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Inheritance of resistance to potyviruses in *Phaseolus vulgaris* L. II. Linkage relations and utility of a dominant gene for lethal systemic necrosis to soybean mosaic virus

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Summary. A single dominant factor, *Hss*, that conditions a rapid lethal necrotic response to soybean mosaic virus (SMV) has been identified in *Phaseolus vulgaris* L. cv. 'Black Turtle Soup', line BT-1. Inoculated plants carrying this factor developed pinpoint necrotic lesions on inoculated tissue followed by systemic vascular necrosis and plant death within about 7 days, regardless of ambient temperature. BT-1 also carries dominant resistance to potyviruses attributed to the tightly linked or identical factors, *I*, *Bcm*, *Cam*, and *Hsw*, so linkage with *Hss* was evaluated. No recombinants were identified among 381 F_3 families segregating for potyvirus susceptibility, thus if *Hss* is a distinct factor, it is tightly linked to *I*, *Bcm*, *Cam*, and *Hsw*. BT-1 was also crossed reciprocally with the line 'Great Northern 1140' ('GN 1140') in which the dominant gene, *Smv*, for systemic resistance to SMV was first identified. *Smv* and *Hss* segregated independently and are co-dominant. The ('GN 1140' × BT-1) F_1 populations showed a seasonal shift of the codominant phenotype. Evaluation of the ('GN 1140' × BT-1) F_2 population under conditions where *Smv* is partially dominant allowed additional phenotypic classes to be distinguished. Pathotype specificity has not been demonstrated for either *Smv* or *Hss*. Genotypes that are homozygous for both dominant alleles are systemically resistant to the virus and in addition show undetectable local viral replication or and no seed transmission. This work demonstrates that a gene which conditions a systemic lethal response to a pathogen may be combined with additional gene(s) to create an improved resistant phenotype.

Key words: Plant virus resistance – *Phaseolus vulgaris* – Soybean mosaic virus – *Smv-Hss-I* gene

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Introduction

Studies of the genetics of plant virus – host interactions have identified a number of individual plant genes that affect the outcome of plant viral infections. These genes may confer resistance to the virus and/or to disease symptoms caused by the virus, or may otherwise alter the reaction of a susceptible host to infection. Further work has begun to reveal relationships among plant virus resistance genes, especially among those that show similar inheritance and determine reactions to related viruses (Provvidenti 1991; Kyle and Provvidenti 1993). We have postulated that the set of viruses affected by the same or similar host genes should share the determinants involved in the resistance mechanism, although they may differ in many other ways including the types of symptoms elicited in susceptible interactions, host range, antigenic properties, etc. (Kyle and Rybicki 1991). In this paper, we describe a gene that conditions a dramatic and rapid lethal response at any temperature to one potyvirus, soybean mosaic virus (SMV), and determine its interactions with dominant potyvirus resistance attributed to *Smv* for soybean mosaic virus with *I* for bean common mosaic virus (BCMV) (Ali 1950), *Bcm* and *Cam* for blackeye cowpea and cowpea aphid-borne mosaic viruses (BICMV and CAMV) (Provvidenti et al. 1983), and *Hsw* for watermelon mosaic virus (WMV) (Kyle and Provvidenti 1987 b). We also evaluate the potential utility of this lethal reaction in the construction of a novel and improved resistant phenotype.

Presently, the potyvirus group includes at least 175 definitive and possible members, which account for approximately 35% of all known plant viruses (Ward and Shukla 1991). Both the size of the group and the extensive interrelationships among members contribute to confused and inconsistent taxonomy (e.g., Frenkel et al.

