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Inheritance of resistance to potyviruses in *Phaseolus vulgaris* L. IV. Inheritance, linkage relations, and environmental effects on systemic resistance to four potyviruses

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Abstract We have examined the genetics of systemic resistance in *Phaseolus vulgaris* to azuki bean mosaic virus (AzMV) and cowpea aphid-borne mosaic virus (CABMV) and the relationship of this resistance to a phenotypically similar resistance to watermelon mosaic virus (WMV) and soybean mosaic virus (SMV). In *P. vulgaris* cv 'Great Northern 1140' (GN1140), resistance to SMV and WMV has been attributed to the genes *Smv* and *Wmv*, respectively, which have been shown to segregate as a unit. Systemic resistance to AzMV is conferred by two incompletely dominant alleles, *Azm1* and *Azm2*, at unlinked loci. At least three resistance alleles must be present at these two loci for systemic resistance to be expressed in the plant. Systemic resistance to CABMV in GN 1140 is conditioned by a dominant allele that has been designated *Cam2*. Under some environmental conditions, a recessive allele at an unlinked locus, *cam3*, also controls a resistant response to CABMV. Resistance to AzMV and CABMV does not assort independently from *Wmv/Smv*, but also does not consistently cosegregate, suggesting that perhaps in each case one of the factors involved in resistance is associated with *Smv/Wmv*.

Key words Plant virus resistance · Azuki bean mosaic virus · Cowpea aphid-borne mosaic virus · Soybean mosaic virus · Watermelon mosaic virus

Introduction

A large number of alleles that condition resistance to viral, bacterial, or fungal pathogens have been identified in plants. Classical genetic analysis has revealed a remarkable redundancy in the host genome for resistance to a single pathogen (Pryor 1987; Bennetzen and Hulbert 1992). Recent plant genome mapping and gene cloning efforts support prior observations that a redundancy of resistance factors within the genome and duplication at host resistance loci are common, even when monogenic inheritance is observed.

Cloning of the *N* locus for resistance to tobacco mosaic virus (Whitham et al., 1994), the *Cf-9* gene for resistance to the fungal pathogen *Cladosporium fulvum* (Jones et al. 1994), and the *Pto* gene for resistance to the bacterial pathogen *Pseudomonas syringae* pv *tomato* (Martin et al. 1993) revealed each to be one member of a multiple gene family, with other members of the family clustered both at the resistance locus and elsewhere in the genome. The function of these other members is not currently known, although it is possible that if functional they condition responses similar to the resistance allele but perhaps with different pathogen or tissue specificities. In addition to the redundancy in the genome for resistance to strains of the same pathogen, clusters of linked resistance genes that condition a phenotypically similar resistance to distinct but related viruses have been identified (Kyle 1988; Kyle and Provvidenti 1993b; Fisher and Kyle 1994; Blauth 1994). Although the structural redundancy of resistance genes has been made clear through both genetic and molecular analyses, the functional and evolutionary significance of this redundancy is still largely unresolved.

The study presented here contributes to a systematic effort to develop a comprehensive picture of the structure, function, and evolution of plant viral resistance genes in a single species. Independent of their role in viral resistance, these genes may also function in host processes involved in viral infection, e.g., RNA metabolism. Thus, variations in plant responses to viruses may reveal genetic variability in important plant pathways. Furthermore, since plant viruses are relatively simple genetic entities that typically express eight or fewer gene products and since infectious clones are increasingly available, the molecular details of host-viral interactions

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appear to be within reach. The types of genetic changes that occur in the pathogen in response to genetic blocks in the host define the evolutionary potential of that pathogen. Ultimately, it is our hope to understand the dynamic genetic interactions between viral parasites and their hosts.

The viruses that are the focus of this study and our previous work in *P. vulgaris* are members of the largest family of plant viruses, the Potyviridae. They are monopartite, positive-sense, single-stranded RNA viruses that regularly cause significant losses in many agriculturally important crop species (Hollings and Brunt 1981). The apparent continuum of variants or strains within the Potyviridae has made it difficult to define sharp boundaries between strains of a virus and between closely related but distinct viruses (Bos 1992). Nevertheless, it is possible to arrange potyviruses into clear subgroups based on coat protein sequence similarity (Shukla and Ward 1988; Rybicki and Shukla 1992; Ward et al. 1992). The subgroup we have focused on includes bean common mosaic virus (BCMV) Serotypes A and B, soybean mosaic virus (SMV), watermelon mosaic virus (WMV), azuki bean mosaic virus (AzMV), cowpea aphid-borne mosaic virus (CABMV), Thailand passionfruit virus (ThPV) (Benscher et al. 1993), zucchini yellow mosaic virus (ZYMV), peanut stripe virus (PStV), passionfruit woodiness virus-K (PWV-K), and blackeye cowpea mosaic virus (BlCMV) (Mink and Silbernagel 1992; McKern et al. 1992; Dijkstra and Khan 1992; Rybicki and Shukla 1992; Tsuchizaki and Omura 1987; Khan et al. 1993).

