Mapping tightly linked genes controlling potyvirus infection at the *Rsv1* and *Rpv1* region in soybean

M.A. Gore, A.J. Hayes, S.C. Jeong, Y.G. Yue, G.R. Buss, and M.A. Saghai Maroof

Abstract: *Soybean mosaic virus* (SMV) and *peanut mottle virus* (PMV) are two potyviruses that cause yield losses and reduce seed quality in infested soybean (*Glycine max* (L.) Merr.) fields throughout the world. *Rsv1* and *Rpv1* are genes that provide soybean with resistance to SMV and PMV, respectively. Isolating and characterizing *Rsv1* and *Rpv1* are instrumental in providing insight into the molecular mechanism of potyvirus recognition in soybean. A population of 1056 F_2 individuals from a cross between SMV- and PMV-resistant line PI 96983 (*Rsv1* and *Rpv1*) and the susceptible cultivar 'Lee 68' (*rsv1* and *rpv1*) was used in this study. Disease reaction and molecular-marker data were collected to determine the linkage relationship between *Rsv1*, *Rpv1*, and markers that target candidate disease-resistance genes. F_2 lines showing a recombination between two of three *Rsv1*-flanking microsatellite markers were selected for fine mapping. Over 20 RFLP, RAPD, and microsatellite markers were used to map 38 loci at high-resolution to a 6.8-cM region around *Rsv1* and *Rpv1*. This study demonstrates that *Rsv1* and *Rpv1* are tightly linked at a distance of 1.1 cM. In addition, resistance-gene candidate sequences were mapped to positions flanking and cosegregating with these resistance loci. Based on comparisons of genetic markers and disease reactions, it appears likely that several tightly linked genes are conditioning a resistance response to SMV. We discuss the specifics of these findings and investigate the utility of two disease resistance related probes for the screening of SMV or PMV resistance in soybean.

Key words: NBS, multigene family, and disease resistance.

Résumé : Le *virus de la mosaïque du soja* (SMV) et le *virus de la marbrure de l'arachide* (PMV) sont deux potyvirus qui causent des pertes de rendement et de qualité des grains dans les champs infectés à travers le monde. Les gènes *Rsv1* et *Rpv1* confèrent au soja (Glycine max (L.) Merr.) la résistance au SMV et au PMV, respectivement. Le clonage et la caractérisation des gènes *Rsv1* et *Rpv1* permettraient de commencer à élucider le mécanisme moléculaire de la reconnaissance des potyvirus chez le soja. Une population de 1 056 individus F_2 issus du croisement entre la lignée résistante PI96983 (*Rsv1* et *Rpv1*) et le cultivar sensible 'Lee 68' (*rsv1* et *rpv1*) a été employée dans ce travail. La réponse à l'infection et le génotype moléculaire ont été déterminés afin de trouver le linkage entre *Rsv1*, *Rpv1* et les marqueurs ciblant des gènes de résistance candidats. Des individus F2 montrant de la recombinaison entre deux de trois microsatellites bordant *Rsv1* ont été choisis pour réaliser une cartographie fine. Plus de 20 marqueurs RFLP, RAPD et microsatellites ont été employés afin de situer 38 locus à haute résolution au sein d'une région chromosomique de 6,8 cM autour de *Rsv1* et *Rpv1*. Cette étude montre que *Rsv1* et *Rpv1* sont étroitement liés à 1,1 cM l'un de l'autre. De plus, des séquences candidates pour des gènes de résistance ont été trouvées en bordure ou en co-ségrégation avec ces locus de résistance. En fonction de la comparaison des marqueurs génétiques et des réactions à l'infection, il semble vraisemblable que plusieurs gènes étroitement liés contribuent à la résistance au SMV. Les auteurs discutent des détails de ces résultats et examinent l'utilité de deux sondes (homologues de gènes R) pour la sélection de la résistance au SMV ou au PMV chez le soja.

Mots clés : NBS, famille multigénique, résistance aux pathogènes.

[Traduit par la Rédaction]

Received 7 September 2001. Accepted 29 January 2001. Published on the NRC Research Press Web site at http://genome.nrc.ca on 25 April 2002.

Corresponding Editor: G.J. Scoles.

M.A. Gore[1], A.J. Hayes[2], S.C. Jeong, Y.G. Yue[3], G.R. Buss, and M.A. Saghai Maroof[4]. Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, U.S.A.

[1]Present address: Department of Analytical Biochemistry, Pioneer Hi-Bred International, Inc., 7300 N.W. 62nd Ave., P.O. Box 1004, Johnston, IA 50131-1004, U.S.A.
[2]Present address: Philip Morris, U.S.A., P.O. Box 26583, Richmond, VA 23261, U.S.A.
[3]Present address: Eli Lilly and Company, BioResearch Technologies and Proteins, Drop Code 0444, Lilly Corporate Center, Indianapolis, IN 46285, U.S.A.
[4]Corresponding author (e-mail: smaroof@vt.edu).

 DOI: 10.1139/G02-009

Introduction

Plants have evolved sophisticated resistance mechanisms to counteract pathogen establishment. The gene-for-gene interaction, first described by Flor (1955), has served as a paradigm to explain many interactions between plants and bacterial, fungal, nematodal, and viral pathogens; a single resistance gene in the plant recognizes signal molecules produced by the corresponding avirulence gene of the invading pathogen, consequently evoking a cellular response that results in pathogen containment and death.

Disease-resistance genes in various plant species have been observed to exist as members of gene clusters. A cluster of disease-resistance genes may convey specific resistance to either variants (e. g., races or strains) of a single pathogen or diverse phytopathogens (Bent 1996; Crute and Pink 1996). One purported evolutionary advantage of clustering is that multiple specificities can be maintained in a single haplotype (Crute and Pink 1996). Moreover, tight linkage between cognate sequences of complex loci may enable the generation of novel specificities through gene conversion, unequal crossing over, gene duplication, and (or) birth and death processes, thus permitting co-evolution with corresponding pathogen populations (Michelmore and Meyers 1998).