1989; Jain et al. 1992; McKern et al. 1992). Soybean mosaic virus (SMV) causes economically important losses in soybean and less frequently losses in beans planted near soybean (Clinton 1915; Gardner and Kendrick 1921). Narrow overlapping host ranges are commonly observed among potyviruses, and SMV typifies this pattern as 1 of at least 14 group members that infects the common bean (Bos 1972; Hollings and Brunt 1981). SMV is transmitted mechanically and non-persistently by a number of aphid species and through seed in both *Glycine max* and *Phaseolus vulgaris* (Costa et al. 1978; Provvidenti et al. 1982). In addition to the striking pinwheel cytoplasmic inclusions found in tissues infected by potyviruses, both scrolls and laminated aggregate inclusions are produced by SMV. Accordingly, it has been assigned to potyviral subgroup III (Christie and Edwardson 1977). Several SMV isolates have been sequenced at the amino acid or nucleotide levels (Gunyuzlu et al. 1987; Eggenberger et al. 1989; Jayaram et al. 1991; Jain et al. 1992). On the basis of high performance liquid chromatography (HPLC) profiles of coat protein, amino acid sequence of the coat protein, and nucleotide sequence of the coat protein and the 3′ untranslated regions it has been proposed that SMV comprises at least two different viruses (Jain et al. 1992). Further, despite widely differing biological properties (Bos 1972; Purcifull and Hiebert 1984), it has been proposed based on HPLC and sequence similarities that WMV should more correctly be considered an isolate of SMV and renamed SMV-WM2 (Frenkel et al. 1989; Yu et al. 1989). This paper demonstrates that host genetics can provide information relevant to this type of virological debate.

The SMV virion is a flexuous rod approximately 750 × 11 nm that consists of a monopartite single-stranded RNA of about 10,000 bases encapsidated by coat-protein molecules (Bos 1972; Eggenberger et al 1989). The genomic RNA is polyadenylated at the 3′ end and contains a genome-linked virion protein (VPg) covalently linked at the 5′ end (Hollings and Brunt 1981). The genome functions as a messenger RNA that is translated to produce a polyprotein subsequently processed to yield mature viral gene products (Vance and Beachy 1984). Potyviruses have been placed in the picornavirus-like supergroup due to marked homologies to poliovirus and related animal viruses (Goldbach and Wellink 1988). The evolutionary mechanisms that underlie genetic variation observed both within the potyvirus group and between potyviruses and other viral groups have been the subject of considerable speculation (e.g., Morozov et al. 1989).

To date, only one gene has been reported that affects the outcome of SMV infection in bean. Systemic resistance to SMV in the common bean is conditioned by an incompletely dominant gene, *Smv*, first identified in cv 'Great Northern 1140' ('GN 1140') (Provvidenti et al. 1982). Lines that are homozygous for this allele (*Smv*/*Smv*) do not develop systemic symptoms, but the virus can be detected and recovered from inoculated leaves, thus providing a reservoir of inoculum for further infection. Under field or summer greenhouse conditions, heterozygous individuals develop local chlorosis followed by systemic symptoms consisting of a mild to moderate mottle, but the plants remain vigorous and only slightly stunted (Provvidenti et al. 1982). Under winter greenhouse conditions, a shift toward full dominance of the allele may be observed. In the study presented here we show that light intensity may be one of the critical factors in this dominance shift. No pathotype specificity was observed when 23 SMV isolates representing the seven soybean pathotypes (Cho and Goodman 1979) were evaluated on *Smv*/*Smv* lines. However, some bean varieties that did not carry the *Smv* allele developed a distinct systemic necrotic response upon inoculation with SMV that differed from the typical susceptible mosaic response and from the reaction conditioned by the *Smv* allele. Pathotype specificity also was not observed for this lethal necrotic reaction (Provvidenti et al. 1982). An earlier study had also noted a necrotic response to SMV on some bean lines (Tamayo et al. 1980); however, neither study determined the genetic basis for this response.

One objective of our investigation was to determine the inheritance of lethal systemic veinal necrosis incited by SMV in the common bean. A second objective was to define the genetic relationship of this necrotic response to incompletely dominant resistance conferred by *Smv* and to dominant temperature-dependent resistance to four related potyviruses presently attributed to *I*, *Bcm*, *Cam*, and *Hsw*. When lines carrying the dominant conditional resistance are inoculated with serogroup B pathotypes of BCMV, BlCMV, CAMV, and WMV, no local or systemic infection occurs, nor is virus recovered from inoculated tissue. However, under particular conditions, such as temperatures above 32 °C, resistance breaks down and a necrotic phenotype develops that is similar to the lethal necrosis incited by SMV at any growing temperature. With BCMV pathotypes NL-3, NL-5, and NL-8, a response occurs on *I*/*I* lines that is phenotypically indistinguishable from that observed with SMV. It has been proposed based on serological and biological criteria that these isolates actually constitute a distinct virus called bean necrosis mosaic virus (McKern et al. 1992) In view of the dominant inheritance of the factor(s) that control the temperature-independent and temperature-dependent responses to potyviruses, allelism has been problematic to discern. In this paper we provide further evidence about the underlying genetic relationships between these reactions to related viral pathogens.