Several phenotypically distinct types of resistant responses to potyviruses have been reported in *P. vulgaris* (Provvidenti 1993a). One response is observed in bean genotypes that possess the dominant allele, *I* (Ali 1950). The *I* allele conditions resistance to BCMV Serotype B. Nevertheless, a necrotic response is observed on *II* genotypes with other viral genotypes and with some BCMV Serotype B isolates under particular environmental conditions. This necrotic response ranges from the development of necrotic local lesions on inoculated leaves to a systemic vascular necrosis resulting in apical death. Resistance to BCMV Serotype B, conferred by the *I* allele, cosegregates with phenotypically similar dominant resistance and/or systemic necrotic response to eight other potyviruses that fall within the BCMV subgroup, including SMV, WMV, AzMV, CABMV, ThPV, ZYMV, PWV-K, and BlCMV (Kyle and Dickson 1988; Fisher and Kyle 1994). Simply-inherited broad-spectrum resistance, exemplified by the *I* locus, suggests that closely related viruses may have evolutionarily conserved structures or processes necessary for pathogenesis that can be interrupted by the product(s) of a single host gene or a tightly linked series of genes.

In this study we examined a second type of resistance to potyviruses that is phenotypically distinct from the response conditioned by the *I* allele and which is found in *P. vulgaris* cv 'Great Northern 1140' (GN 1140) (Provvidenti 1974; Provvidenti et al. 1982). In this case, the virus can be recovered from inoculated tissue of resistant genotypes, but it does not move systemically and vascular necrosis is never observed. Resistance to SMV in GN 1140 is conferred by a single incompletely dominant gene, *Smv* (Provvidenti et al. 1982; Kyle and Provvidenti 1993a); resistance to WMV is conditioned by a single dominant gene, *Wmv* (Provvidenti 1974; Kyle and Provvidenti 1987). Both genes segregate independently from the *I* locus. In view of the phenotypic similarity of responses to SMV and WMV, the first objective of the present study was to examine whether *Smv* and *Wmv* were linked. In addition to possessing resistance to SMV and WMV, GN 1140 has a similar non-necrotic systemic resistance to several other potyviruses belonging to the BCMV subgroup, including AzMV, CABMV, and ThPV (Taiwo et al. 1982; Provvidenti 1993b). While previous work attributed resistance to ThPV to a single dominant gene (Provvidenti 1993b), our preliminary results showed considerably more complexity in the response by 3 or more weeks post-inoculation. For this reason, ThPV was not included in the present study. Further objectives were to extend our understanding of resistance to potyviruses in *P. vulgaris* by elucidating the genetic basis of the non-necrotic systemic resistance to AzMV and CABMV and to determine the relationship, if any, of the genes controlling these responses to the genes *Smv* and *Wmv*.

Materials and methods

Germ plasm and genetic populations

Parental lines included BT-2, a selection from *P. vulgaris* cv 'Black Turtle Soup' which is uniformly susceptible to the four potyviruses used in this study, and 'Great Northern 1140' (GN 1140), which is resistant to SMV, WMV, AzMV, and CABMV (Provvidenti 1974; Provvidenti et al. 1982; Provvidenti 1983; Kyle and Provvidenti 1987, 1993a). Reciprocal crosses between BT-2 and GN 1140 were made to produce the F_1, F_2 and F_2-derived F_3 families and backcross populations.

Linkage relationships among resistance responses to SMV, WMV, AzMV, and CABMV were evaluated with 28 F_3 families derived from one F_2 population. A set of at least 12 individuals from each F_3 family was planted and inoculated with one of the above-mentioned four viruses. In this manner, a set of at least 12 seedlings from each F_3 family was screened with each of these four viruses.

Viral isolates and inoculation

SMV isolate NY 76–6 and WMV isolate NY 62–76 were provided by R. Provvidenti, NYSAES, Cornell University, Geneva, N.Y. and maintained on *P. vulgaris* 'BT-2'. The Moroccan isolate of CABMV was obtained from R.O. Hampton, Oregon State University, Corvallis, Ore. and maintained in *Vigna ungiculata* 'California blackeye cowpea'. AzMV was provided by M. Silbernagel, USDA WSU-IAREC, Prosser, Wash. and was maintained on *P. vulgaris* 'BT-2'. The purity of the viral isolates was monitored routinely with ELISA, host index tests, and evaluation of characteristic symptomatology on a range of susceptible host genotypes.

Inoculum for each of the viruses was prepared by grinding systemically infected tissue in 50 m*M* potassium phosphate buffer, pH 8.4, containing carborundum and then straining it through cheesecloth. Primary leaves of 7- to 10-day-old seedlings were mechanically inoculated using a pestle dipped in inoculum. Uninoculated and

susceptible controls were routinely included. Test plants were scored weekly for the presence of viral symptoms for at least 30 days post-inoculation (dpi) and in some cases up to 90 dpi. If symptoms were ambiguous, plants were tested for the presence of virus using back inoculations to *P. vulgaris* 'BT-2', and/or direct double antibody sandwich ELISA (Clark and Adams 1977).

