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Inheritance of resistance to watermelon mosaic virus in the cucumber line TMG-1: tissue-specific expression and relationship to zucchini yellow mosaic virus resistance

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Abstract The inbred cucumber (*Cucumis sativus* L.) line TMG-1 is resistant to three potyviruses: zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), and the watermelon strain of papaya ringspot virus (PRSV-W). The genetics of resistance to WMV and the relationship of WMV resistance to ZYMV resistance were examined. TMG-1 was crossed with WI-2757, a susceptible inbred line. F_1, F_2 and backcross progeny populations were screened for resistance to WMV and/or ZYMV. Two independently assorting factors conferred resistance to WMV. One resistance was conferred by a single recessive gene from TMG-1 (*wmv-2*). The second resistance was conferred by an epistatic interaction between a second recessive gene from TMG-1 (*wmv-3*) and either a dominant gene from WI-2757 (*Wmv-4*) or a third recessive gene from TMG-1 (*wmv-4*) located 20–30 cM from *wmv-3*. The two resistances exhibited tissue-specific expression. Resistance conferred by *wmv-2* was expressed in the cotyledons and throughout the plant. Resistance conferred by *wmv-3* + *Wmv-4* (or *wmv-4*) was expressed only in true leaves. The gene conferring resistance to ZYMV appeared to be the same as, or tightly linked to one of the WMV resistance genes, *wmv-3*.

Key words Plant virus resistance · Potyvirus · *Cucumis sativus* L.

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Introduction

Potyviruses are the most economically important group of plant viruses (Hollings and Brunt 1981). Most crops are infected by one, if not several, members of this group. At least three distinct potyviruses, zucchini yellow mosaic virus (ZYMV) (Lisa and Lecoq 1984), watermelon mosaic virus (WMV) (Purcifull et al. 1984), and the watermelon strain of papaya ringspot virus (PRSV-W) (Purcifull and Gonsalves 1984), cause severe losses in cucurbit crops (e.g., Nameth et al. 1985, 1986; Davis and Mizuki 1985; Sammons et al. 1989; Perring et al. 1992). Provvidenti (1985) identified resistance to all three of these viruses in a single plant selection from the Chinese cucumber cultivar 'Taichung Mau Gau' (TMG-1). Cultivars that are resistant to these three cucurbit potyviruses would be very valuable, especially because of the frequent occurrence of mixed infections (Nameth et al. 1985; Davis and Mizuki 1985).

Multiple potyvirus resistance can be conditioned by several independent genes, by linked genes, or by a gene at a single locus. In *Phaseolus vulgaris*, the dominant *I* allele confers a systemic necrotic resistance at temperatures below 30 °C to five potyviruses: bean common mosaic virus, blackeye cowpea mosaic virus, cowpea aphid-borne mosaic virus, soybean mosaic virus, watermelon mosaic virus, and possibly passion fruit woodiness virus (Kyle and Dickson 1988; Provvidenti et al. 1983; Provvidenti 1993). To data, it has not been possible to break the linkage among these resistances. Alleles conferring resistance to more than one virus also exist in *Pisum sativum* (Schroeder and Provvidenti 1971) and *Solanum stoloniferum* (Cockerham 1970), and a single dominat gene in *Curcurbita moschata* was recently reported to confer resistance to both ZYMV and WMV (Gilbert-Albertini et al. 1993). Tightly clustered arrays of multiple potyvirus resistance genes have been identified in pea; one cluster is located on linkage group 6, another on linkage group 2 (Provvidenti 1987b, 1990, 1991; Provvidenti and Alconero 1988). Linked, but

separable genes have been identified in soybean for resistance to peanut stripe virus and soybean mosaic virus (Choi et al. 1989).