Materials and methods

Germplasm and genetic populations

Parental lines included BT-1, a selection from *P. vulgaris* cv 'Black Turtle Soup' that develops lethal systemic veinal necrosis upon inoculation with SMV, and a second closely related selection, BT-2, that develops typical mosaic symptoms (Provvidenti 1983). Reciprocal crosses were made between BT-1, BT-2 and 'Great Northern 1140' ('GN 1140') to produce the F_1, F_2, F_3, and backcross populations.

For evaluation of linkage between the gene that confers lethal necrosis to SMV and the gene(s) that confer temperature-dependent potyvirus resistance, the line BT-2, uniformly susceptible to the five viruses, was crossed with BT-1. (BT-2 × BT-1) F_1 individuals were randomly selected and selfed to produce the F_2 population. Selfed seed was harvested from F_2 plants generating 381 F_3 families with at least 100 seeds per family. Five sets of 15 seeds per F_3 family were planted and inoculated with either SMV or one of four potyviruses related to SMV, BCMV (Bos 1971), BlCMV (Purcifull and Gonsalves 1985), CAMV (Bock and Conti 1974), and WMV (Purcifull and Hiebert 1984). Thus, each F_3 family was evaluated independently with each of five viruses to determine whether the F_2 parent plant was homozygous dominant, heterozygous, or homozygous recessive for the reaction to the given virus. In the case of BCMV, BlCMV, CAMV, and WMV, the expression of necrosis only occurs at high temperatures after mechanical inoculation, so these tests were performed in a greenhouse held at approximately 33 °C.

For evaluation of linkage between the two genes that alter the susceptible mosaic reaction to SMV, ('GN 1140' × BT-1) populations were screened under summer greenhouse conditions in Geneva, N.Y. Plants were inoculated at the primary leaf stage, and pruned approximately 10 days later leaving only the inoculated leaves. The regrowth was evaluated daily for up to 2 weeks after pruning, at which point phenotypes were no longer changing. More limited evaluations of F_1 populations were conducted under winter greenhouse conditions, which consisted of fluorescent supplemental light and a constant temperature of about 25 °C. Growth chamber tests were conducted during the winter. Plants were germinated in the chamber, which was held at 25 °C with 12 h of full fluorescent and incandescent illumination, inoculated and held in the chamber until evaluation.

Viral cultures and inoculation

Inocula from mechanical transmissions were prepared from infected foliar tissue homogenized in 0.1 *M* K_2HPO_4 buffer pH 8.8, diluted tenfold, strained through cheesecloth, and held on ice. SMV isolate NY 76-6 (Provvidenti et al. 1982) was maintained on *Glycine max* cv 'Altona' and increased on *P. vulgaris* BT-2. The BCMV NY 15 Zaumeyer strain was obtained from M.J. Silbernagel, Prosser, Wash., and both this isolate and BCMV NY15 isolate NY 68-95 (Kyle and Provvidenti 1987a) were maintained on *P. vulgaris* cv 'California Light Red Kidney'. BlCMV-Fla and CAMV-Mor were maintained on *Vigna unguiculata* cv 'California Blackeye No. 5', and WMV isolate NY 62-76 on *P. vulgaris* BT-2. The purity of the viral cultures was monitored routinely with ELISA, immunodiffusion, host index tests, and the evaluation of characteristic symptomatology on a range of susceptible host genotypes and species. Antisera were prepared by Uyemoto et al. (1972), BCMV; Taiwo and Gonsalves (1982), BlCMV and CAMV; H.A. Scott, University of Arkansas, WMV; and J.B. Sinclair, University of Illinois, SMV.

For mechanical inoculation, plants at the primary leaf stage were dusted with 400 mesh Carborundum, rubbed with inoculum, rinsed with water, and held either in the greenhouse at 25°–28 °C with supplemental lighting or, for high temperature treatment, in a greenhouse or growth chamber at 33°–35 °C. Mock-inoculated and uninoculated controls were included routinely. When an individual plant was to be evaluated for reaction to more than one virus, a detached tissue test was used to rapidly determine genotype (Quantz 1961; Kyle and Dickson 1988). To evaluate the effect of the mode of inoculation on expression of the necrotic phenotype, BT-2 and BT-1 plants were grown in the same pot and approach-grafted by slicing and binding the stems to each other and allowing the graft to heal.