Growth conditions

Plants used in the inheritance and linkage analyses were held in the greenhouse under supplemental lighting at temperatures that ranged between 20° and 25 °C in the winter (February - March) or 25° and 30 °C in the summer (June-September). The phenotypic response of BT-2, GN 1140, and reciprocal (BT-2 × GN1140) F_1 populations to inoculation with each of the viruses was tested under controlled environmental conditions. Three growth chambers were used to examine the individual contribution of light intensity and temperature on the phenotypic response of (BT-2 × GN 1140) F_1 plants inoculated with SMV, WMV, AzMV, or CABMV. Plants were inoculated at the primary leaf stage as previously described and then held at 21 °C, 237 $uEs^{-1}m^{-2}$; 30°/27 °C day/night, 257 $uEs^{-1}m^{-2}$; or 30 °/27 °C day/night, 85 $uEs^{-1}m^{-2}$. All chambers had 16 h of illumination. Plants were scored weekly for the presence of viral symptoms. At 26 dpi, the first and third trifoliates above the primary leaves and the top newly expanded trifoliate were assayed for the presence of virus using back inoculation to BT-2 plants and/or double-antibody sandwich ELISA.

Results

Conditional shifts in dominance

Inoculation of the susceptible parental line, BT-2, with SMV, WMV, AzMV, or CABMV resulted in a systemic infection with severe mosaic, stunting, and distortion of upper trifoliate leaves by 14 dpi, regardless of ambient temperature. When the parental line GN 1140 was inoculated with any of these potyviruses, inoculated leaves became infected, but the virus never moved to uninoculated tissue at either 25 °C or 33 °C up to 30 dpi (Table 1a). The appearance of the symptoms on inoculated primary leaves was different with each virus and also varied with changes in temperature or light intensities.

In contrast to the parental genotypes, (BT-2 × GN 1140) F_1 plants inoculated with WMV showed a seasonally dependent shift in phenotype. When F_1 plants were inoculated and held in the greenhouse during summer months, they developed symptoms intermediate between the parental phenotypes. The first three to four trifoliates above the primary leaves developed chlorotic patches and vein clearing symptoms by 26 dpi. The virus could be recovered by back inoculation from these leaves and viral coat protein was detected using ELISA, but the plants were not stunted and the uppermost trifoliates remained free of virus. Under winter greenhouse conditions, however, there was a shift toward full dominance, and (BT-2 × GN 1140) F_1 plants were phenotypically identical to the GN 1140 parent. This seasonal shift in the phenotypic response of F_1 plants inoculated with WMV had not been previously reported. We observed similar seasonal phenotypic shifts for F_1 plants inoculated with SMV, as has been previously described by Kyle and Provvidenti (1993a).

In order to examine the environmental components of the seasonal shift, the effect of temperature and light intensity on the phenotypic response of (BT-2 × GN 1140) F_1 plants inoculated with SMV, WMV, AzMV, or CABMV was examined under controlled growth chamber conditions (Table 1b). Both temperature and light intensity influenced the rate of the systemic infection of F_1 plants inoculated with SMV, WMV, or AzMV. The virus moved most rapidly to uninoculated tissues at 30 °C and 257 $uEs^{-1}m^{-2}$, the maximum light intensity possible in the chamber. Systemic infection was also observed in plants maintained in the growth chambers at 30 °C and 85 $uEs^{-1}m^{-2}$, but the systemic movement of SMV and WMV was delayed under these conditions

Table 1a Local and systemic response of *P. vulgaris* parental lines to mechanical inoculation with SMV, WMV, AzMV, or CABMV at 30 days post-inoculation

P. vulgaris	Temperature	SMV	WMV	AzMV	CABMV
BT-2	25 °C or 35 °C	+/+[a]	+/+	+/+	+/+
GN 1140	25 °C or 35 °C	+/−	+/−	+/−	+/−

[a] Local/systemic: + = virus recovered; − = no virus recovered

Table 1b Phenotypic shift of (BT-2 × GN) F_1 plants inoculated with SMV, WMV, AzMV, or CABMV

	Temperature day/night	Light $uEs^{-1}m^{-2}$	12 dpi[a]		26 dpi[b]	
			Lower trifoliates	Upper trifoliates	Lower trifoliates	Upper trifoliates
SMV	30/27 °C	257	+	−	+	+
	30/27 °C	85	−	−	+	+
	21 °C	237	−	−	−	−
WMV	30/27 °C	257	+	−	+	+
	30/27 °C	85	−	−	+	−
	21 °C	237	−	−	−	−
AzMV	30/27 °C	257	+	−	+	+
	30/27 °C	85	+	+	+	+
	21 °C	237	−	−	+	−
CABMV	30/27 °C	257	−	−	−	−
	30/27 °C	85	−	−	−	−
	21 °C	237	−	−	−	−