Most characterized disease resistance genes encode a putative protein for the defense signal transduction cascade. Structural domain similarities shared between resistance genes have allowed their categorization into different classes. A major class of cloned disease resistance genes includes those that encode N-terminal nucleotide-binding site (NBS) and C-terminal leucine-rich repeat (LRR) domains (Bent et al. 1994). Conserved NBS-encoding sequences have been amplified from numerous plant species using degenerate PCR primers. Many of these resistance-gene candidate NBS sequences have been shown to either map near or cosegregate with resistance-gene loci (Kanazin et al. 1996; Leister et al. 1996; Yu et al. 1996*a*).

Rsv1, a *soybean mosaic virus* (SMV) resistance gene, is a constituent of the diverse disease resistance gene cluster on soybean molecular linkage group (MLG) F. Tightly linked loci of this cluster include *Rpv1* (conferring resistance to *peanut mottle virus*), *Rps3* (resistance to *Phytophthora* root rot), and *Rpg1* (resistance to bacterial blight) (Roane et al. 1983; Diers et al. 1992; Ashfield et al. 1996). Quantitative trait loci for resistance to root-knot nematode, corn earworm, and *Sclerotinia* stem rot are also present in this chromosomal region (Tamulonis et al. 1997*a*, 1997*b*; Rector et al. 1999; Arahana et al. 2001).

Our research has focused on cloning and characterizing disease-resistance genes from this economically important disease-resistance gene cluster in soybean. Yu et al. (1996*a*) isolated two classes of NBS sequence, classes b and j, that map to the resistance gene cluster on MLG F. The full-length NBS5 (class b) gene is highly homologous to the Toll–Interleukin-1 cytoplasmic receptor (TIR) subclass of NBS–LRR disease-resistance genes and maps to a single locus on MLG F (Hayes et al. 2000). Conversely, NBS61 (class j) resembles a second subclass of NBS–LRR disease-resistance genes lacking a TIR domain (non-TIR) in the transcribed product. This NBS sequence is one member of a high-copy NBS–LRR gene family mapping to MLG F (Yu et al. 1996*a*). R14, another member of this gene family, was isolated using a modified AFLP approach (Hayes and Saghai Maroof 2000). Twelve non-TIR–NBS class j members from the *Rsv1* resistant line, PI 96983, have been characterized and map to a single locus (or several tightly linked loci) flanking the *Rsv1* gene (Jeong et al. 2001). Some of these sequences may encode portions of functional resistance-gene products.

In this study, we describe the development of a high-resolution genetic map at the soybean *Rsv1* and *Rpv*1 loci. The map is saturated with disease resistance related markers that are tightly linked to *Rsv1* and *Rpv1*. Most importantly, these disease resistance related markers will facilitate cloning of *Rsv1*, *Rpv1*, and other multigene family members on linkage group F of soybean.

Materials and methods

Plant genetic materials

A soybean F_2 mapping population of 243 individuals from PI 96983 (*Rsv1*, *Rpv1*) × Lee 68 (*rsv1*, *rpv1*) was used for constructing a genetic map at the *Rsv1* chromosomal region. The number of F_2 individuals in this mapping population was extended to 1056 by incorporating a newly generated subpopulation of 813 F_2 individuals. Therefore, a total of 1056 F_2 individuals were used to develop a high-resolution map around the *Rsv1* and *Rpv1* chromosomal region.

Eleven soybean cultivars and breeding lines were used as differentials for confirming virus reaction and for additional genotyping using resistance gene specific markers. PI 96983 and 'York' (*Rsv1-y*) each have a resistance allele at both the *Rsv1* and *Rpv1* loci (Roane et al. 1983; Chen et al. 1991; Bagade 1998). L81-4420 is a near-isogenic line (NIL) of 'Williams' carrying *RSV1* and *Rpv1* derived from PI 96983. 'Buffalo' and 'Kwanggyo' (*RSV1-k*) each contain a resistance allele at the *Rsv1* locus and a PMV resistance gene at an undefined locus (Bays et al. 1986; Chen et al. 1991; Yu et. al 1994, 1996*b*; Bagade 1998). PI 507389 (*Rsv1-n*), 'Ogden' (*RSV1-t*), and 'Marshall' *(RSV1-m*) each carry an *Rsv*1 resistance allele and are susceptible to PMV-P1 (Bays et al. 1986; Chen et al. 1991; G.R. Buss, unpublished data). 'CNS' has a single, dominant PMV resistance gene (not *Rpv1*) and gives a susceptible reaction to SMV-G1 (Bagade 1998). Both 'Williams' and 'Lee 68' are susceptible to SMV and PMV.

Recombinant screen at the *Rsv1* locus

The high-resolution map is based on a genetic map containing 38 mapped marker loci around *Rsv1* on linkage group F, which was initially developed using a mapping population of 243 F_2 individuals. From this mapping experiment, we identified three microsatellite markers tightly linked to *Rsv1* and thus also linked to other members of the resistance gene cluster on MLG F (e.g., *Rpv1*, *Rpg1*, *Rps3*, etc.). Microsatellite markers Hsp176, 64-A8C, and Satt120 were employed to screen the additional 813 new F_2 individuals used to extend the original mapping population. Based on the marker data, 91 out of 813 F_2 lines carry a chromosome with a crossover between two of the three *Rsv1*-flanking markers. These 91 informative F_2 lines were used for

fine mapping the region around the *Rsv1* and *Rpv1* loci, and a high-resolution map based on the full population of 1056 F_2 individuals was constructed.