Results

Reaction of parental lines to SMV

The parental line, BT-1, developed pinpoint necrotic lesions on leaves mechanically inoculated with SMV isolate NY 76-6 within 3 or 4 days of inoculation, this was followed by systemic veinal necrosis resulting in dark streaks along petioles and stems that culminated in apical death within approximately 1 week (Table 1). A similar response was seen in BT-1 plants inoculated via approach graft to infected BT-2 plants. The parental line, BT-2, developed mosaic on inoculated leaves that moved systemically and resulted in severe mosaic, stunting, and distortion of the plant. Veinal necrosis was never observed in BT-2 plants, even when grafted to collapsed BT-1 plants, suggesting that necrosis is only expressed in

Table 1. Segregation data for lethal systemic veinal necrosis to SMV NY 76-6 in populations derived from *Phaseolus vulgaris* cvs 'BT-1' and 'BT-2'

Populations	Number of plants[a]			Expected ratio	Goodness of fit ($P_\alpha = 0.05$)
	SN	M			
BT-1	12[b]	0			
BT-2	0	14			
(BT-2 × BT-1) F_1	12	0			
(BT-1 × BT-2) F_1	14	0			
(BT-2 × BT-1) F_2	49	12		3:1	0.35
BT-2 × (BT-2 × BT-1)	23	18		1:1	0.45
BT-1 × (BT-2 × BT-1)	46	0			
	Number of F_3 families				
	SN	Segregating	M		
(BT-2 × BT-1) F_3	109	178	94	1:2:1	0.25

[a] SN, Lethal systemic veinal necrosis; M, systemic mosaic

[b] BT-1 is uniformly resistant at <30 °C to BCMV NY 15, BlCMV, CAMV, and WMV. BT-2 is uniformly susceptible to these four viruses. Resistance is inherited as a block, which cosegregated in the F_3 with the systemic necrotic reaction to SMV

Table 2. Response of *Phaseolus vulgaris* cv. 'GN 1140', BT-2 and BT-1 parental lines, and F_1 populations to inoculation with SMV NY 76-6 in the greenhouse

Populations	Genotype	Number of plants Phenotype[a]					Total
		O/R	NLL/R	NLL/SN	CLL/MM	CLL/SM	
a) December, 1989–February, 1990							
BT-1	*Hss/Hss smv/smv*	0	0	15	0	0	15
GN 1140	*hss/hss Smv/Smv*	15	0	0	0	0	15
BT-2	*hss/hss smv/smv*	0	0	0	0	14	14
(BT-2 × BT-1) F_1	*Hss/hss smv/smv*	0	0	15	0	0	15
(BT-1 × BT-2) F_1	*Hss/hss smv/smv*	0	0	14	0	0	14
(GN 1140 × BT-2) F_1	*hss/hss Smv/smv*	15	0	0	0	0	15
(BT-2 × GN 1140) F_1	*hss/hss Smv/smv*	15	0	0	0	0	15
(GN 1140 × BT-1) F_1	*Hss/hss Smv/smv*	15	0	0	0	0	15
(BT-1 × GN 1140) F_1	*Hss/hss Smv/smv*	15	0	0	0	0	15
b) July, 1990–August, 1990							
BT-1	*Hss/Hss smv/smv*	0	0	15	0	0	15
GN 1140	*hss/hss Smv/Smv*	15	0	0	0	0	15
BT-2	*hss/hss smv/smv*	0	0	0	0	15	15
(BT-2 × BT-1) F_1	*Hss/hss smv/smv*	0	0	15	0	0	15
(BT-1 × BT-2) F_1	*Hss/hss smv/smv*	0	0	15	0	0	15
(GN 1140 × BT-2) F_1	*hss/hss Smv/smv*	0	0	0	15	0	15
(BT-2 × GN 1140) F_1	*hss/hss Smv/smv*	0	0	0	15	0	15
(GN 1140 × BT-1) F_1	*Hss/hss Smv/smv*	9	6	0	0	0	15
(BT-1 × GN 1140) F_1	*Hss/hss Smv/smv*	11	4	0	0	0	15