[a] Plants were scored for the presence of viral symptoms: + = mosaic and/or chlorotic vien clearing; − = no symptoms

[b] Recovery tests were performed: + = virus recovered; − = no virus recovered

(Table 1b). The phenotypes of SMV-, WMV-, or AzMV-inoculated F_1 plants that are observed under winter greenhouse conditions could be mimicked in a growth chamber held at 21°C and 257 $uEs^{-1}m^{-2}$. Under these conditions, SMV and WMV never moved from the inoculated primary leaves up to 26 dpi and the systemic movement of AzMV in F_1 plants was drastically slowed. CABMV could not be recovered from uninoculated tissue of any of the F_1 plants, regardless of temperature or light intensity (Table 1b).

Inheritance of resistance to AzMV

Infection of the susceptible parental line, BT-2, by AzMV caused chlorotic lesions on inoculated leaves followed by a severe mosaic, stunting, and distortion of upper trifoliate leaves. Inoculated primary leaves of the resistant parent line, GN 1140, developed chlorotic spots and dark green vein banding, and the virus could be recovered from these leaves. However, the infection remained localized to the inoculated leaves, and the virus could not be recovered from uninoculated tissue. The phenotypic response of reciprocal (BT-2 × GN 1140) F_1 populations to inoculation with AzMV was intermediate between the two parental lines. The F_1 plants developed systematic mottle that was mild and delayed compared to that developed by the susceptible parent, and F_1 plants were not stunted.

(BT-2 × GN 1140) F_2 populations were screened with AzMV in the greenhouse under winter and summer conditions. In both seasons, the segregation of F_2 plants was consistent with a ratio of 1 resistant plant: 3 susceptible plants ($P_{\alpha=0.05} = 0.17$ summer; $P_{\alpha=0.05} = 0.06$ winter), however a ratio of 5 resistant individuals: 11 susceptible individuals better explained the data in both cases ($P_{\alpha=0.05} = 0.88$ summer; $P_{\alpha=0.05} = 0.73$ winter). Thus, our hypothesis is that two genes may be involved in determining the resistant phenotype (Table 2).

All of the inoculated testcross [BT-2 × (BT-2 × GN 1140)] progeny became systemically infected (Table 2). The severity of symptoms in these plants varied from plants similar to the BT-2 parents that had stunting and severe mosaic symptoms on upper trifoliate leaves to plants resembling the F_1 with mild systemic mosaic symptoms. It was not possible to assign these plants to distinct classes based on symptoms, therefore all individuals with systemic symptoms were grouped into one class. The inoculated backcross [GN 1140 × (BT-2 × GN 1140)] population segregated approximately 3 systemically resistant plants: 1 systemically infected plant that resembled the F_1 (Table 2). The 3:1 ratio in the backcross population again was most consistent with the hypothesis that two genes were involved in conferring resistance to AzMV (Table 2).

The segregation ratios observed in the backcross and F_2 populations support the hypothesis that resistance to AzMV in GN 1140 is controlled by two unlinked loci at which incompletely dominant resistance alleles occur. We have given these alleles the symbols *Azm1* and *Azm2*. While these alleles clearly segregate independently and are therefore distinct genetically, the phenotype conditioned by each allele is indistinguishable from the others. In the backcross and F_2 populations, we propose that plants are resistant to systemic infection if they are homozygous at one locus and homozygous or heterozygous at the other locus, and thus genotypes *Azm1/Azm1 Azm2/Azm2*; *Azm1/Azm1 Azm2/azm2*; and *Azm1/azm1 Azm2/Azm2* are resistant (Table 2). The remaining genotypes all became systemically infected,

Table 2 Segregation data for resistance to AzMV in populations derived from *P. vulgaris* cv 'Great Northern 1140' and 'Black Turtle-2' inoculated in the winter and screened 30 days post-inoculation

Populations	Genotypes and proposed phenotypes									Observed number of plants		Expected ratio R:S	$P_{\alpha=0.05}$
	A1/A1[a] *A2/A2* R[b]	*A1/A1 A2/a2* R	*A1/a1 A2/A2* R	*A1/A1 a2/a2* S[c]	*A1/a1 A2/a2* S	*A1/a1 a2/a2* S	*a1/a1 A2/A2* S	*a1/a1 A2/a1* S	*a1/a1 a2/a2* S	R	S		
GN	1	0	0	0	0	0	0	0	0	6	0	1:0	
BT-2	0	0	0	0	0	0	0	0	1	0	5	0:1	
(BT-2 × GN) F_1	0	0	0	0	1	0	0	0	0	0	6	0:1	
(GN × BT-2) F_1	0	0	0	0	1	0	0	0	0	0	3	0:1	
BT-2 × (BT-2 × GN)	0	0	0	0	1	1	0	1	1	0	31	0:1	
GN × (BT-2 × GN)	1	1	1	0	1	0	0	0	0	29	8	3:1	0.65
(BT-2 × GN) F_2	1	2	2	1	4	2	1	2	1	38	78	5:11	0.73
(BT-2 × GN) F_2[d]	1	2	2	1	4	2	1	2	1	37	84	5:11	0.88
	Expected segregation of F_3 families given the above F_2 genotypes									Number of families Seg		R:Seg:S	
(BT-2 × GN) F_3	1R	2Seg[e]	2Seg	1S	4Seg	2S	1S	2S	1S	3	15 10	1:8:7	0.48

