Theor Appl Genet (1995) 91:907–914

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Genetic characteristics of two genes for resistance to soybean mosaic virus in PI486355 soybean

Received and accepted: 20 February 1995

Abstract Soybean [*Glycine max* (L.) Merr.] PI486355 is resistant to all the identified strains of soybean mosaic virus (SMV) and possesses two independently inherited resistance genes. To characterize the two genes, PI486355 was crossed with the susceptible cultivars 'Lee 68' and 'Essex' and with cultivars 'Ogden' and 'Marshall', which are resistant to SMV-G1 but systemically necrotic to SMV-G7. The F_2 populations and $F_{2:3}$ progenies from these crosses were inoculated with SMV-G7 in the greenhouse. The two resistance genes were separated in two $F_{3:4}$ lines, 'LR1' and 'LR2', derived from Essex × PI486355. F_1 individuals from the crosses of LR1 and LR2 with Lee 68, Ogden, and 'York' were tested with SMV-G7 in the greenhouse; the F_2 populations were tested with SMV-G1 and G7. The results revealed that expression of the gene in LR1 is gene-dosage dependent, with the homozygotes conferring resistance but the heterozygotes showing systemic necrosis to SMV-G7. This gene was shown to be an allele of the *Rsv1* locus and was designated as *Rsv1-s*. It is the only allele identified so far at the *Rsv1* locus which confers resistance to SMV-G7. *Rsv1-s* also confers resistance to SMV-G1 through G4, but results in systemic necrosis with SMV-G5 and G6. The gene in LR2 confers resistance to strains SMV-G1 through G7 and exhibits complete dominance. It appears to be epistatic to genes at the *Rsv1* locus, inhibiting the expression of the systemic necrosis conditioned by the *Rsv1* alleles. SMV-G7 induced a pin-point necrotic reaction on the inoculated primary leaves in LR1 but not in LR2. The unique genetic features of the two resistance genes from PI486355 will facilitate their proper use and identification in breeding and contribute to a better understanding of the interaction of SMV strains with soybean resistance genes.

Key words *Glycine max* · Soybean mosaic virus (SMV) · Gene-dosage dependent resistance · Epistasis · Inheritance

Communicated by G. Wenzel

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Introduction

Soybean [*Glycine max* (L.) Merr.] mosaic, caused by soybean mosaic virus (SMV), occurs worldwide and is an important soybean disease in many areas (Dunleavy 1973; Sinclair 1982). The disease can cause serious yield losses and seed-quality deterioration (Dunleavy 1973; Ross 1983; Hill et al. 1987). Cho and Goodman (1979, 1982) classified 98 isolates of SMV from seeds in the USDA soybean germplasm collections into seven strain groups (G1–G7), based on the symptoms caused in a set of soybean differential cultivars. Various and/or more-virulent SMV strains or isolates were reported in China (Xu et al. 1986) and Japan (Takahashi et al. 1980; Shigemori 1988). SMV induces three distinct systemic reactions in soybean: resistance (symptomless), necrosis, and susceptible (mosaic), depending on the virus strains and soybean genotypes (Cho and Goodman 1979, 1982).

Various sources of SMV resistance have been identified in soybean and most of them are not resistant to all of the SMV strains (Cho and Goodman 1979, 1982; Kwon and Oh 1980; Lim 1985; Gai et al. 1989). The inheritance of soybean resistance to SMV has been studied by many investigators. In most of the sources, the resistance was conferred by single dominant genes (Koshimizu and Iizuka 1963; Kiihl and Hartwig 1979; Buzzell and Tu 1984; Lim 1985; Yan and Ma 1985; Shigemori 1988; Buss et al. 1989a; Zhang et al. 1989; Chen et al. 1991; Bowers et al. 1992). Kwon and Oh (1980) crossed resistant cultivars with "susceptible" cultivars which showed necrotic bud blight to a severe

strain (SMV-N) of SMV in Korea and concluded that necrosis was dominant to resistance and conditioned by a single gene. Tu and Buzzell (1987) found that stem-tip necrosis was controlled by a single gene in the 'Columbia' cultivar which was dominant to mosaic reaction. Chen et al. (1994) reported similar results with more virulent strains of SMV and postulated that the necrosis could be the expression either of heterozygotes of resistance alleles or homozygotes of alleles which are necrotic to specific SMV strains. Koshimizu and Iizuka (1963) found the F_1 to be susceptible and the F_2 to segregate in a ratio of 7 resistant:9 susceptible in 2 of 13 resistant × susceptible crosses they studied.