In cucumber (*Cucumis sativus* L.), several monogenic resistance have been characterized. Resistance to PRSV-W (formerly called WMV-1) in the cultivar 'Surinam Local' is controlled by a single recessive gene (*wmv-1-1*, Wang et al. 1984; renamed *prsv*, Wehner 1993). Resistance to WMV in the cultivar 'Kyoto 3 feet long' is due to a single dominant gene (*Wmv*, Cohen et al. 1971). In TMG-1, the resistance to ZYMV is coferred by a single recessive gene (*zym*, Provvidenti 1987a), and resistance to PRSV-W is due to a single dominant gene (*Prsv-2*, Wai and Grumet 1995); inheritance of resistance to WMV in TMG-1 was not characterized. In the investigation presented here we sought to determine the genetics of WMV resistance in TMG-1 and to determine the relationship between the resistances to WMV and ZYMV.

Materials and methods

Maintenance of virus inocula

ZYMV (Connecticut strain, Provvidenti et al. 1984) and WMV (ATCC PV379) were propagated in zucchini squash plants (*Cucurbita pepo* cv 'Blackjack', Petoseed Co, Saticoy, Calif.) maintained in a growth chamber (16-h day, 26 °C constant temperautre, ca. 300 µmol photons $M^{-2}s^{-1}$). Cotyledons of 1-week-old seedlings were lightly dusted with 320-grift Carborundum (Fisher Scientific, Pittsburgh, Pa.) and mechanically inoculated using sponge plugs. Virus-infected tissue (lyophilized, frozen, or fresh) was macerated in ice-cold 20 m*M* sodium phosphate buffer, pH 7.0, in a pre-chilled mortar and pestle. All non-biological materials were sterilized prior to use. Young, symptomatic virus-infected leaves were harvested for use as inocula sources at the time when symptoms were expressed optimally (2–4 weeks). ZYMV and WMV were differentiated using *Phaseolus vulgaris* cv 'Black Turtle 2' (Provvidenti et al. 1984). WMV elicits prominent, systemic mosaic symptoms in approximately 2–3 weeks, while ZYMV causes red, necrotic, local lesions on the inoculated leaves.

Cucumber genotypes

The inbred cucmber (*Cucumis sativus* L.) lines TMG-1, resistant to ZYMV, WMV, and PRSV-W, (Provvidenti 1985), and WI-2757, susceptible to all three viruses (Peterson et al. 1982), were provided by Dr. J. Staub (US Department of Agriculture, University of Wisconsin-Madison). The F_1 progeny (WI-2757 × TMG-1) were either self- or sib-pollinated to produce the F_2 generation or crossed to parents to produce reciprocal backcross families (WI-2757 × F_1 and F_1 × TMG-1). The inbred line 'Straight 8' (Stokes Seeds, Buffalo, N.Y.) was used as an additional control genotype that is susceptible to all three viruses.

Propagation of rooted cuttings

Rooted cuttings of TMG-1, WI-2757, and their F_1 and F_2 progeny were made by cutting plants two nodes below the terminal whorl with an ethanol-sterilized razor blade. After removing the leaf at the lowest node, each cutting was dipped in fungicide (Captan, Zeneca Agricultural Products, Wilmington, Del.) and placed in an 1 and 1/4 X 1 X 1 and 1/2-inch rooting cube (Smithers-Oasis; Kent, Ohio). Trays were filled with tap water to a depth of 2 cm, and the cuttings were covered with plastic wrap to maintain high humidity for 5 days. The plastic was peeled back slowly on a daily basis until rootlets emerged through the rooting cubes (approximately 2 weeks). Plantlets were transplanted to wet Baccto Professional Planting Mix (The Michigan Peat Company, Tex.), and allowed to grow for 2–3 weeks prior to inoculation with virus.

Experimental designs and data analysis

Plants were mechanically inoculated with virus-infected sap (approximately 1 : 4 dilution leaf material : buffer) at either the cotyledonary stage and/or the true leaf stage. Rows of susceptible 'Straight 8' plants were evenly spaced throughout each experiment in order to detect any possible vairation in inoculation technique and symptom expression. For the F_2 populations, 10 rows of F_2 individuals with 10 plants/row were interspersed with 5 internal control rows consisting of inoculated and mock-inoculated TMG-1, WI-2757, and F_1 plants. Backcross populations of 20–120 individuals (10 plants/row) also contained evenly spaced control rows. Each experiment was performed two to five times. The number of times each experiment was performed is included in each table; experiments termed as independent were performed at different times in the greenhouse. Chi-square analyses were performed on data from each experiment individually, and on the pooled data from repeated experiments. In each case there was agreement among individual experiments (see Table footnotes). Genetic models proposed are the simplest ones that explained the collective data sets.