[a] 0/R, No apparent symptoms on inoculated leaves, no systemic symptoms; NLL/R, necrotic local lesions on inoculated leaves, no systemic symptoms; NLL/SN, necrotic local lesions on inoculated leaves, systemic veinal necrosis; CLL/MM, chlorotic local lesions, mild systemic mottle; CLL/SM, chlorotic local lesion, severe systemic mottle

tissue that carries the gene contributed by BT-1 and not by a transmissible substance. This lethal necrotic response to SMV in BT-1, the expression of an extreme lethal hypersensitivity to the virus, was independent of ambient temperature. When the parental line, 'GN 1140' (*Smv/Smv*), was inoculated with SMV, virus could be recovered from symptomless inoculated leaves, but systemic movement did not occur. Veinal necrosis was never apparent on 'GN 1140' after inoculation with SMV.

Segregation of lethal hypersensitivity to SMV in F_1, F_2 and reciprocal backcross populations

The response of F_1 individuals from the cross (BT-2 × BT-1) was identical to that of the parental line BT-1, indicating complete dominance of lethal necrotic response to SMV (Table 1). No maternal effect was noted in reciprocal F_1 populations. The inoculated F_2 populations showed a segregation consistent with a 3 lethal necrotic : 1 mosaic ratio suggesting the hypothesis that lethal hypersensitivity to SMV is conferred by a single dominant gene that we have designated *Hss* for hypersensitivity to SMV (Table 1). Data from reciprocal backcross populations provided further confirmation of the hypothesis. Individuals derived from the backcross, [BT-2 × (BT-2 × BT-1)] segregated approximately 1 hypersensitive (*Hss/hss*) : 1 mosaic (*hss/hss*), while [BT-1 × (BT-2 × BT-1)] populations uniformly developed lethal veinal necrosis upon inoculation with SMV, as the two expected genotypic classes, *Hss/Hss* and *Hss/hss*, are phenotypically indistinguishable.

Linkage analysis of dominant hypersensitivity to five potyviruses

To evaluate the relationship between *Hss* and dominant conditional potyvirus resistance presently attributed to the factors *I*, *Bcm*, *Cam*, and *Hsw*, BT-1 was crossed with the closely related uniformly susceptible line BT-2 and then selfed to generate 381 families with sufficient seed. No recombinant F_3 families were identified that differed in response to one or more of the five viruses. *Hss*, *I*, *Cam*, *Bcm*, and *Hsw* segregated as a unit consistent with a 1:2:1 ratio (Table 1). These results imply identity, but do not rule out tight linkage of the genetic determinant(s) of this phenotypically similar reaction to related patho-

Table 3. Response of *Phaseolus vulgaris* ('GN 1140' and BT-1) F_2 populations to inoculation with SMV NY 76-6 in the greenhouse, July, 1990–August, 1990

Population	Phenotype[c]					Total
	O/R	NLL/R	NLL/SN	CLL/MM	CLL/SM	
	Proposed genotypes based on F_1 phenotypes					
(GN 1140 × BT-1) F_2	*hss/hss Smv/Smv*	*Hss/- Smv/-*	*Hss/- smv/smv*	*hss/hss Smv/smv*	*hss/hss smv/smv*	
Expected frequency in F_2	0.0625	0.5625	0.1875	0.125	0.0625	1.00
	Number of plants					
Observed no. F_2 plants[a]	17	22[d]	7	7	4	57
Expected no. F_2 plants	3.56	32.06	10.70	7.12	3.56	57.00
	Chi-square 3 *df*= 1.626		$P_{\alpha 0.05}=0.66$			
Observed no. F_2 plants[b]	12	17[d]	8	5	3	45
Expected no. F_2 plants	2.81	25.31	8.44	5.63	2.81	45.00
	Chi-square 3 *df*= 0.107		$P_{\alpha 0.05}=>0.99$			

[a] Plants were inoculated on primary leaves at the first true leaf stage on June 21, 1990, at Geneva, N.Y., pruned on June 29, 1990, leaving only inoculated primary leaves, and the final reading was taken on July 13, 1990

[b] Plants were inoculated on primary leaves at the first true leaf stage on July 24, 1990, at Geneva, N.Y., pruned on August 2, 1990, leaving only inoculated primary leaves, and the final reading was taken on August 13, 1990

[c] 0/R, No apparent symptoms on inoculated leaves, no systemic symptoms; NLL/R, necrotic local lesions on inoculated leaves, no systemic symptoms; NLL/SN, necrotic local lesions on inoculated leaves, systemic veinal necrosis; CLL/MM, chlorotic local lesions, mild systemic mottle; CLL/SM, chlorotic local lesions, severe systemic mottle

[d] The first two classes are combined for the chi-square statistic based on results in Table 2 showing that the genotype *Hss/hss Smv/smv* frequently fails to develop local necrotic lesions

gens. Proof of identity is not absolute via Mendelian analysis, but experiments are in progress to more precisely define the nature of the relationship between response to these five potyviruses.