Symbols have been assigned for the identified SMV resistance genes. Kiihl and Hartwig (1979) designated the dominant resistance gene in PI96983 as *Rsv* (now *Rsv1*) and the resistance gene in the Ogden cultivar as *rsvt* (changed to *Rsv1-t*, Chen et al. 1991). The single resistance genes in the cultivars York, Marshall and Kwanggyo were found to be alleles at the *Rsv1* locus and were assigned the gene symbols *Rsv1-y*, *Rsv1-m*, and *Rsv1-k*, respectively (Chen et al. 1991). These five alleles confer differential reactions to SMV strains G1 through G7. *Rsv2* was assigned to the resistance gene derived from the 'Raiden' cultivar (Buzzell and Tu 1984). A dominant gene for the stem-tip necrosis derived from the Columbia cultivar was designated as *Rsv3* (Buzzell and Tu 1989).

Shigemori (1988) found that F_2 populations segregated in the ratio 15 resistant:1 mosaic in two resistant × resistant crosses, indicating the presence of two independent resistance genes in different cultivars. Bowers et al. (1992) also found that 'Buffalo' and 'HLS' have single dominant genes for SMV resistance at different loci. No gene symbols, however, were assigned for these resistance genes because of the lack of allelism tests with the known loci.

PI486355, an accession of soybean germplasm in the USDA obtained from Korea, is resistant to strains SMV-G1 through G7 and to 23 SMV isolates from China, some of which are more virulent than G1 through G7 (Lim 1985; Xu et al. 1986). Chen et al. (1993) reported that SMV resistance in PI486355 was conferred by two independent dominant genes and one of the resistance genes appeared to be at the *Rsv1* locus. However, the two genes were not separated to determine their individual reactions to different SMV strains and therefore their genetic differences were not distinguished. In that study, plants in segregating populations were classified as resistant, necrotic, or susceptible, but necrotic plants were combined with resistant plants for genetic analysis as in other investigations with resistant × susceptible crosses (Kiihl and Hartwig 1979; Shigemori 1988; Chen et al. 1991; Bowers et al. 1992). The experiments presented in the present paper revealed the unique genetic features of the two resistance genes in PI486355 by separating them into different lines and treating resistance and systemic necrosis as separate classes for the purpose of genetic analysis.

Materials and methods

Parental materials and genetic populations

PI486355 was crossed with the SMV-susceptible cultivars 'Lee 68' and 'Essex' as well as the resistant cultivars 'Ogden' and 'Marshall' which contain *Rsv1* alleles (Chen et al. 1991). Ogden and Marshall show resistance to SMV-G1 but systemic necrosis to SMV-G7 (Cho and Goodman 1979, 1982). The F_2 populations and $F_{2:3}$ progenies were tested with SMV-G7 in the greenhouse at Blacksburg, Virginia. An average of 20 (a minimium of 12) plants for each $F_{2:3}$ progeny were inoculated. Individual plant reactions in F_2 populations and $F_{2:3}$ progenies were examined about 2 weeks and 4 weeks after inoculation and classified into three distinct types: resistant (R) (symptomless or pin-point necrotic spots only on the inoculated leaves), systemic necrotic (N) (necrotic lesions and spots on both inoculated and non-inoculated leaflets, petioles, and stems), and susceptible (S) (mosaic). The three types of plants were treated as separate classes for genetic analysis. Reaction ratios of segregating $F_{2:3}$ progenies were classified based on chi-square comparisons to the expected ratios.

$F_{2:3}$ progenies derived from Essex × PI486355 were also tested with SMV-G1 in the field at Blacksburg, Virginia. A number of individual resistant plants were selected from the progenies which appeared to be segregating for a single resistance gene and their derived $F_{3:4}$ progenies were tested with SMV-G1. Eight homogeneous resistant $F_{3:4}$ progenies which were derived from seven different $F_{2:3}$ progenies were selected. Plants from remnant seeds of those seven $F_{2:3}$ progenies were inoculated with SMV-G7 in the greenhouse. Four of them contained necrotic plants and provided good fits to a 1R:2N:1S segregation. No necrotic plants were observed in the other three $F_{2:3}$ progenies which exhibited a 3R:1S segregation. Therefore, these two groups were postulated to possess different resistance genes. One $F_{3:4}$ progeny from each group, hereafter referred to as LR1 and LR2, respectively, were selected for crossing to further characterize the two resistance genes derived from PI486355.