Secondary inoculation of resistant plants and F_2 cutting experiments

To test for the relationship between the resistances to ZYMV and WMV (i.e., does a common gene confer resistance to both viruses?) we sought to compare the response of a given individual to inoculation by both ZYMV and WMV. Experiments were performed in two ways. (1) Clonally propagated pairs of genetically identical F_2 individuals were prepared as described above; one member of the pair was inoculated with ZYMV, the other with WMV. Rooted cuttings of TMG-1, WI-2757, and their F_1 progeny were included as controls. (2) Sequential inoculations of ZYMV followed by WMV were performed on F_2 and BC (F_1 × TMG-1) progeny. Individuals were inoculated with ZYMV; those with symptoms were discarded while those without symptoms were assayed by ELISA to verify that they were free of virus. In some experiments, the plants were inoculated with ZYMV a second time to ensure that there were no escapes prior to inoculation with WMV. Half-fully expanded leaves of the virus-free individuals then were inoculated with WMV. Additional control rows composed of plants at the same developmental stage as those used for sequential inoculations were added to experiments at the time that they were inoculated with WMV.

Scoring of symptoms

Plants were scored when the symptoms were most clearly expressed, generally 7–14 days after inoculation. Susceptibility of an individual plant to virus infection was scored visually and/or by ELISA. Symptoms caused by cucurbit potyviruses include the presence of mosaic, severe leaf distortion, or rugosity. Symptoms were rated using a scale from 0 to 4, where: 0 = no symptom expression; 1 = light mosaic on at least one leaf; 2 = moderate mosaic on one or more leaves; 3 = prominent mosaic on one or more leaves; 4 = severe mosaic on several leaves, symptoms spread to terminal leaves, often severe stunting. Many experiments were scored by two people, and there was agreement to within one point for the ratings given to each plant. When assigning a simple classification of resistant or susceptible, any score of 1 or greater (any symptom expression) was classified as susceptible.

ELISA analyses

One or two leaves at the half- to first fully-expanded stage were harvested from each plant and stored at either 4 °C or −80 °C. ELISAs were performed either using standard sandwich methods as described by Clark and Adams (1977) or using a modified version of the leaf disk procedure of Romaine et al. (1981) as described below. The two methods were verified to give comparable results. At least four or more healthy controls were included on each plate. Healthy and mock-inoculated controls of all the genotypes (TMG-1, WI-2757, their F_1 progeny, 'Straight 8', and 'Blackjack' zucchini squash) gave comparable readings. Buffers were prepared according to Clark et al. (1986). ZYMV and WMV were both detected with anti-ZYMV (CT strain) polyclonal rabbit IgG antibody (Hammar and Grumet, unpublished). For the sandwich assays, the anti-ZYMV antibody was conjugated with alkaline phosphatase (Sigma, St. Louis, Mo.) as per Clark and Adams (1977). Samples were reacted with *p*-nitrophenyl phosphate (Sigma, St. Louis, Mo.), and absorbance (405 nm) was monitored using an EIA Reader Model EL-307 (Bio-Tek Instruments, Laboratory Division, Winooski, Vt.). To perform the leaf disk assays, 6-mm disks (prepared with a paper hole puncher) were placed immediately into microtiter plate wells contianing 200 µl coating buffer and either incubated directly or frozen and thawed prior to incubation (either method worked equally well). Samples then were reacted with 100 µl per well of 1 µg/ml anti-virus-specific antibody in virus buffer at pH 7.4. The virus-specific antibody was indirectly detected using alkaline phosphatase conjugated goat anti-rabbit IgG (Sigma, St. Louis, Mo.) and *p*-nitrophenyl phosphate as described above.