[a] *A1* = *Azm1*; *A2* = *Azm2*

[b] R = plants are resistant to systemic infection, although virus can be recovered from inoculated tissue

[c] S = plants become systemically infected, and virus can be recovered from upper trifoliate leaves

[d] This population was inoculated in midsummer in the greenhouse and scored 30 dpi for the presence of symptoms on upper trifoliate leaves

[e] Seg = individuals in the family are segregating for resistance to AzMV

although the onset of symptoms and symptom severity varied considerably, presumably due to the presence of different combinations of *Azm1* and *Azm2* alleles. We were unable to distinguish the different phenotypic classes corresponding to genotypes heterozygous at both the loci or homozygous susceptible at one or the other, therefore, we grouped all individuals that became systemically infected into one class. The additive nature of these two genes, however, is consistent with the clearly intermediate phenotype of F_1 (*Azm1/azm1 Azm2/azm2*) plants, and also with the range of symptom severity observed in the testcross population. The segregation of 28 (BT-2 × GN 1140) F_3 families followed a ratio of 1 resistant family: 8 segregating families: 7 susceptible families, as expected for the two-gene hypothesis proposed above (Table 2).

Inheritance of resistance to CABMV

A similar study was done to determine the genetic basis for systemic resistance to CABMV in GN 1140. In this case, the susceptible parent, BT-2, developed irregular light-brown necrotic lesions surrounded by bright-yellow chlorotic zones on inoculated primary leaves. Systemic infection of BT-2 by CABMV resulted in severe mottle and bright-yellow chlorotic spots on upper trifoliates, followed by plant death. The resistant parent, GN 1140, developed chlorotic lesions on primary inoculated leaves. The virus could be recovered from inoculated leaves but was never recovered from any uninoculated tissue up to 30 dpi. Reciprocal (BT-2 × GN 1140) F_1 populations inoculated with CABMV were phenotypically identical to the GN 1140 parent (Table 3). Virus could be recovered from the inoculated primary leaves but was never recovered from uninoculated tissue.

In (BT-2 × GN 1140) F_2 populations screened 30 dpi in March, 137 plants remained free of systemic symptoms while 27 plants became systemically infected, consistent with a two-gene ratio of 13 resistant plants : 3 susceptible plants (Table 3). When a second F_2 population was inoculated and screened 14 dpi in midsummer, a ratio of approximately 13 resistant plants : 3 susceptible plants was also observed. However, when the same population was re-evaluated at 30 dpi, 9 additional plants had become systemically infected. These 9 plants were not stunted, had milder mosaic symptoms, flowered, and set seed, unlike plants susceptible at 14 dpi, which remained very severely affected. A segregation ratio of 12 resistant plants : 1 plant with delayed mild systemic infection : 3 plants with severe systemic infection can account for these data. Fifty-one F_3 seeds from 6 of the 9 F_2 plants that exhibited delayed systemic infection to CABMV were grown and inoculated with CABMV in late summer. Unlike the inoculated susceptible BT-2 parent, all these F_3 individuals were uniformly delayed in developing systemic infection, with the exception of 1 individual that never became infected (data not shown).

Together, these F_2 data are consistent with the hypothesis that two unlinked genes confer resistance to CABMV in GN 1140 (Table 3). CABMV resistance that cosegregates with the *I* locus has been assigned the gene symbol *Cam* (cowpea aphid-borne mosaic) (Provvidenti et al. 1983). We propose that *Cam* be re-

Table 3 Segregation data for resistance to CABMV in populations derived from *P. vulgaris* cv 'Great Northern 1140' and 'Black Turtle-2' inoculated in the winter and screened 30 days post-inoculation