LR1 and LR2 were crossed with each other and with Lee 68, Ogden, and 'York'. York possesses *Rsv1-y* which gives a resistant reaction to SMV-G1 but a susceptible mosaic reactioin to SMV-G7 (Chen et al. 1991). The F_1 individuals and F_2 populations were inoculated with SMV-G7 in the greenhouse and F_2 populations were also tested with SMV-G1 in the greenhouse and/or in the field. $F_{2:3}$ progenies from Ogden × LR1 were inoculated with SMV-G1 in the field. LR1 and LR2 were tested with SMV-G1 through G7 in the greenhouse to compare their differential reactions.

Viral cultures and inoculation

The strain SMV-G1 used in this study was originally from 'Lee' soybean in Virginia and is analogous to the SMV-G1 of Cho and Goodman (1979, 1982) with respect to reactions induced on the soybean differentials (Hunst and Tolin 1982). This isolate has also been referred to as SMV-VA (Hunst and Tolin 1982). The SMV-G7 was the type isolate of its strain group (Cho and Goodman 1979) and was originally obtained from Dr. R. M. Goodman in 1984, then at the University of Illinois. Cultures of these isolates have been deposited in the American Type Culture Collection (12301 Parklawn Drive, Rockville, Maryland 20852, USA) as PV-571(SMV-G1/VA) and PV-613 (SMV-G7). SMV-G2 through G6 were also originally obtained from Dr. R. M. Goodman and were described previously by Cho and Goodman (1979).

The viral cultures used in this study were maintained by continuous passage in Lee 68 for SMV-G1 through G4, and York for SMV-G5 through G7, in the greenhouse and/or in soybean callus culture (Chen et al. 1988).

For the greenhouse tests, inocula were prepared from infected trifoliolate leaf tissue homogenized in 0.01 M sodium phosphate buffer solution, pH 7.0, at an approximate rate of 1g of infected tissue per 10 ml of buffer. Unifoliate leaves were inoculated before trifoliolate leaves emerged, approximately 10 days after planting. A small amount of 600-mesh carborundum was dusted on the leaves to be

inoculated. Inoculum was applied by rubbing both unifoliate leaves of each plant with a pestle dipped in the inoculum. Inoculated leaves were rinsed with tap water. In each batch of inoculation, the differential cultivars were also included to confirm the identity and purity of the strain used. Tests in the greenhouse were conducted from October to May. A daylength of approximately 14 h was maintained by using both fluorescent and incandescent supplemental lighting during winter months. Greenhouse temperatures ranged from 24–30 °C during daylight hours to 15–20 °C at night.

For field inoculation with SMV-G1, the procedure described by Roane et al. (1983) was used except that susceptible and systemic necrotic plants in populations from R × R crosses were tested by the dot-blotting immunoassays (Srinivasan and Tolin 1992) for detecting possible infection of alien viruses in the field.

Results and discussion

Segregation of reactions to SMV-G7 in F_2 populations and $F_{2:3}$ progenies from crosses involving PI486355

The F_2 population from PI486355 × Lee 68 showed a two-gene segregation that fits a 13R:2N:1S ratio when inoculated with SMV-G7 (Table 1). Three F_2 populations from the crosses between PI486355 and the systemic necrotic cultivars Ogden and Marshall exhibited a 13R:3N segregation and were homogeneous (Table 1). The combined data of these F_2 populations also gave a good fit to the 13R:3N ratio for two-gene segregation but did not fit a 3R:1N monogenic ratio (Table 1).

When inoculated with SMV-G7, the overall segregation of the $F_{2:3}$ progenies from PI486355 × Essex gave a satisfactory fit to ratio of 7 (all R):4 (13R:2N:1S): 2(1R:2N:1S): 2 (3R:1S): 1 (all S) (Table 2). Interestingly, in the progenies that appeared to segregate for single genes, some showed a 3R:1S ratio and others showed a 1R:2N:1S ratio (Table 2). The fact that no $F_{2:3}$ progenies had all systemic necrotic plants indicated that systemic necrosis was not the expression of any homozygous genotypes in this cross.