Results

Resistance to WMV appeared to be controlled by recessive factors. When inoculated with WMV, plants of the TMG-1 parent remained symptom free while plants of the WI-2757 parent developed prominent symptoms (ratings of 3–4). Plants of the F_1 progeny developed symptoms comparable to those of the susceptible parent. The observed segregation ratios in the F_2 and backcross progeny populations, however, differed depending on how the experiments were performed. When the plants were inoculated at the cotyledon stage, simple segregation ratios were observed (Table 1). The F_2 progeny segregated in a 3:1 susceptible:resistant (S:R) ratio. The $F_1 \times$ TMG-1 backcross progeny segregated in a 1:1 (S:R) ratio, and the WI-2757 × F_1 backcross progeny were all susceptible. These data suggest that resistance is controlled by a single recessive gene [proposed gene designation: *wmv-2* (to distinguish it from the dominant *Wmv-1* in cv 'Kyoto 3 feet long')].

When true leaves were inoculated, however, the observed segregation ratios suggested that the inheritance of resistance to WMV was more complex (Table 2).

Table 1 Response of TMG-1, WI-2757, and their progeny to inoculation with WMV at the cotyledon stage (*ns* non-significant χ^2 value)

Parent or Progeny	Number of plants		Expected ratio (R:S)[a]	χ^2
	Resistant	Susceptible		
TMG-1	104	0		
WI-2757	0	86		
F_1	0	72		
F_2[b]	117	402	1:3	1.85 ns
$F_1 \times$ TMG-1[c]	198	195	1:1	0.011 ns
WI-2757 × F_1	0	22	0:1	

[a] Expected ratios based on a single recessive gene model, R = resistant, S = susceptible

[b] Data pooled from two independent experiments. Each experiment fits the predicted ratios: $\chi^2_{exp1} = 0.74$, $\chi^2_{exp2} = 0.81$, χ^2 homogeneity = 0.053

[c] Data pooled from two independent experiments. Each experiment fits the predicted ratios. $\chi^2_{exp1} = 0.152$, $\chi^2_{exp2} = 0.170$, χ^2 homogeneity = 0.44

Table 2 Response of TMG-1, WI-2757, and their progeny to inoculation with WMV at the true leaf stage

Parent or Progeny	Number of plants		Genetic models[a]							
	R[b]	S	One-gene model		Two-gene model		Three-gene model A		Three-gene model B	
			R:S	χ^2	R:S	χ^2	R:S	χ^2	R:S	χ^2
TMG-1	41	0								
WI-2757	0	34								
F_1	0	20								
F_2[c]	124	167	1:3	47.2**	7:9	0.11 ns	25:39	1.39 ns	26:38	0.43 ns
$F_1 \times$ TMG-1[d]	132	79	1:1	12.82**	3:1	16.70**	5:3	0.003 ns	43:21	1.95 ns
WI-2757 × F_1	0	22	0:1		0:1		0:1		0:1	

*, **, ns significant χ^2 values indicate that the observed data do not support the proposed genetic model: $*P < 0.05$; $**P < 0.01$; ns, not significant, $P \geq 0.05$

[a] Expected ratios are presented for four different models: (1) resistance conferred by a single recessive gene; (2) resistance conferred by two independently assorting recessive genes; and, (3) two, separate independently assorting resistance factors conferred by three genes. The first resistance factor is due to a single recessive gene; the second factor results from either: (A) an epistatic interaction between a single recessive gene from TMG-1 and a single dominant gene from 2757, or (B) two linked recessive genes from TMG-1 at a distance of approximately 20 cM–30 cM. See also Table 4A for a further description of the three-gene model

[b] R = resistant, no symtom expression; S = susceptible, symptom expression of 1 or greater, as described in Methods

[c] Data pooled from three independent experiments. Each experiment fits the predicted ratios for the three-gene model: $\chi^2_{exp1} = 1.54$, $\chi^2_{exp2} = 0.029$, and $\chi^2_{exp3} = 0.095$. χ^2 homogeneity = 1.68