Populations	Genotypes and proposed phenotypes									Observed *number of plants*			Expected *ratio* R:S	$P_{\alpha=0.05}$
	C2/C2[a] *C3/C3* R[b]	*C2/C2 C3/c3* R	*C2/c2 C3/C3* R	*C2/C2 c3/c3* R	*C2/c2 C3/c3* R	*C2/c2 c3/c3* R	*c2/c2 C3/C3* R/SS[c]	*c2/c2 C3/c3* S[d]	*c2/c2 c3/c3* S	R	R/SS	S		
GN	1	0	0	0	0	0	0	0	0	11	0	0	1:0:0	
BT-2	0	0	0	0	0	0	0	0	1	0	0	7	0:0:1	
(BT-2 × GN) F_1	0	0	0	0	1	0	0	0	0	6	0	0	1:0:0	
(GN × BT-2) F_1[e]	0	0	0	0	1	0	0	0	0	2	0	0	1:0:0	
BT-2 × GN) × BT-2	0	0	0	0	1	1	0	1	1	22	0	19	1:0:1	0.65
((BT-2 × GN) × GN	1	1	1	0	1	0	0	0	0	21	0	0	1:0:0	
(BT-2 × GN) F_2	1	2	2	1	4	2	1	2	1	137	0	27	13:0:3	0.47
(BT-2 × GN) F_2[e]	1	2	2	1	4	2	1	2	1	126	9	37	12:1:3	0.60
	Expected segregation of F_3 families given the F_2 genotype									Number of families R	Seg	S	R:Seg:S	
(BT-2 × GN) F_3	1 R	2 R	2 R	1 R	4 Seg[f]	2 Seg	1 R	Seg	1 S	10	15	3	7:8:1	0.50

[a] *C2* = *Cam2*; *C3* = *Cam3*

[b] R = plants are resistant to systemic infection, although virus can be recovered from inoculated tissue

[c] R/SS = resistant/slow susceptibility. In winter, plants are resistant to systemic infection by CAMBV; in summer, plants develop systemic symptoms, but the appearance of symptoms is delayed relative to when they appear in BT-2 plants

[d] S = plants become systemically infected, and virus can be recovered from upper trifoliate leaves

[e] This population was inoculated in midsummer in the greenhouse and scored 4 weeks post-inoculation for the presence of symptoms on upper trifoliate leaves

[f] Seg = individuals in the family are segregating for resistance to CABMV

designated *Cam1* and that the two genes for resistance to CABMV in GN 1140 be assigned the gene symbols *Cam2* and *cam3*. One of the genes in GN 1140, we thus have designated *Cam2*. This resistance allele confers monogenic dominant systemic resistance to CABMV. The other gene, designated *cam3*, provides conditional recessive resistance. The *cam3* allele appears to slow the rate of systemic infection, however its expression is influenced by environmental conditions. In winter greenhouse tests, plants with the genotype *cam2/cam2 cam3/cam/3* remained free of systemic symptoms up to 30 dpi, giving an F_2 segregation ratio of 13 resistant plants : 3 systemically infected plants, which indicates the combined action of the dominant gene, *Cam2*, and the recessive gene, *cam3*. In summer tests, *cam2/cam2 cam3/cam3* genotypes did become systemically infected by 30 dpi, although the appearance of systemic symptoms was delayed and these symptoms were less severe than those of the susceptible parent.

[(BT-2 × GN 1140) × BT-2] testcross populations segregated approximately 1 resistant plant : 1 systemically infected plant, while the [(BT-2 × GN 1140) × GN 1140] backcross populations were completely resistant, consistent with the two-gene hypothesis proposed above. The 28 (BT-2 × GN 1140) F_3 families were screened in the winter with CABMV and segregated approximately 7 resistant families : 8 segregating families : 1 susceptible family, as is expected if one dominant gene and one recessive gene control resistance to CABMV (Table 3).

Association of resistance responses to four potyviruses in (BT-2 × GN 1140) F_3 families

The phenotypic similarities in the resistance responses to SMV and WMV, conditioned by *Smv* and *Wmv*, respectively, prompted us to investigate linkage between these responses by screening 28 (BT-2 × GN 1140) F_3 families. No recombinant families were identified, i.e., no families were found that differed in their responses to this pair of viruses. Resistance to SMV and WMV cosegregated as a unit (Table 4). The segregation of F_3 families was consistent with a 1:2:1 ratio expected for a single incompletely dominant gene conditioning resistance.

Nevertheless, individuals in the susceptible class were not phenotypically uniform. The timing and severity of systemic infection varied considerably within a family and between families, suggesting that there are other genetic factors that modify the symptom expression and/or systemic infection of plants inoculated with SMV or WMV. Of the 19 F_3 families that were segregating for resistance to SMV and WMV, 2 did not segregate in the expected ratio for a single dominant gene of 3 resistant plants : 1 susceptible plant. Instead, there were many more susceptible individuals in sets of plants screened with either WMV or SMV. The cause of this deviation from the expected ratio is being explored.

Seeds from each of the 28 F_3 families were also grown and screened for their response to inoculation with AzMV and CABMV. The resistance response to AzMV, which is conditioned by two unlinked incompletely dominant genes, did not segregate independently of *Smv/Wmv* (Table 4). The association between resistance to SMV/WMV and resistance to AzMV can be explained by linkage of one of the *Azm* loci to *Smv* and *Wmv*. Although we cannot phenotypically distinguish *Azm1* from *Azm2*, we have arbitrarily designated *Azm1* as the factor possibly linked to *Smv/Wmv*. In 5 F_3 families, inconsistencies between the segregation patterns of AzMV and SMV/WMV were observed. Four families were uniformly susceptible to AzMV, but segregated for resistance to SMV and WMV (Table 4). These families could possess the *Azm1* allele, which is linked to *Smv/Wmv*, but not the *Azm2* allele, which is necessary to condition resistance to AzMV. All of the individuals in F_3 family no. 30 were susceptible to AzMV but resistant to SMV and WMV. To check this result, we re-screened F_4 families from F_3 individuals that were susceptible to AzMV and found them to be resistant to SMV and WMV. Conversely, F_4 families from F_3 plants resistant to WMV became systemically infected when inoculated with AzMV (data not shown).