DNA extraction

Young trifoliolate leaf tissue from the 813 field-grown F_2 individuals was collected. Soybean DNA was isolated by the cetyltrimethylammonium bromide (CTAB) extraction method described by Doyle and Doyle (1987).

Two-week-old trifoliolate leaf tissue was collected from individual F_2 plants for each of the 243 individuals. Similarly, two-week-old trifoliolate leaf tissue was collected and bulked from 15–20 $F_{2:3}$ plants for each of the 91 recombinant F_2 individuals to make DNA representative of the F_2 plant for RFLP analysis. Soybean genomic DNA was extracted from powdered freeze-dried tissue with CTAB extraction buffer, adhering to the protocol described previously by Saghai Maroof et al. (1984). The DNA from the 243 F_2 and 91 $F_{2:3}$ lines was used for genotyping one RAPD, four SSRs, and 19 RFLPs.

RFLP analysis

RFLP was achieved following previously published procedures (Yu et al. 1994; Hayes et al. 2000).

RFLP probes gG and eG were derived from a genomic clone isolated from a lambda library of 'Williams 82' (susceptible to SMV and PMV) hybridized with the class j probe NBS61 (Yu et al. 1996*a*; A.J. Hayes and M.A. Saghai Maroof, unpublished data). Analysis of gG and eG determined they were both from regions flanking a class j candidate disease-resistance gene (A.J. Hayes and M.A. Saghai Maroof, unpublished data). RFLP probes T3G and gGsp were developed from genomic clones isolated from a lambda library of 'Williams NIL' L81-4420 (containing *Rsv1* and *Rpv1* from PI 96983) hybridized with gG. T3G was used as a probe to isolate an additional genomic clone from the above L81-4420 lambda library. RFLP probe 3T3G1 was developed from this clone (M.A. Saghai Maroof, unpublished data). T3G and 3T3G1 are derived from the 5′ untranslated region and gG, eG, and gGsp are derived from the 3′ untranslated region of class j candidate disease-resistance genes (M.A. Sagahi Maroof, unpublished data). Additional framework soybean RFLP clones were provided by Dr. Randy Shoemaker (Iowa State University – United States Dept. of Agriculture – Agriculture Research Service, Ames, Iowa).

Microsatellite analysis

Four microsatellite markers (Hsp176, 64-A8C, Satt510, and Satt120) were employed for high-resolution map construction. The microsatellite procedure was as described by Yu et al. (1994, 1996*b*) and Hayes et al. (2000). Drs. Perry Cregan (USDA–ARS, Beltsville, Md.) and Roger Innes (Indiana University, Bloomington, Ind.) provided primer sequences for microsatellite markers used in this study with the exception of Hsp176.

RAPD analysis

RAPD analysis was performed adhering to the protocol described previously by Williams et al. (1990). PCR amplification was performed in a 25-μL reaction containing 25 ng of $F_{2:3}$ genomic DNA; 0.2 mM primer (OPN O-11; Operon Technologies Inc., Alameda, Calif.); 200 μM each of dGTP, dTTP, dATP, and dCTP; 2.5 mM $MgCl_2$; 1× reaction buffer (20 mM Tris-HCl (pH 8.4) – 50 mM KCl), and 1.0 U *Taq* DNA polymerase (Gibco-BRL Life Technologies, Rockville, Md.). The reaction mixture was denatured at 94°C for 3 min, followed by 42 cycles of 94°C for 1 min, 37°C for 1 min, and 72°C for 2 min, with a final extension step of 72°C for 3 min. Li et al. (1998) initially demonstrated a linkage relationship between *Rsv1* and OPN O-11.

SMV and PMV assays: *Rsv1* and *Rpv1* genotyping procedures

Progeny tests were done to determine the *Rsv1* genotype for each of the 243 F_2 individuals and 91 recombinant F_2 individuals. Between 15 and 20 10-day-old seedlings from each of the 334 $F_{2:3}$ lines were inoculated with an SMV-G1 strain Virginia isolate (Hunst and Tolin 1982) as previously described by Chen et al. (1991). Similarly, progeny tests were done to determine the *Rpv1* genotype for each of the 91 recombinant F_2 individuals. For this purpose, an additional 15–20 10-day-old seedlings from each of the 91 $F_{2:3}$ lines were inoculated using PMV-P1 (Bays et al. 1986) following the procedure developed by Tolin and Ford (1983). Symptoms for SMV and PMV were recorded 7 days after inoculation. Reactions of individual plants to SMV-G1 and PMV-P1 were recorded as resistant (symptomless), susceptible (systemic mosaic or mottling symptoms), or necrotic (systemic necrosis). Three additional observations were made at one-week intervals after the initial reading. Susceptible plants inoculated with SMV-G1 displayed transient systemic vein clearing, followed by a rolling or distorting mosaic in the younger leaves. Eventually, leaves at the third or fourth node above the inoculated leaf became dark green and crinkled. The disease symptoms induced in leaves of susceptible plants by PMV-P1 ranged from systemic mild mottle to severe mottle accompanied by leaf crinkling. Systemic necrosis caused by either PMV-P1 or SMV-G1 was expressed as plants showing bud blight, systemic veinal necrosis, and (or) systemic necrotic lesions on the leaves. The growth of necrotic plants was often severely stunted, and necrotic plants sometimes died. SMV-G1 and PMV-P1 were propagated separately on the susceptible soybean cultivar Lee 68 in the greenhouse. PI 96983, 'Lee 68', and a set of soybean differentials ('York', 'Marshall', 'CNS', and PI 507389) were inoculated to confirm the effectiveness of inoculation and verify the identities of the SMV and PMV strains used in this study. F_2 lines that possessed a resistance genotype (*Rsv1* or *Rpv1*) not in concordance with their marker data or unusual disease reaction data (SMV or PMV) were retested by inoculating a further 15–20 $F_{2:3}$ progeny to confirm the resistance genotype.