[d] Data pooled from four independent experiments. Each experiment fits the predicted ratios for the three-gene model: $\chi^2_{exp1} = 0.48$, $\chi^2_{exp2} = 0.066$, $\chi^2_{exp3} = 0.005$, $\chi^2_{exp4} = 0.019$. χ^2 homogeneity = 0.981. Two of these experiments (exp. 3 and 4) were performed simultaneously with cotyledon inoculations

Segregation ratios in the F_2 population were consistent with a model proposing that either of two independently assorting recessive genes could confer resistance to WMV. However, the F_1 × TMG-1 backcross progeny gave ratios that more closely fit a 5:3 (R:S) segregation rather than the 3:1 (R:S) that would be expected for two independent recessive genes. The simplest models that best fit the data from the true leaf inoculation experiments propose the involvement of a third gene that acts epistatically to one of the two recessive resistance genes. In these models one resistance would be conferred by a single recessive gene from TMG-1 (proposed genotype *wmv-2wmv-2*). The second resistance would be conferred by an epistatic interaction between a second recessive gene from TMG-1 and either (A) a dominant gene from WI-2757 (proposed genotype: *wmv-3wmv-3*, *Wmv-4-*; Model 3A) or (B) a third recessive gene from TMG-1 located 20–30 cM from *wmv-3* (proposed genotype: *wmv-3wmv-3*, *wmv-4wmv-4*; Model 3B).

Possible explanations for the variant segregation ratios in the two types of experiments (Table 1 vs. Table 2) inlcude: different environmental conditions when the experiments were performed, different ages of the plants at the time of inoculation, or tissue-specific expression of the resistances (cotyledon vs. true leaf). To differentiate between these possibilities, we performed concurrent sets of experiments where only cotyledons, cotyledons and true leaves, or only true leaves were inoculated. All plants were the same age (two true leaf stage). Different segregation ratios again were observed depending on whether true leaves or cotyledons were inoculated. The inoculation of cotyledons alone indicated a single recessive gene (ratios of 50:144 R:S for the F_2 generation $\chi^2_{1:3} = 0.015$, ns; and 82:85 R:S for the backcross to TMG-1 $\chi^2_{1:1} = 0.024$, ns). The inoculation of true leaves indicated two resistances as described earlier (ratios of 88:151 R:S for the F_2 generation, $\chi^2_{\text{model 3A}} = 0.414$, ns; or $\chi^2_{\text{model 3B}} = 1.25$, ns; and 68:38 R:S for the backcross generation $\chi^2_{\text{model 3A}} = 0.63$, ns or $\chi^2_{\text{model 3B}} = 0.27$, ns). The inoculation of both true leaves and cotyledons gave the same segregation ratios as when cotyledons alone were inoculated (data not shown). Inoculation of either the second true leaf or the eighth true leaf gave similar results (64:41 R:S for eighth true leaf vs. 68:38 for the second true leaf in the F_1 × TMG-1 backcross). These results suggest that the observed difference is due to the tissue that is being inoculated, and not differences in plant age at the time of inoculation or different environmental conditions.

Consistent with the possibility of tissue-specific expression of the two resistance factors were observations made on segregating populations inoculated at the cotyledon stage. Upon closer inspection of the susceptible individuals in cotyledon-inoculated experiments, two levels of symptom expression were detected. In experiments using F_1 × TMG-1 backcross progeny, the individuals again segregated as a single gene trait, 1:1 resistance:susceptible (90R:84S). Approximately one quarter of the susceptible class (20 plants) showed mild symptoms, while the remainder (64 plants) exhibited more severe symptoms. An approximately 3:1 (susceptible:partially resistant) segregation within the susceptible class would be predicted if the second gene could confer only partial resistance once an infection became established in the cotyledons. In one of the experiments there were differences in symptom spread as well as severity. About one quarter of the susceptible class showed symtom spread approximately one-tenth of the way down the leaf, while the remainder of the susceptible class exhibited a more extensive moasic. These observations gave further evidence for two separable resistances and supported the hypothesis that the second resistance was not expressed until the true leaf stage.