Table 4 (BT-2 × GN) F_3 families were inoculated with SMV, WMV, AzMV, or CABMV to determine if the family was homozygous-resistant, segregating, or homozygous-susceptible to each of the viruses

Virus	F_3 family number													
	20	25	26	5	12	7	23	11	28	22	29	15	19	3
SMV	S[a]	S	S	S	S	Seg[b]	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg
WMV	S	S	S	S	S	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg
AzMV	S	S	S	S	S	S	S	S	S	Seg	Seg	Seg	Seg	Seg
CABMV	S	S	S	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg

Virus	F_3 family number													
	6	10	14	4	9	16	18	21	24	27	30	8	1	13
SMV	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	R[c]	R	R	R
WMV	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	R	R	R	R
AzMV	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	S	R	R	R
CABMV	Seg	Seg	Seg	Seg	Seg	R	R	R	R	R	R	R	R	R

[a] S, all individuals in the family are susceptible
[b] Seg, individuals in the family are segregating for resistance
[c] R, all individuals in the family are resistant

If *Azm1* is linked to *Smv/Wmv*, these results can be explained by the absence of the *Azm2* allele. Alternatively, F_3 family no. 30 might represent a recombinational event between the *Azm1* locus and *Smv* and *Wmv*.

Using the same 28 F_3 families, we cannot demonstrate independent assortment between resistance to CABMV and resistance to WMV or to SMV (Table 4). The genetic basis of the deviation from independent assortment is not known. It is possible that one of the factors conditioning resistance to CABMV, either *Cam2* or *cam3*, is linked to *Smv* and *Wmv*. The screening of F_3 families with CABMV was done under winter greenhouse conditions when *cam3* would be expected to provide resistance to CABMV in addition to *Cam2*. Because we could not distinguish resistance provided by *Cam2* from resistance provided by *cam3*, we cannot demonstrate linkage unequivocally on the basis of our F_3 data.

Discussion

The first objective of this study was to assess linkage between two phenotypically similar resistance responses to the closely related viruses, SMV and WMV, conditioned by the genes, *Smv* and *Wmv*, respectively (Provvidenti 1974; Provvidenti et al. 1982, Kyle and Provvidenti 1987, 1993a). *Smv* and *Wmv* were linked to each other and uniformly cosegregated in a small population of F_3 families. Phenotypically similar resistance to AzMV and CABMV was not monogenically inherited. In both cases, two unlinked loci determined the resistant response. Nevertheless, there was an association between resistance to SMV and WMV and systemic resistance responses to AzMV and CABMV.

Inheritance of systemic resistance to AzMV

While resistance to AzMV in GN 1140 is phenotypically similar to systemic resistance responses to SMV and WMV, resistance to AzMV was conferred by two unlinked, incompletely dominant alleles designated *Azm1* and *Azm2*. These genes are phenotypically indistiguishable from each other and function in an additive manner. The presence of at least three dominant alleles at the two loci was necessary to observe the resistance response. Heterozygosity at each locus gave an F_1 phenotype intermediate between that of the susceptible parent and resistant parent. While it is relatively unusual that resistance to a viral pathogen requires an interaction between alleles at different loci (Fraser 1992), a well-documented example of an interaction between alleles at two unlinked resistance loci is observed in GN 1140 for systemic resistance to some strains of BCMV.

Resistance to isolates of BCMV in GN 1140 is controlled by two recessive alleles, *bc-u* and *bc-1*^{2} (Drijfhout 1978). Full systemic resistance to viral symptoms is expressed only if the plant is homozygous for both alleles. However, viral accumulation is greatly reduced in *bc-u*$^+$*bc-u/bc-1*$^+$*bc-1*^{2} heterozygotes when compared to the susceptible parent. With regard to viral accumulation, *bc-u* and *bc-1*^{2} could be considered to be incompletely dominant alleles (Day 1984). Given that the resistance responses to AzMV and BCMV in GN 1140 are phenotypically similar, that AzMV is closely related to BCMV (McKern 1992; Tsuchizaki and Omura 1987), and that *Azm1*, *Azm2*, *bc-u*, and *bc-1*^{2} are, under some conditions, all incompletely dominant alleles, it is possible that there may be an association or identity between *Azm1* or *Azm2* and the *bc* loci.