Response of F_1 populations heterozygous at Smv and/or Hss

To evaluate the interaction between *Hss* and the other dominant gene in bean, *Smv*, that affects the outcome of infection with SMV, BT-1 (*Hss/Hss smv/smv*) and BT-2 (*hss/hss smv/smv*) were crossed with 'GN 1140' (*hss/hss Smv/Smv*) to obtain F_1 populations heterozygous at one or both loci in order to assign phenotypes to known genotypes. Groups of 15 seedlings of each of the three parental lines and reciprocal F_1 populations were inoculated with SMV NY 76-6 and held in the greenhouse under summer and winter conditions. Under winter greenhouse conditions, F_1 results confirmed that each line, 'GN 1140' and BT-1, has a fully dominant gene that alters the typical susceptible mosaic response to systemic resistance and lethal necrosis, respectively (Table 2a). When (BT-2 × 'GN 1140') F_1 (*hss/hss Smv/smv*) plants were grown during the winter but held in a growth chamber with full supplemental lighting at approximately the same temperature, the *Smv* allele became incompletely dominant in contrast to full dominance observed in the greenhouse at the same time of year. This suggests that high light intensity or some other condition provided in the growth chamber is responsible for the shift from full dominance during winter greenhouse tests (Table 2a) to incomplete dominance observed during the summer months (Table 2b).

The dihybrid ('GN 1140' × BT-1) F_1 plants did not develop necrotic lesions on inoculated leaves, and no virus was recovered from inoculated or systemic tissue in the winter greenhouse tests (Table 2a). Thus, the F_1 phenotype is different from each of the parents. We hypothesize that lack of systemic spread is contributed by *Smv*, and incapacity to express mosaic and replicate virus in leaf tissue to recoverable levels is conditioned by the *Hss* allele. No maternal effects were noted. This result establishes that the alleles *Hss* and *Smv* were codominant.

As expected, a shift in the codominant ('GN 1140' × BT-1) F_1 phenotype was noted during the summer months. While inoculated primary leaves remained symptomless in the winter, during summer tests some plants developed clear necrotic local lesions that were limited to inoculated tissue (Table 2b). All ('GN 1140' × BT-1) F_1 individuals possess an identical genotype (*Hss/hss Smv/smv*), yet two phenotypes were observed that share the common feature of systemic resistance. In theory, under these conditions, all plants should develop

local necrotic lesions. In fact, only a relatively small number expressed local lesions, indicating incomplete penetrance of this aspect of the phenotype. Thus, the genotypes (*hss/hss Smv/Smv*) and (*Hss/-* Smv/-) cannot reliably be distinguished. We have used these results as the basis for combining the two phenotypic classes, designated O/R and NLL/R respectively, in the analysis of segregating ('GN 1140' × BT-1) F_2 populations shown in Table 3.

Response of F_2 populations segregating at Smv and Hss

F_2 segregation data summarized in Table 3 confirmed that the two genes in bean, *Smv* and *Hss*, segregated independently and were codominant. As mentioned above, the phenotypic class where no local or systemic symptoms were observed was comprised of both (*hss/hss Smv/Smv*) and (*Hss/- Smv/-*) genotypes, as the necrotic local lesions expected for the second genotype were not always observed. Thus, the phenotypic class designated O/R is significantly larger than expected. If plants were not pruned, the class comprised of individuals that developed necrotic local lesions followed by systemic necrosis was also smaller than expected (data not shown). Segregation in the F_2 population was consistent with a 10 systemic resistant : 3 systemic necrotic : 2 mild systemic mosaic : 1 severe mosaic phenotypic ratio that was expected according to hypotheses developed on the basis of F_1 phenotypes (Tables 1 and 2a, b) corresponding to a genotypic ration of 10 (9 *Hss/- Smv/--* + 1 *hss/hss Smv/Smv*) : 3 *Hss/- smv/smv* : 2 *hss/hss Smv/smv* : 1 *hss/hss smv/smv*.