In the $F_{2:3}$ progenies from R × N crosses of PI486355 with Ogden and Marshall, two different patterns of single-gene segregation, 1R:3N and 3R:1N, were observed(Table 3). The overall segregation of $F_{2:3}$

Table 1 Segregation of F_2 populations from the crosses of PI486355 with susceptible-mosaic and systemic-necrotic cultivars when inoculated with SMV-G7 in the greenhouse

Cross and parents	Number of plants[a]			Expected ratio[a]	χ^2	Goodness of fit (P)
	R	N	S			
PI486355 × Lee 68	288	43	18	13R:2N:1S	0.744	0.69
PI486355	18	0	0			
Lee 68	0	0	20			
Ogden × PI486355	144	36	0	13R:3N	0.185	0.67
Ogden	0	41	0			
PI486355	61	0	0			
PI486355 × Ogden	114	31	0	13R:3N	0.645	0.42
PI486355	26	0	0			
Ogden	0	39	0			
Marshall × PI486355	153	37	0	13R:3N	0.068	>0.75
Marshall	1	30	0			
PI486355	45	0	0			
Total for F2s from 3 R × N crosses	411	104	0	13R:3N(pooled)	0.698	0.40
				(heterogeneity)	0.200	0.91
				3R:1N(pooled)	6.344	0.01

[a] R, resistant(symptomless or pin-point necrotic spots on the inoculated leaves); N, systemic necrotic; S, susceptible(mosaic)

Table 2 Reaction patterns of $F_{2:3}$ progenies from PI486355 × Essex when inoculated with SMV-G7 in the greenhouse

Reaction class[a]	Number of progenies		Total number of plants[a]			χ^2	P
	Observed	Expected	R	N	S		
All R	18	23.6	359	0	0		
13R:2N:1S	15	13.5	237	48	19	3.037	0.22
1R:2N:1S	12	6.8	65	125	55	0.919	0.63
3R:1S	5	6.8	86	0	22	1.235	0.27
All S	4	3.4	0	0	70		
Total	54	54					
χ^2 (7:4:2:2:1)		6.042					
P		0.20					

[a] R, resistant (symptomless or pin-point necrotic spots on the inoculated leaves); N, systemic necrotic; S, susceptible (mosaic)

Table 3 Reaction patterns of $F_{2:3}$ progenies from the crosses of PI486355 with Ogden and Marshall when inoculated with SMV-G7 in the greenhouse

Reaction class[a]	Number of progenies		Total number of plants[a]			χ^2	P
	Observed	Expected	R	N	S		
PI486355 × Ogden							
All R	33	33.3	671	0	0		
(13R:3N) + (3R:1N)	29	28.5	431	121	0	0.395[c]	0.53
1R:3N	11	9.5	53	163	0	0.025	>0.75
All N	3	4.8	0	55	0		
Total	76	76					
χ^2 (7:6:2:1)[b]		0.893					
P		0.83					
Marshall × PI486355							
All R	32	32.4	570	0	0		
(13R:3N) + (3R:1N)	29	27.8	393	106	0	0.049[c]	>0.75
1R:3N	7	9.3	19	86	0	2.670	0.10
All N	6	4.6	0	99	0		
Total	74	74					
χ^2 (7:6:2:1)[b]		1.192					
P		0.76					

[a] R, resistant (symptomless or pin-point necrotic spots on the inoculated leaves); N, systemic necrotic; S, susceptible (mosaic)
[b] The explanation for the expected ratio of $F_{2:3}$ progenies is shown in Fig. 2
[c] The weighted average ratio of 12 2/3 R:3 1/3 N for the 13R:3N and 3R:1N classes was used for the chi-square test since the two classes could not be distinguished from each other statistically

progenies from the two crosses fit a ratio of 7 (all R):6 [4(13R:3N) + 2(3R:1N)]:2 (1R:3N):1 (all N) (Table 3).

Genetic models explaining segregation patterns of reactions to SMV-G7 in crosses involving PI486355

Based on the above results, we proposed genetic models for the interaction of genes at two loci (Figs. 1 and 2). PI486355 possesses two resistance genes, temporarily referred to as R1 and R2 hereafter. In the R × S crosses of PI486355 with Lee 68 or Essex, the systemic necrosis is postulated to be an expression of the heterozygotes of the R1 gene in the absence of the R2 gene (*R1r1 r2r2*). The R1 gene confers resistance to SMV-G7 only in its homozygous state. The R2 gene confers completely dominant resistance; any plants possessing the R2 gene always show resistance (Fig. 1). Plants with the *R1r1 r2r2* genotype produce progenies showing 1R:2N