Inheritance of systemic resistance to CABMV

Like resistance to AzMV, resistance to CABMV in GN 1140 was controlled by more than one genetic factor. An allele for monogenic dominant resistance to CABMV has been designated *Cam2*. In addition, a second unlinked recessive allele, *cam3*, can independently provide systemic resistance to CABMV under winter conditions. The *cam3* allele appears to affect the timing of the systemic movement of CABMV. Under winter greenhouse conditions, *cam2/cam2 cam3/cam3* plants inoculated with CABMV did not become systemically infected by 30 dpi. However, when *cam2/cam2 cam3/cam3* plants were inoculated in summer greenhouse tests, virus could be recovered from uppermost trifoliate leaves by 30 dpi. A similar situation was reported in *Lactuca sativa* where one dominant gene and a second unlinked recessive gene independently provided resistance to lettuce mosaic potyvirus (Pink et al. 1992).

Environmental effects on phenotype

Environmental conditions have been shown to have an important influence on the expression of *cam3* and *Smv* (Kyle and Provvidenti 1993a). In light of these results, the role that environmental conditions have on the expression of *Wmv* was re-examined. Under winter greenhouse conditions, *Wmv* is dominant; however, in summer greenhouse tests, F_1 plants became systemically infected, indicating that *Wmv*, similar to *Smv*, is incompletely dominant under these conditions. In controlled environments, both light and temperature influenced the phenotype of F_1 plants inoculated with AzMV, SMV, or WMV.

It is important to note that resistance to these viruses can be described as dominant, incompletely dominant, or even recessive, depending on the environmental conditions of the screen, how long after inoculation the plants were scored, and the classifications used to score phenotype. In preliminary studies, plant age at time of inoculation was also observed to influence the outcome of infection. Phenotypic shifts due to changes in season, light intensity, temperature, or plant growth have been observed in other plant-viral systems and are probably

quite common, explaining in part the problems applied breeding programs encounter in doing off-season seedling screens for virus resistance (e.g., Fraser 1986; Leisner et al. 1993, Bijaisoradat and Kuhn 1985; Wintermantel et al. 1993).

Linkage of *Smv* to *Wmv*

A similar inheritance and phenotype suggested the possibility of some genetic relationship between the genes *Smv* and *Wmv*. In a small study of 28 F_3 families, *Smv* and *Wmv* cosegregated. Screening for the presence of *Smv* and *Wmv* in F_3 families was difficult, presumably due to the presence of additional genes in GN 1140 that modified the expression of *Smv* or *Wmv*. Some plants became systemically infected within 7 dpi, while others had just begun to develop mild symptoms after 30 dpi. It is possible that genetic factors controlling resistance to AzMV, CABMV, or BCMV may also influence the infection of plants by WMV or SMV. Examples of a major gene for resistance to one pathogen that also has minor effects on a second related pathogen have been described for fungi (Ellingboe 1981).

Linkage of resistance responses to AzMV and CABMV with *Smv* and *Wmv*

While resistance to AzMV and CABMV was not monogenically inherited, independent assortment of resistance to SMV and WMV was not observed, although neither was consistent cosegregation observed. The association observed between resistance to AzMV and resistance to SMV and WMV can be explained by the linkage of one of the *Azm* loci to *Smv* and *Wmv*. As mentioned before, we were unable to distinguish the *Azm* alleles phenotypically, therefore we have arbitrarily assigned *Azm1* as the locus linked to *Smv/Wmv*. Likewise, the association of resistance to CABMV with resistance to SMV and WMV might be due to the linkage of one of the genetic factors conditioning resistance to CABMV with *Smv/Wmv*. In the winter screens of F_3 families, we were unable to distinguish *Cam2* from *cam3*, thus it is still unclear which one might account for the association of the resistance responses. A possible association between *Azm2* and either *Cam2* or *cam3* has not been not been explored.

Using plant-virus interactions to assess genetic variability in the virus and host

Plant resistance genes are defined specifically by their effect on the fate of a viral pathogen as it moves through the infection process. The absence of an immune system in plants suggests that some of these genes could directly block critical steps in viral infection either by the presence or absence of their product. In view of the extremely intimate intracellular nature of the parasitic interaction of viruses with their hosts, these host genes or gene clusters could be used to assess genetic variation in the pathogenic processes of pathogens. For example, in *P. vulgaris*, a single locus, the *I* locus, conditions dominant resistant and/or systemic necrotic responses to nine closely related potyviruses (Kyle and Dickson 1988; Fisher and Kyle 1994). Because of similar phenotypes, identical inheritance, and linkage, we believe that the gene or genes at or near this locus may interrupt a highly conserved function of viral pathogenesis which is shared by these viruses due to common ancestry or recombinational events. In GN 1140, systemic resistance responses to the four potyviruses in this study are also phenotypically similar to one another. However, in this case, resistance responses are not identically inherited, although there may be a genetic association between some of the factors involved. Because of the differences in inheritance, we would predict that the viral structures or processes interrupted by genes in GN 1140 are more variable than viral structures or processes involved in *I* locus resistance. By understanding the genetic diversity that exists within a viral family, we might be able to make predictions about the stability of different types of resistance genes.

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