Linkage analysis

The marker data generated from scoring 1 RAPD, 4 SSRs, and 19 RFLPs (24 markers detect 38 loci) on the 243 F_2 and 91 $F_{2:3}$ lines was combined with the SMV (from 243 $F_{2:3}$ and 91 $F_{2:3}$) and PMV (91 $F_{2:3}$) disease reaction data. We inferred genotypes for each of the 722 non-recombinant F_2 lines using marker loci Hsp176, 64-A8C, and Satt120. The most probable order and map distances were ascertained by

multiple linkage analysis with the computer program Mapmaker 3.0 at LOD = 3.0 and a maximum Haldane distance of 50 cM (Lander et al. 1987). We executed the genotypes command and then visually scanned the data to identify unusual marker orders.

Results and discussion

Construction of the high-resolution map

The 91 F_2 individuals having a recombination breakpoint between two of three *Rsv1* flanking markers were separately scored for their *Rsv1* and *Rpv1* genotypes (see detailed description in Materials and methods under Recombinant screen at the *Rsv1* locus). Subsequently, 38 loci detected by 24 markers were scored on the 91 recombinant $F_{2:3}$ lines. These markers include one RAPD, four SSRs, and 19 RFLPs. Twelve of the 24 markers were anchors to aid in construction of the high-resolution map. The 38 marker loci scored on the 243 F_2 and 91 recombinant $F_{2:3}$ lines were placed with respect to *Rsv1* and *Rpv1*. These two resistance genes map 1.1 cM from one another in a single mapping population of 1056 F_2 individuals (Fig. 1). In all, the 40 loci mapped to a defined chromosomal interval of 6.8 cM.

We employed NBS probes to tag members of the multigene resistance family on linkage group F that have similarities to NBS–LRR-type disease resistance genes. Seven RFLP probes used in this study are homologous to NBS-encoding sequences. NBS61, R14, NTN1, NTN5, NTN8, and Z40a belong to NBS class j (non-TIR-NBS), whereas NBS5 represents the class b TIR–NBS (for a review see Jeong et al. 2001). NTN1 detects four loci (NTN1a to NTN1d), and the remaining six NBS probes detect one locus each (Fig. 1). The seven NBS class b and j markers cluster within a 1.7-cM region around *Rsv1* and *Rpv1*, but most notably the locus NTN1d cosegregates with *Rpv1*. Also, NBS5, R14, and NTN5 cosegregate, demonstrating a tight linkage relationship between TIR–NBS and non-TIR–NBS sequences in soybean.

Five RFLP probes used for mapping are derived from genomic DNA flanking a class j resistance gene candidate. The RFLP probes gG and eG were isolated from a genomic clone of 'Williams 82', whereas gGsp, T3G, and 3T3G1 were derived from genomic clones of the NIL L81-4420 (see Materials and methods for further details). The gG and eG probes detect seven (gGa–g) and four (eGa–d) dominant polymorphic RFLP bands on a Southern blot of *Hin*dIII-digested genomic DNA from PI 96983 and 'Lee 68', respectively, that map within a 1-cM region around *Rsv*1 and *Rpv*1 (Fig. 1). Most notably, gGb, an RFLP band detected in PI 96983, cosegregates with *Rpv1*. Probe gGsp is derived from the same 3′ region as gG (M.A. Saghai Maroof, unpublished data). This probe encompasses a 500-bp insertion–deletion (indel) present in some members of the class j gene family (M.A. Saghai Maroof, unpublished data), thus detecting a subset of the bands detected by gG (See Fig. 2 legend). RFLP bands hybridized by probes gGsp and T3G cosegregate with *Rsv1*, and a single recombination event (0.1 cM) separates them from 3T3G1 (Fig. 1). All of the polymorphic bands detected by gG, eG, and gGsp were scored as dominant markers because of the ambiguity in associating allelic bands. T3G and 3T3G1 detect one locus each. The clones gG, eG, gGsp, T3G, and 3T3G1 are unique compared with most class j NBS probes, because they target class j candidate disease-resistance genes, but do not have NBS-encoding sequences. To varying degrees, these five clones do not cross-hybridize to 10–15 restriction fragments in a genomic Southern blot like class j NBS probes. Therefore, these five probes could be useful for differentiating resistance gene candidates between soybean cultivars and breeding lines.

Fig. 1. High-resolution genetic map of the chromosomal region around the *Rsv1* and *Rpv1* loci on MLG F, encoding for resistance to SMV and PMV, respectively. Thirty-eight loci detected by 24 markers were scored on 243 F_2 and 91 $F_{2:3}$ lines of the cross PI 96983 × 'Lee 68'. Map distances are given in centimorgans (cM). Fine map distances for Mapmaker have a minimum value of 0.1 cM. For this particular genetic map, a genetic distance of 0.1 cM represents a single recombination event between two loci.