Discussion

A single dominant factor designated *Hss* located in *P. vulgaris* cv 'Black Turtle Soup' selection BT-1 has been identified that confers a temperature-insensitive lethal systemic necrotic reaction to SMV. *Hss/-* genotypes lacking modifier genes developed pinpoint necrotic lesions followed by systemic vascular necrosis that resulted in dark stem streaks and apical death within a week post-inoculation, regardless of the ambient temperature. This distinctive necrotic response conditioned by *Hss* was also expressed in mechanically inoculated detached leaves held in a moist chamber for 3–4 days and in *Hss/-* plants approach-grafted to an infected susceptible plant. The approach graft inoculation data suggest that only tissue of plants carrying the *Hss* allele is capable of expressing the rapid and distinctive necrotic response to SMV; thus, expression of the *Hss* allele probably does not involve an unspecific transmissible signal.

In a previous study, each of 23 SMV isolates selected to represent the seven soybean SMV pathotypes uniformly incited systemic necrosis in BT-1 and systemic mosaic in BT-2 (Provvidenti et al. 1982). In another study, a number of bean varieties were inoculated with 11 Colombian SMV isolates, and again no isolate-specificity was observed on lines that developed systemic necrosis (Tamayo et al. 1980). Thus, there is no conclusive evidence for distinct SMV pathotypes on bean. It appears that the seven soybean SMV pathotypes (Cho and Goodman 1979) constitute only one bean pathotype, underscoring that it is the genetics of the host–pathogen interaction, not solely the genetic variability of the pathogen, that defines viral pathotypes.

The co-occurrence of dominant lethal hypersensitivity to SMV and temperature-dependent resistance to systemic mosaic mottle symptoms incited by related potyviruses was explored. A previous study using a small F_2 population of large-seeded, determinate near-isogenic lines segregating for potyvirus resistance indicated linkage between dominant resistance to BCMV NY 15, BlCMV, CAMV, WMV, and lethal necrosis to SMV (Kyle and Dickson 1988). The present study included larger populations derived from a cross between small-seeded indeterminate black bean lines, BT-2 and BT-1 that were completely unrelated to the germ plasm used in the previous work. Our results provided further evidence consistent with identity, but did not rule out the close linkage of genetic factor(s) that condition necrotic reactions to SMV and dominant resistance to BCMV, BlCMV, CAMV and WMV. Until conclusive evidence of identity is obtained, we propose the use of a distinct gene symbol for response to SMV. It should be emphasized that unlike *I*, *Cam*, *Bcm*, and *Wmv*, *Hss* is not a resistance gene against SMV except when present with additional genes, but it is rather a gene that confers true hyper-sensitivity to the virus, resulting in the complete death of unprotected *Hss/-* plants within just a few days of inoculation with SMV. Because of the inherent problem in determining allelism of dominant factors and because these viral isolates that induced necrosis represent a distinct virus with a distinctive reaction on bean, we have given the dominant factor controlling this response to SMV in bean the designation *Hss* for lethal hypersensitivity to SMV. Further experiments are in progress to define the relationship suggested by the tight linkage, similarity of inheritance, and the tissue-specific necrotic phenotype conferred by *Hss*, and factors previously designated *I*, *Bcm*, *Cam*, and *Hsw*, now mapped to Lamprecht's linkage group III (Ali 1950; Provvidenti et al. 1983; Kyle and Provvidenti 1987b, Kyle and Dickson 1988).

There are at least two genetic configurations that could account for the observed cosegregation of *Hss* and dominant temperature-dependent resistance to related potyviruses. First, a tightly linked cluster of distinct genes, and second, a single multifunction locus, either

complex or simple, could confer this phenotype. There is a precedent for the first possibility in pea where two tightly linked clusters of potyviral resistance genes have been identified, with each locus in the cluster conferring resistance to a clearly distinct but related potyvirus (Provvidenti 1991). Lines carrying some but not all of these recessive factors were readily identified when germ plasm from the center of origin of the species was evaluated. Similar experiments in bean have not yet revealed a genotype that possesses one or more but not the full set of dominant factor(s) (M. M. Kyle in preparation). Thus, there is no evidence to support a cluster of distinct genes in bean, but this alternative cannot be conclusively ruled out with the information available.