Fig. 1 The genetic model explaining segregation patterns and gene interactions at two loci in the R × S crosses of PI486355 with Lee 68 or Essex. PI486355: genotype *R1R1 R2R2*, phenotype resistant to SMV-G1 and G7. Lee 68 or Essex: genotype *r1r1 r2r2*, phenotype susceptible to SMV-G1 and G7

F_2 genotype[a]	Frequency	F_2 reaction to SMV-G7[b]	$F_{2:3}$ reaction to SMV-G7[b]	F_2 reaction to SMV-G1[b]
R1R1 R2R2	1	R	All R	R
R1R1 R2r2	2	R	All R	R
R1R1 r2r2	1	R	All R	R
R1r1 R2R2	2	R	All R	R
R1r1 R2r2	4	R	13R:2N:1S	R
R1r1 r2r2	2	N	1R:2N:1S	R + N
r1r1 R2R2	1	R	All R	R
r1r1 R2r2	2	R	3R:1S	R
r1r1 r2r2	1	S	All S	S
	16	13R:2N:1S	All R:7/16 13R:2N:1S:4/16 1R:2N:1S:2/16 3R:1S:2/16 All S:1/16	15(R + N):1S

[a] *r1* = *rsv1*
[b] R, resistant (symptomless or pin-point necrotic spots on the inoculated leaves); N, systemic necrotic; S, susceptible (mosaic)

Fig. 2 The genetic model explaining segregation patterns and gene interactions at two loci in the R × N crosses of PI486355 with Ogden and Marshall. PI486355: genotype *R1R1 R2R2*, phenotype resistant to SMV-G1 and G7. Ogden or Marshall: genotype *R1tR1tr2r2*, phenotype resistant to SMV-G1 but systemic necrotic to G7

F_2 genotype[a]	Frequency	F_2 reaction to SMV-G7[b]	$F_{2:3}$ reaction to SMV-G7[b]	F_2 reaction to SMV-G1[b]
R1R1 R2R2	1	R	All R	R
R1R1 R2r2	2	R	All R	R
R1R1 r2r2	1	R	All R	R
R1R1t R2R2	2	R	All R	R
R1R1t R2r2	4	R	13R:3N	R
R1R1tr2r2	2	N	1R:3N	R
R1tR1t R2R2	1	R	All R	R
R1tR1t R2r2	2	R	3R:1N	R
R1tR1tr2r2	1	N	All N	R
	16	13R:3N	All R:7/16 13R:3N:4/16 3R:1N:2/16 1R:3N:2/16 All N:1/16	All R

[a] *R1t* = *Rsv1-t* or *Rsv1-m*; *Rsv1-t* and *Rsv1-m* function similarly in response to SMV-G1 and G7

[b] R, resistant (symptomless or pin-point necrotic spots on the inoculated leaves); N, systemic necrotic; S, susceptible (mosaic)

:1S segregation, while plants with *r1r1 R2r2* produce progenies that segregate 3R:1S (Fig. 1).

In the R × N crosses of PI486355 with Ogden and Marshall, the systemic necrotic plants include the heterozygotes of the R1 gene and the homozygotes of the *Rsv1* alleles from Ogden or Marshall in the absence of the R2 gene (*R1R1t r2r2* and *R1tR1t r2r2*) (Fig. 2). In the presence of the R2 gene, plants always show resistance regardless of genotypes at the R1 locus (Fig. 2). Plants with *R1R1t r2r2* produce progenies showing the 1R:3N segregation, while the 3R:1N progenies were derived from plants with *R1tR1t R2r2* (Fig. 2). A 13R:3N ratio is expected as the result of the cosegregation of genes at two loci (Fig. 2).

Segregation of reactions to SMV-G1 of F_2 populations from the crosses of LR1 and LR2 with Lee 68, York, and Ogden

LR1 and LR2 were postulated to possess different resistance genes, R1 and R2, respectively, because they were derived from two $F_{2:3}$ progenies which exhibited different responses to SMV-G7. When tested with SMV-G1 through G7, LR2 showed resistance to all the strains. LR1 gave a resistant response to SMV-G1 through G4 and G7, but a systemic necrotic response to SMV-G5 and G6. When LR1 and LR2 were inoculated with SMV-G7 in the greenhouse, pin-point necrotic spots on the inoculated primary leaves were consistently observed on LR1 but not on LR2 which invariably remained symptomless.