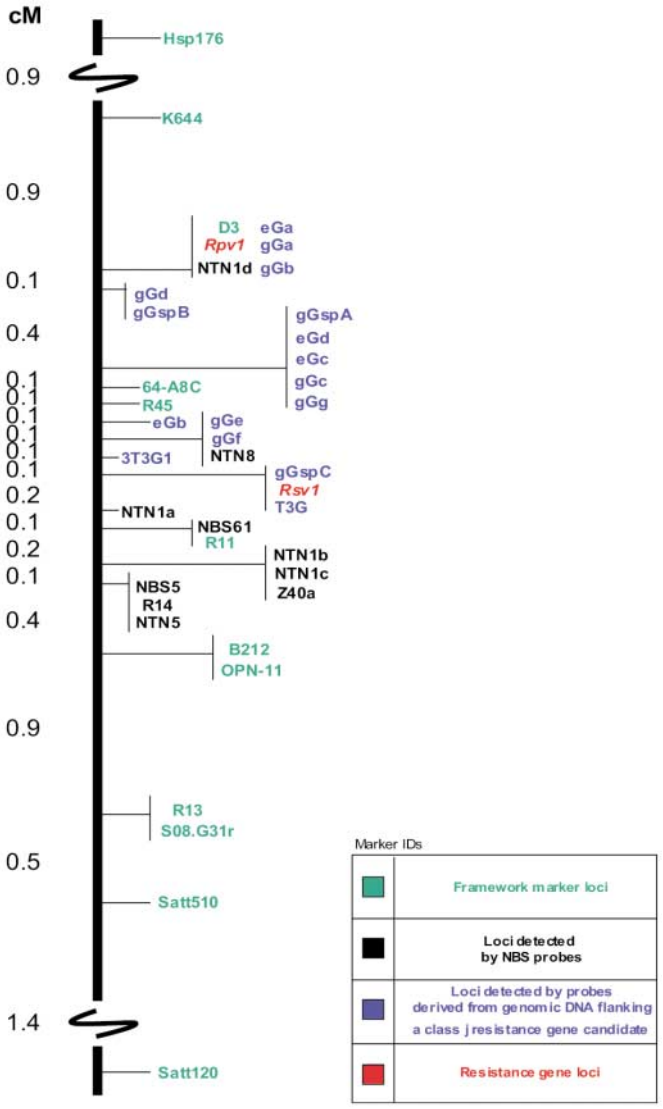


Fig. 2. Comparison of *Rsv1* and *Rpv1*-linked RFLP bands among 11 differential soybean cultivars and breeding lines. DNA size standards (MW) are indicated as kilobase pairs. Lanes 1 and 2 are 'Lee 68' and 'Williams', respectively (both susceptible to all strains of SMV and PMV). Lane 3 is L81-4420, a near-isogenic line (NIL) of 'Williams', carrying *Rsv1* and *Rpv1* from PI 96983 (lane 4). Lane 5 is 'Buffalo' (containing an allele of *Rsv1* and an uncharacterized PMV resistance gene). Lanes 6 and 7 are 'Ogden' and 'Marshall' (each carry a different allele of *Rsv1* and both are susceptible to PMV-P1). Lane 8 is PI 507389 (exhibiting a necrotic reaction to SMV-G1 conferred by an allele of *Rsv1* and is susceptible to PMV-P1). Lane 9 is 'Kwanggyo' (containing an allele of *Rsv1* and an uncharacterized PMV resistance gene). Lane 10 is 'York' (which has a resistance allele at both the *Rsv1* and *Rpv1* loci). Lane 11 is CNS (which exhibits a resistance reaction to PMV as mediated by a single, dominant PMV resistance gene, but is susceptible to SMV). (A) Autoradiograph of the *Hin*dIII-digested DNA samples separated on an agarose gel and probed with gG. (B) Autoradiograph of the same Southern blot, but hybridized with RFLP probe, gGsp. The gG and gGsp probes are derived from genomic sequences that exclusively flank class j NBS genes, and thus specifically tag a group of class j NBS resistance gene candidates on linkage group F. RFLP probe gGsp detects a subset of the bands detected by gG. Therefore, gGsp and gG hybridize an identically sized RFLP band in PI 96983 (gGspA and gGc, respectively) and 'Lee 68' (gGspB and gGd, respectively). The signal intensity of gGd, gGspB, and gGf suggest that each band is actually two comigrating *Hin*dIII restriction fragments. Further research will be required to validate this hypothesis.

Table 1. Presence (+) or absence (–) of RFLP bands detected by probes gG and gGsp in $F_{2:3}$ DNA from seven F_2 lines.

	RFLP bands detected in PI 96983						Segregation (3(R + N): 1S)[a]				
F_2 ID	gGb	gGspA[b]	gGc	gGg	gGf	gGspC	R	N	S	Total	%*N*[c]
647	+	+	+	+	–	–	7	9	10	26	35
709	–	–	–	–	+	+	30	3	4	37	8
896	–	–	–	–	+	+	24	4	5	33	12
938	+	–	–	–	–	–	5	7	4	16	44
943	+	+	+	+	+	–	4	22	10	36	61
982	+	–	–	–	–	–	5	10	7	22	46
1044	–	+	+	+	+	+	13	9	8	30	30

Note: The gG and gGsp probes detect four (gGb, gGc, gGf, and gGg) and two (gGspA and gGspC) dominant polymorphic RFLP bands on a Southern blot of *Hin*dIII-digested genomic DNA from PI 96983, respectively. These two probes target candidate disease-resistance genes that are members of class j NBS on linkage group F. SMV-G1 reaction data were collected from $F_{2:3}$ families derived from F_2 individuals. These seven F_2 lines have a high percentage of progeny developing systemic necrosis after inoculation with SMV-G1.

[a]R, resistant (symptomless); N, necrotic (systemic necrosis); S, susceptible (mosaic symptoms).

