik AR, Livak KJ, Rafalski J, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wolko B, Weeden NF (1990) Additional markers for chromosome 6. *Pisum* Newslett 22:71–74

Yu ZH, Mackill DJ, Bonman JM, Tanksley SD (1991) Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor Appl Genet 81:471–476