To confirm the hypothesis that LR1 and LR2 possess single resistance genes at different loci, the F_2 populations were tested with SMV-G1. This is the least virulent strain of the virus and can be used for detecting resistance genes which could be defeated by more virulent strains. The F_2 population from LR1 × Lee 68 gave a 3(R + N):1S segregation to SMV-G1, whereas LR2 × Lee 68 F_2 exhibited a 3R:1S segregation without any necrotic plants (Table 4). These results show that LR1 and LR2 carry single resistance genes. When the F_2 populations from the R × R crosses of LR1 with Ogden and York were tested with SMV-G1, no susceptible segregants were observed (Table 4), indicating that the R1 gene in LR1 is allelic to *Rsv1-t* in Ogden and *Rsv1-y* in York. Since none of the reported *Rsv1* alleles shows resistance to SMV-G7, the R1 gene can be considered a new allele. A few systemic necrotic plants were observed in these F_2 populations (Table 4). They did not appear to result from genetic segregation since their frequency was very low and one necrotic plant was also observed in York. A similar phenomenon in R × R crosses involving the resistance genes at the *Rsv1* locus was discussed by Chen et al. (1991). Seventy-one $F_{2:3}$ progenies from Ogden × LR1 were further tested with SMV-G1 in the field. Of a of total 1606 plants, 16 (occurring in 11 progenies) showed systemic necrosis. Again, no susceptible plants were observed and no single progeny had all necrotic plants, which further supports the hypothesis that the R1 gene is allelic to *Rsv1*. This confirms the result of Chen et al. (1993) that one of the genes in PI486355 was at the *Rsv1* locus. They found no segregation in crosses of PI486355 with Ogden, Marshall, and York when inoculated with SMV-G1. The F_2 population from Ogden × LR2 gave a good fit to a two-gene segregation ratio of 15(R + N):1S (Table 4), indicating that the R2 gene is non-allelic to *Rsv1*.

The F_2 population from LR1 × Lee 68 inoculated with SMV-G1 produced much less than the expected 50% of necrotic plants (Table 4), suggesting that not all of the heterozygotes between the R1 gene and *rsv1* develop

Table 4 Reactions of F_2 populations from the crosses of LR1 and LR2 with Lee 68, York, and Ogden when inoculated with SMV-G1 in the greenhouse or field

Cross and parents	Number of plants[a]			Expected ratio[a]	χ^2	Goodness of fit (P)
	R	N	S			
LR1 + Lee 68 F_2	43	44	25	3(R + N):1S	0.429	0.51
LR1	7	0	0			
Lee 68	0	0	5			
LR2 × Lee 68 F_2	62	0	28	3R:1S	1.792	0.18
LR2	28	0	0			
Lee 68	0	0	26			
LR2 × Lee 68[b] F_2	89	0	27	3R:1S	0.184	0.67
LR2	24	0	0			
Lee 68	0	0	24			
York × LR1[b] F_2	147	4	0	All R		
York	29	1	0			
LR1	29	0	0			
Ogden × LR1 F_2	365	5	0	All R		
Ogden	16	0	0			
LR1	16	0	0			
Ogden × LR2 F_2	203	4	15	15(R + N):1S	0.097	>0.75
Ogden	25	0	0			
LR2	28	0	0			
Ogden × LR2[b] F_2	67	3	6	15(R + N):1S	0.351	0.55
Ogden	26	0	0			
LR2	24	0	0			

[a] R, resistant (symptomless or pin-point necrotic spots on the inoculated leaves); N, systemic necrotic; S, susceptible (mosaic)
[b] Data from field inoculations

necrosis to SMV-G1. It has also been reported in previous studies that, when inoculated with SMV-G1 or G2, the proportions of necrotic plants in segregating F_2 populations did not fit any meaningful genetic ratios, but when necrotic plants were combined with resistant plants for genetic analysis, the data provided good fits to monogenic or digenic ratios (Kiihl and Hartwig 1979; Chen et al. 1991, 1993; Bowers et al. 1992). A few necrotic plants in the F_2 population from the R × R cross of Ogden with LR2 were observed after the inoculation with SMV-G1 (Table 4). The necrosis could be the expression of plants heterozygous for *Rsv1-t* in the absence of the R2 gene, but not all such heterozygotes expressed necrosis to SMV-G1.