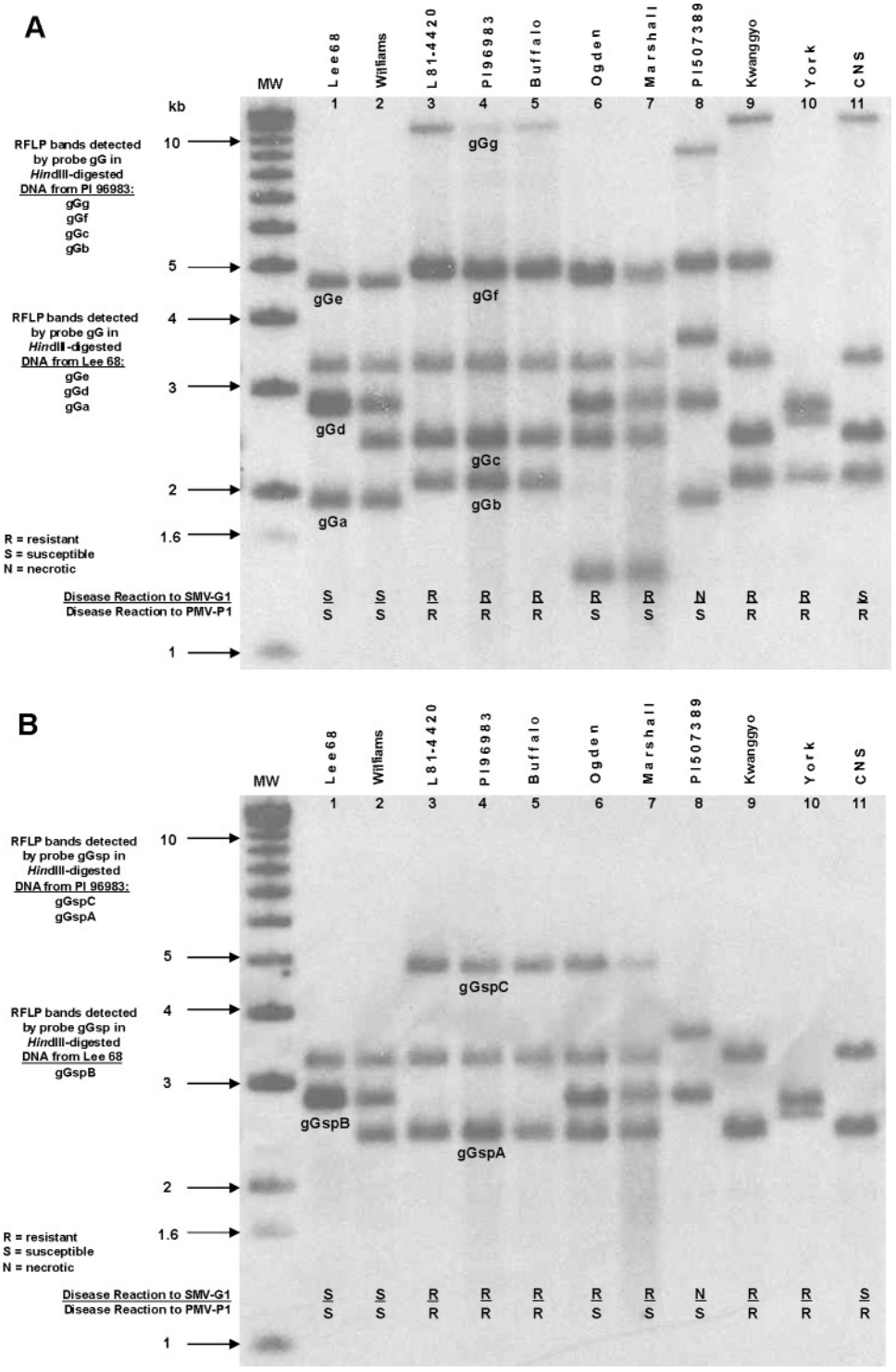
[b]RFLP probes gG and gGsp detect an identical-sized RFLP band (gGc and gGspA, respectively) in PI 96983, because gGsp detects a subset of the bands detected by gG.

[c]%*N* = (No. necrotic plants/No. total plants).

Evidence for several tightly linked genes controlling SMV infection

Seven of the 19 F_2 lines that had a recombination within the *Rsv1* chromosomal region (i. e., intrachromosomal region flanked by *Rsv1* and *Rpv1*) show an *Rsv1* genotype not in concordance with their molecular marker data. $F_{2:3}$ families derived from F_2 lines 647, 938, 943, and 982 segregated for the SMV-G1 reaction (see Table 1), indicating that they have a heterozygous genotype for an SMV resistance gene. However, according to their marker data, the four F_2 lines are homozygous susceptible at the mapped *Rsv1* locus. Similarly, F_2 lines 128, 248, and 957 should have a heterozygous genotype for *Rsv1* based on marker data, but all $F_{2:3}$ plants tested show resistance to SMV-G1. Taken together, the disease reaction and marker data from these seven F_2 lines imply that one or more tightly linked genes besides *Rsv1* conditions resistance to SMV in PI 96983. These observations contradict all previous genetic data, which indicate that a single, dominant gene mediates SMV resistance in PI 96983. However, in most previous cases the F_2 population used to characterize or map the *Rsv1* locus in PI 96983 was not large enough to rule out the possibility of other tightly linked genes controlling SMV infection. The occurrence of several tightly linked genes conferring resistance to a specific pathogen also exists in flax (*Linum usitatissimum*) (*M*), lettuce (*Lactuca sativa*) (*Dm3*), tomato (*Lycopersicon esculentum*) (*Cf-9*), and rice (*Oryza sativa) (Xa21*) (Ellis et al. 1995; Meyers et al. 1998; Parniske and Jones 1999; Song et al. 1997).

Results of studies conducted by Chen et al. (1991; 1994) suggest that in many soybean crosses of *Rsv1*-resistant and -susceptible lines, F_1s (i.e., heterozygotes) often show systemic necrosis upon viral infection. In the cross between PI 96983 and 'Lee 68', however, systemic necrosis is rarely observed in F_1s when inoculated with SMV-G1. Indeed, in most of the $F_{2:3}$ families tested in the present study, no systemic necrosis was observed in $F_{2:3}$ families segregating for SMV-G1 reaction. By contrast, a high percentage of $F_{2:3}$ plants grown from F_2 lines 647, 709, 896, 938, 943, 982, and 1044 developed systemic necrosis after inoculation with SMV-G1 (Fig. 3; Table 1). Each of these seven F_2 lines has undergone a crossover near *Rsv1* that has recombined the class j multigene NBS family (i.e., they lack an RFLP band detected in PI 96983 by probes gG and gGsp; Table 1). Presumably, recombination of this multigene resistance family has somehow disrupted the molecular mechanisms of SMV-G1 recognition and containment (i.e., hypersensitive response), allowing systemic necrosis to develop. Interestingly, there appears to be a relationship between presence or absence of specific RFLP bands detected by probes gG and gGsp in these seven F_2 lines and the percentage of their progeny developing necrosis after inoculation with SMV-G1