If one locus in bean confers a temperature-dependent reaction to some potyviral genotypes and a temperature-independent reaction to others, a gene for resistance could also be a lethal gene. Proof awaits the determination of the fine structure at this locus in bean. Several groups have targeted the *I* gene for map-based cloning efforts as its location on the bean linkage map has been determined (Nodari et al. 1992; E. Vallejos, personal communication).

These associations among host resistance genes are reflected by a similarity among the nucleotide sequences of viral genomes whose infection cycles are interrupted by the same or similar host genes (Kyle and Rybicki 1991). Presumably, if the same or similar host genes interact with more than one virus, those viruses should share critical feature(s) involved in the resistant reaction. In the case of SMV, considerable debate surrounds whether SMV and WMV are distinct viruses or isolates of the same virus that differ by a single 16 amino acid deletion at the coat-protein N terminus (Eggenberger et al. 1989; Frenkel et al. 1989; Quemada et al. 1990; Jayaram et al. 1991; Ward and Shukla 1991; Jain et al. 1992). We interpret the host genetic data presented in this paper to be consistent with the proposition that these are indeed closely related viruses, despite large differences in standard biological criteria of relationship including host range, inclusion bodies, seed transmissibility, etc. However, no isolate assigned to SMV shows the conditional reaction on *I*/*I* genotypes observed for all WMV isolates tested in this and previous work (Kyle and Provvidenti 1987b). Thus, there is a clear demarcation between isolates assigned to WMV and those assigned to SMV with regard to host response conditioned by *Hss* and related factors. If similarity of host response is closely correlated with sequence relationships, another case may provide a test. There are BCMV isolates belonging to pathotypes NL-3, NL-5, and NL-8 that incite a reaction on *I*/*I* genotypes that is identical to that of SMV isolates on *Hss*/- genotypes. However, mounting evidence suggests that these BCMV isolates are quite different at the sequence level from most BCMV pathotypes. The name bean necrosis mosaic virus has been suggested recently to reflect this fact (McKern et al. 1992). Again, host response and sequence information point to the possibility that the necrotic BCMV isolates comprise a distinct set that may share some key features with SMV.

We also evaluated the relationship between *Hss* and the only other gene in bean, *Smv*, known to affect the outcome of infection with SMV (Provvidenti et al. 1982). *Hss* and *Smv* are codominant and segregate independently. The difference in phenotype and lack of genetic association suggest that these two allelic pairs operate through independent mechanisms. In light of this, the combined action of both genes in the homozygous and heterozygous conditions was assessed by screening a segregating F_2 population in an effort to identify an improved resistant phenotype. This strategy is similar to that employed with the *I* gene protected from necrotic BCMV pathotypes by the *bc-x* genes (Drifhout et al. 1978), except that from a breeding standpoint, a single dominant gene is much more easily handled than a series of recessive alleles at unlinked loci.

Homozygous *Hss* plants without modifying genes die quickly and are therefore not resistant at all. Homozygous *Smv* plants lacking *Hss* develop local infection, but that infection is limited to inoculated tissue. The homozygous dihybrid (*Hss*/*Hss Smv*/*Smv*) shows systemic resistance but does not develop local infection and therefore cannot serve as a reservoir for the virus in the field. Occasionally, these plants develop necrotic local lesions on inoculated leaves, but the virus cannot be detected with ELISA or recovery tests, and the necrosis never moves systemically. Thus, the dihybrid genotype constitutes and improvement in type and level of resistance when compared with either parent. Moreover, the use of the two genes (*Hss*/*Hss Smv*/*Smv*) theoretically increases the probability of a more stable long-lasting resistance. If *Hss* is present in most bean varieties, as our data and that of others suggest, either because it is identical or linked to the *I* gene, the addition of *Smv* could prevent the major losses that can occur when an *I*/*I* variety is grown near a soybean field.

These experiments have provided a base for a general strategy we are using in several breeding programs where a necrotic response, even a lethal reaction, is combined with another gene that involves some type of localization mechanism that may or may not involve necrosis. Using this strategy exemplified by *Hss* and *Smv* in *P. vulgaris*, we have combined phenotypes that are not fully resistant to create a novel resistant genotype that limits both loss to disease and spread of the disease within and between crops.

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