Reactions to SMV-G7 of F_1 individuals and F_2 populations from the crosses of LR1 and LR2 with Lee 68, York, and Ogden

When inoculated with SMV-G7, the F_1 individuals from the R × S crosses of LR1 with Lee 68 and York gave a systemic necrotic response (Table 5). Both F_2 popuplations from LR1 × S crosses fit the single-gene segregation ratio of 1R:2N:1S (Table 5). These results provide additional evidence that LR1 carries only one of the resistance genes from PI486355 and demonstrate that the R1 gene confers resistance in its homozygous state but systemic necrosis in the heterozygous state.

The F_1 individuals from LR2 × Lee 68 exhibited a resistant response and the F_2 population showed a 3R:1S single-gene segregation (Table 5). The F_1 plants and resistant F_2 plants were symptomless without any pin-point necrotic spots even on the inoculated leaves. The results show that LR2 contains a single, completely dominant resistance gene derived from PI486355.

When inoculated with SMV-G7, the F_1 individuals from the N × R cross of Ogden with LR1 showed a systemic necrotic reaction similar to the necrotic symptom induced in Ogden. The F_2 population segregated with a good fit to a 1R:3N ratio (Table 5). The lack of susceptible segregants indicates that the R1 gene is allelic to *Rsv1-t*. When the F_1 and F_2 data are considered together, it is clear that the systemic necrotic plants in the F_2 population include the homozygotes of *Rsv1-t* and the heterozygotes between *Rsv1-t* and the R1 allele. In contrast, the inoculated F_1 individuals from the N × R cross of Ogden with LR2 were completely resistant and the F_2 population segregated in a ratio of 12R:3N:1S (Table 5), which is expected from the segregation of two non-allelic genes, *Rsv1-t* and the R2 gene, according to the model in Fig. 2. The results indicate that the R2 gene is at a locus other than *Rsv1* and is epistatic to *Rsv1-t* since the R2 gene inhibited the necrotic expression of *Rsv1-t*.

When inoculated with SMV-G7, the F_2 population from LR2 × LR1 segregated in a two-gene ratio consistent with the expected 13R:2N:1S (Table 5, Fig. 1), which confirmed that LR1 and LR2 possess single resistance genes at different loci.

The expression of the necrotic reaction on the heterozygotes of the R1 gene clearly is strain-dependent. In

Table 5 Reactions of F_1 individuals and F_2 populations from the crosses of LR1 and LR2 with Lee 68, York, and Ogden when inoculated with SMV-G7 in the greenhouse

Cross and parents	Number of plants[a]			Expected ratio[a]	χ^2	Goodness of fit (P)
	R	N	S			
LR1 × Lee 68 F_1	0	5	0			
LR1 × Lee 68 F_2	46	110	54	1R:2N:1S	1.086	0.58
LR1	13	0	0			
Lee 68	0	0	13			
York × LR1 F_1	0	6	0			
York × LR1 F_2	17	41	14	1R:2N:1S	1.639	0.44
York	0	0	16			
LR1	20	0	0			
LR2 × Lee 68 F_1	13	0	0			
LR2 × Lee 68 F_2	121	0	37	3R:1S	0.211	0.65
LR2	23	0	0			
Lee 68	0	0	27			
Ogden × LR1 F_1	0	8	0			
Ogden × LR1 F_2	48	167	0	1R:3N	0.820	0.37
Ogden	0	12	0			
LR1	15	0	0			
Ogden × LR2 F_1	13	0	0			
Ogden × LR2 F_2	136	34	13	12R:3N:1S	0.240	0.89
Ogden	0	19	0			
LR2	14	0	0			
LR2 × LR1 F_2	163	20	12			
LR2	7	0	0	13R:2N:1S	0.930	0.63
LR1	8	0	0			

[a] R, resistant (symptomless or pin-point necrotic spots on the inoculated leaves); N, systemic necrotic; S, susceptible (mosaic)