(Table 1). For example, F_2 lines 709 and 896 both have similar percentages of necrotic progeny and lack RFLP bands gGb, gGspA, gGc, and gGg. A comparable relationship also is seen for F_2 lines 938 and 982, each of which only possess RFLP band gGb. F_2 line 943 is the only line out of 1056 F_2 lines that possesses RFLP band gGf without gGspC and has the highest incidence (61%) of progeny showing necrosis from SMV-G1 infection. The common response of these lines suggests that both gene dosage effects and presence or absence of certain members of the multigene resistance family may alter the host–virus interaction. This lends further credence to the hypothesis that several tightly linked genes recognize and respond to SMV infection and that these genes work in concert.

Variability of resistance-gene candidates

The utility of RFLP probes gG and gGsp for the screening of SMV or PMV resistance in eleven differential soybean cultivars and breeding lines was investigated. These eleven soybean accessions were chosen because of their differential

Fig. 3. Systemic response. An $F_{2:3}$ plant derived from F_2 line 982 expressed characteristics of systemic necrosis two weeks after inoculation with SMV-G1. Necrotic lesions and veinal necrosis on trifoliolate leaves at the second node above the leaf inoculated are shown. Eventually, the necrotic trifoliolate leaves became desiccated and fell from the plant.

disease reactions to SMV and PMV (see Materials and methods for further details). RFLP probes gGsp and gG were selected for the genotypic analysis because these two low-copy probes target a group of class j NBS resistance gene candidates and hybridize to RFLP bands that cosegregate with either *Rsv1* or *Rpv1*. Both probes detect multiple RFLP bands in a Southern blot of *Hin*dIII-digested genomic DNA from each accession (Figs. 2A and 2B). gGb is only observed in accessions having PMV resistance (Fig. 2A, lanes 3, 4, 5, 9, 10, and 11) and gGspC is only detected in accessions with resistance to SMV mediated by *Rsv1* (Fig. 2B, lanes 3, 4, 5, 6, and 7). Cultivar 'York' (Fig. 2B, lane 10) appears to have a unique allele of *Rsv1*, and the source of SMV resistance in 'Kwanggyo' (Fig. 2B, lane 9) might be a class j NBS family member detected by probe gG (Fig. 2A, lane 9) and not gGsp.

Together, gGsp and gG detect 11 unique banding patterns in the nine differential soybean cultivars and breeding lines resistant to either SMV and (or) PMV. This implies that an extremely high level of polymorphism exists within the disease-resistance gene cluster on linkage group F. The apparent resistance gene candidate variability between accessions having an allele of *Rsv1* might explain why some accessions are resistant to more virulent strains of SMV (G2-G7), whereas others are necrotic or susceptible (for a review of phenotypic responses of differential soybean genotypes to SMV strains see Chen et al. 1994). However, a similarity in banding patterns exists between virus-resistant accessions and PI 96983, thus providing evidence for the tight linkage of their *Rsv1* and (or) *Rpv1* loci with bands detected by gG and gGsp. For example, resistant NIL L81-4420 has the same RFLP pattern as PI 96983 (donor parent), but a different pattern from its recurrent parent, 'Williams' (Figs. 2A and 2B, lanes 2, 3, and 4). 'Buffalo' and PI 96983 have identical banding patterns (Figs. 2A and 2B, lanes 4 and 5), indicating that they share the same linkage block around *Rsv1*; furthermore, this observation suggests that PMV resistance in 'Buffalo' is likely conferred by an allele of *Rpv1*.

The high-resolution map saturated with markers targeting resistance genes and F_2 lines that carry a chromosome with a crossover near *Rsv1* (recombinant lines) will facilitate the next phase of research concentrating on cloning *Rsv1*. We have identified resistance-gene candidates and developed recombinant lines that incorporate one or several of these gene candidates. Complementation studies of gene candidates selected using class j NBS and related genomic probes and SMV reaction studies of fixed recombinant lines should further elucidate the role of class j genes in conditioning resistance to SMV and PMV.

Acknowledgements

This study was supported in part by the USDA National Research Initiative Competitive Grants Program grant No. 96-35300-3648, the United Soybean Board, and the Virginia Soybean Board.

References

Arahana, V.S., Graef, G.L., Specht, J.E., and Steadman, J.R. 2001. Identification of QTLs for *Sclerotinia* stem rot resistance in soybean using SSR markers. Crop Sci. **41**: 180–188.

Ashfield, T., Danzer, J.R., Held, D., Clayton, K., Keim, P., Saghai Maroof, M.A., Webb, D.M., and Innes, R.W. 1996. *Rpg1*, a soybean gene effective against races of bacterial blight, maps to a cluster of previously identified disease resistance genes. Theor. Appl. Genet. **96**: 1013–1021.

Bagade, P. 1998. Genetics of resistance to peanut mottle virus in soybean. M.Sc. thesis. Virginia Polytechnic Institute and State University, Blacksburg, Va.

Bays, D.C., Tolin, S.A., and Roane, C.W. 1986. Interactions of *peanut mottle virus* strains and soybean germplasm. Phytopathology, **76**: 764–768.

Bent, A.F. 1996. Plant disease resistance genes: function meets structure. Plant Cell, **8**: 1757–1771.

Bent, A.F., Kunkel, B.N., Dahlbeck, D., Brown, K.L., Schmidt, R.L., Giraudat, J., Leung, J.L., and Staskawicz, B.J. 1994. *Rps*2 of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. Science (Washington, D.C.), **265**: 1856–1859.

Chen, P., Buss, G.R., Roane, C.W., and Tolin, S.A. 1991. Allelism among genes for resistance to soybean mosaic virus in strain-differential soybean cultivars. Crop Sci. **31**: 305–309.

Chen, P., Buss, G.R., Roane, C.W., and Tolin, S.A. 1994. Inheritance in soybean of resistant and necrotic reactions to *soybean mosaic virus* strains. Crop Sci. **34**: 414–422.

Crute, I.R., and Pink, D.A.C. 1996. Genetics and utilization of pathogen resistance in plants. Plant Cell, **8**: 1747–1755.

Diers, B.W., Mansur, L., Imsande, J., and Shoemaker, R.C. 1992. Mapping *Phytophthora* resistance loci in soybean with restriction fragment length polymorphism markers. Crop Sci. **32**: 377–383.

Doyle, J.J., and Doyle, J.V. 1987. A rapid DNA isolation procedure for small amounts of leaf tissue. Phytochem. Bull. **19**: 810–815.

Ellis, J.G., Lawrence, G.J., Finnegan, E.J., and Anderson, P.A. 1995. Contrasting complexity of two rust resistance loci in flax. Proc. Natl. Acad. Sci. U.S.A. **92**: 4185–4188.

Flor, H.H. 1955. Host–parasite interactions in flax rust—its genetics and other implications. Phytopathology, **45**: 680–685.

Hayes, A.J., and Saghai Maroof, M.A. 2000. Targeted resistance gene mapping in soybean using modified AFLPs. Theor. Appl. Genet. **100**: 1279–1283.

Hayes, A.J., Yue, Y.G., Saghai Maroof, M.A. 2000. Expression of two soybean resistance gene candidates shows divergence of paralogous single-copy genes. Theor. Appl. Genet. **101**: 789–795.

Hunst, P.L., and Tolin, S.A. 1982. Isolation and comparison of two strains of soybean mosaic virus. Phytopathology, **72**: 710–713.

Jeong, S.C., Hayes, A.J., Biyashev, R.M., and Saghai Maroof, M.A. 2001. Diversity and evolution of a non-TIR–NBS sequence family that clusters to a chromosomal "hotspot" for disease resistance genes in soybean. Theor. Appl. Genet. **103**: 406–414.

Kanazin, V., Marek, L.F., and Shoemaker, R.C. 1996. Resistance gene analogs are conserved and clustered in soybean. Proc. Natl. Acad. Sci. U.S.A. **93**: 11 746 – 11 750.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, J.M., Lincoln, S.E., and Newberg, L. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, **1**: 174–181.

Leister, D., Ballvora, A., Salamini, F., and Gebhardt, C. 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat. Genet. **14**: 421–429.

Li, Z., Bernard, R.L., and Nelson, R.L. 1998. A RAPD marker with a heterozygous form linked to the *Rsv1* locus in soybean. *In* 1998 Agronomy abstracts. ASA, Madison, Wis. p. 157.

Meyers, B.C., Chin, D.B., Shen, K.A., Sivaramakrishnan, S., Lavelle, D.O., Zhang, Z., and Michelmore, R.W. 1998. The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. Plant Cell, **10**: 1817–1832.

Michelmore, R.W., and Meyers, B.C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. **8**: 1113–1130.

Parniske, M., and Jones, J.D.G. 1999. Recombination between diverged clusters of the tomato *Cf-9* plant disease resistance gene family. Proc. Natl. Acad. Sci. U.S.A. **96**: 5850–5855.

Rector, B.G., All, J.N., Parrott, W.A., and Boerma, H.R. 1999. Quantitative trait loci for antixenosis resistance to corn earworm in soybean. Crop Sci. **39**: 531–538.

Roane, C.W., Tolin, S.A., and Buss, G.R. 1983. Inheritance of reaction to two viruses in the soybean cross 'York' × 'Lee 68'. J. Hered. **74**: 289–291.

Saghai Maroof, M.A., Soliman, K.M., Jorgensen, R.A., and Allard, R.W. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. U.S.A. **81**: 8014–8018.

Song, W.Y., Pi, L.-Y., Wang, G.-L., Gardner, J., Holsten, T., and Ronald, P.C. 1997. Evolution of the rice *Xa21* disease resistance gene family. Plant Cell, **9**: 1279–1287.

Tamulonis, J.P., Luzzi, B.M., Hussey, R.S., Parrott, W.A., and Boerma, H.R. 1997*a*. DNA markers associated with resistance to javanese root-knot nematode in soybean. Crop Sci. **37**: 783–788.

Tamulonis, J.P., Luzzi, B.M., Hussey, R.S., Parrott, W.A., and Boerma, H.R. 1997*b*. DNA marker analysis of loci conferring resistance to peanut root-knot nematode in soybean. Theor. Appl. Genet. **95**: 664–670.

Tolin, S.A., and Ford, R.H. 1983. Purification and serology of peanut mottle virus. Phytopathology, **73**: 899–903.

Williams, J.G.K., Kubelik, A.R., Kivak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531–6535.

Yu, Y.G., Saghai Maroof, M.A., Buss, G.R., Maughan, P.J., and Tolin, S.A. 1994. RFLP and microsatellite mapping of a gene for soybean mosaic virus resistance. Phytopathology, **84**: 60–64.

Yu, Y.G., Buss, G.R., and Saghai Maroof, M.A. 1996*a*. Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc. Natl. Acad. Sci. U.S.A. **93**: 11 751 – 11 756.

Yu, Y.G., Saghai Maroof, M.A., and Buss, G.R. 1996*b*. Divergence and allelomorphic relationship of a soybean virus resistance gene based on tightly linked DNA microsatellite and RFLP markers. Theor. Appl. Genet. **92**: 64–69.