contrast to the result with SMV-G1 where only a portion of the heterozygotes expressed necrosis, the heretozygotes of the R1 gene all exhibited systemic necrosis to SMV-G7. Several crosses used by Chen et al. (1993) were included in the present study. In the F_2 population from the R × S cross of PI486355 with Lee 68, the data presented by Chen et al. (1993) were a good fit to a 15 (R + N):1S ratio, but they are also a good fit to the 13R:2N:1S ratio ($\chi^2 = 0.481$, 2 *df*, $P = 0.786$) when inoculated with SMV-G7. The data of Chen et al. (1993) from the same F_2 population inoculated with SMV-G1 were not a good fit to the 13R:2N:1S ratio because there were fewer necrotic plants than expected. The data presented by Chen et al. (1993) from the F_2 population of the same cross inoculated with SMV-G6 were not a fit to the 13R:2N:1S ratio ($\chi^2 = 9.441$, 2 *df*, $P = 0.009$) but did fit a ratio of 12R:3N:1S ($\chi^2 = 2.734$, 2 *df*, $P = 0.255$) as would be expected with our current model and with the knowledge that the R1 gene conditions systemic necrosis to SMV-G6, even in its homozygous state. Separation of the R1 and R2 genes into different lines, and a characterization of their reactions to SMV strains, has allowed us to more fully understand the host-pathogen genetic interactions.

Implications and applications of the results from this investigation

In this study, one of the resistance genes (R1) derived from PI486355 has been shown to be gene-dosage dependent and allelic to *Rsv1*. The homozygotes of the R1 gene show resistance, and the heterozygotes express systemic necrosis, to SMV-G7. With SMV-G1, only a portion of heterozygotes of the R1 gene showed the systemic necrosis, as do those of other previously reported alleles at the *Rsv1* locus (Chen et al. 1991). Homozygotes of five previously reported *Rsv1* alleles are all resistant to SMV-G1 (Chen et al. 1991). However, *Rsv1-y* from York conditions a susceptible (mosaic) reaction to SMV-G7, and *Rsv1* from PI96983, *Rsv1-m* from Marshall, *Rsv1-t* from Ogden and *Rsv1-k* from 'Kwanggyo' all confer systemic necrotic reactions to SMV-G7 (Chen et al. 1994). The R1 gene is the only allele identified so far at the *Rsv1* locus which confers resistance in its homozygous state to SMV-G7. The gene symbol *Rsv1-s* is proposed for the R1 gene since it is derived from 'SS74185', the original designation of PI486355.

The other resistance gene (R2) from PI486355 is completely dominant and is epistatic to genes at the *Rsv1* locus. It appears to be a valuable source of resistance since it not only confers resistance to all the strains of SMV-1 through G7 but also none of these strains induces necrosis in the line with the R2 gene. All the identified resistance alleles at the *Rsv1* locus, including *Rsv1-s*, can confer systemic necrosis to one or more of these strains (Chen et al. 1991, 1994), which poses a risk when these resistance genes are utilized. The necrotic reaction can be much more deleterious to the plant than the mosaic symptom since necrotic plants produce virtually no seeds and eventually die (Buss et al. 1989b). An

outbreak of the necrotic disease, caused by a severe strain of SMV, was reported in Korea on cultivars that were resistant to the endemic common SMV (Know and Oh 1980).

The observed differences between the two resistance genes from PI486355 will facilitate their proper use and identification in breeding. For example, if a line derived from PI486355 shows pin-point necrosis on leaves inoculated with SMV-G7 or systemic necrosis to SMV-G5 or G6, it probably possesses only the *Rsv1-s* gene. A breeding line that possesses the R2 gene should remain symptomless after inoculation with SMV-G7. A breeder that uses backcrossing to transfer the R2 gene can inoculate F_1 plants to select those carrying the resistance gene without the risk of causing necrosis that could prevent seed set.

The revealed unique features of the two resistance genes from PI486355 and the lines with the separate resistance genes will help further research such as investigating the mechanism of SMV resistance. Gene-dosage-dependent and completely dominant resistance genes have been reported in several host plant-virus systems and were reviewed by Fraser (1986, 1990). Gene-dosage-dependent resistance is strongly associated with mechanisms that give only partial localization of the virus, such as restriction of the virus to the inoculated leaf or inhibition of overall virus multiplication, even though the virus has become systemic (Fraser1990). The pin-point necrosis on the inoculated leaves observed in the line LR1 (*Rsv1-s*) appears to be characteristic of gene-dosage-dependent resistance. We also observed a few plants of LR1 developing systemic necrotic lesions when inoculated with SMV-G7 in hot greenhouse conditions in the summer.

The R2 gene appeared to confer immunity to SMV. The mechanism of this kind of apparent immunity might involve inhibition of virus replication at a very early stage or an ineffective viral-coded movement protein that prevents the initial cell-to-cell movement of the virus (Fraser 1990; Matthews 1991).

Acknowledgements The authors appreciate the technical assistance of Lloyd Flinchum. The research was funded in part by grants from the Virginia Soybean Board.

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