We next sought to determine the relationship between resistance to WMV and resistance to ZYMV. Consistent with the results of Provvidenti (1987a), resisitance to ZYMV was conferred by a single recessive gene (Table 3). The ratios observed for ZYMV were the same whether true leaves or cotyledons were inoculated (data not shown). To test the possibility that the single recessive gene that confers resistance to ZYMV is also one of the recessive genes that confers resistance to WMV, we used two approaches. In the first set of experiments, young true leaves of the individual members of clonal pairs of vegetatively propagated, genetically identical F_2 plants were inoculated with either WMV or ZYMV. Four possible models were tested: (1) the ZYMV resistance gene is the same recessive gene that idependently confers resistance to WMV (*zym* = *wmv-2*); (2) the ZYMV resistance gene is the second recessive gene that is involved in resistance to WMV (*zym* = *wmv-3*); (3) the ZYMV resistance is conferred by the second resistance to WMV involving the epistatic interaction between two genes, either a single recessive resistance gene from TMG-1 and a dominant gene from WI-2757 (*zym* = *wmv-3* + *Wmv-4*) or two linked recessive genes from TMG-1 (*zym* = *wmv-3* + *wmv-*

Table 3 Response of TMG-1, WI-2757, and their progeny to inoculation with ZYMV

Parent or Progeny	Number of plants		Expected ratio (R:S)[b]	χ^2
	R[a]	S		
TMG-1	58	0		
WI-2757	0	57		
F_1	0	42		
F_2[c]	134	390	1:3	0.06 ns
F_1 × TMG-1[d]	105	115	1:1	0.37 ns
WI-2757 × F_1	0	44	0:1	

[a] R = resistant, no symptom expression; S = susceptible, symptom expression of 1 or greater as described in Methods

[b] Expected ratios based on a single recessive gene model

[c] Data pooled from six independent experiments. Each experiment fits the predicted ratios: $\chi^2_{\text{exp1}} = 0.12$, $\chi^2_{\text{exp2}} = 0.27$, $\chi^2_{\text{exp3}} = 0.52$, $\chi^2_{\text{exp4}} = 0.042$, $\chi^2_{\text{exp5}} = 0.23$, and $\chi^2_{\text{exp6}} = 0.000$, χ^2 homogeneity = 1.12

[d] Data pooled from four independent experiments. Each experiment fits the predicted ratios. $\chi^2_{\text{exp1}} = 0.31$, $\chi^2_{\text{exp2}} = 0.093$, $\chi^2_{\text{exp3}} = 0.085$, and $\chi^2_{\text{exp4}} = 0.18$. χ^2 homogeneity = 1.2

4); and (4) four independently assorting genes confer resistance to WMV and ZYMV (three genes that confer resistance to WMV and a fourth one that confers resistance to ZYMV). The expected phenotypic and genotypic ratios for these models are presented in Table 4.

Models 1 and 3A and 3B predict that there would be no individuals that are resistant to ZYMV but susceptible to WMV (Tables 4, 5). These models proved unacceptable since this class of individuals was indeed observed (Table 5). Model 3A and 3B also can be ruled out because the ZYMV segregation data are not consistent with the involvement of two genes. The $F_1 \times$ TMG-1 backcross generation segregated 1:1 (R:S) for ZYMV (Table 3), and not 1:3 or 2.75:5.25 (R:S). Model 4, which proposes that the ZYMV resistance is completely independent of the WMV resistances also was not supported by the observed segregation ratios from the clonal pairs experiments (Table 5). The model that best fits the observed data (Model 2) predicts that *zym* is actually *wmv-3*, or that *zym* is tightly linked with *wmv-3*.

The second test of the relationship between the resistance to ZYMV and WMV was performed by sequential inoculations. F_2 and $F_1 \times$ TMG-1 backcross progeny were first inoculated with ZYMV, and then resistant individuals were tested for susceptibility to WMV. The results from these experiments (Table 6) closely paralleled the results from the clonal pairs experiments. Again,

Table 4 Predicted genotypes and phenotypes for resistance to WMV. (A) Summary of expected WMV resistance phenotype ratios for the three-gene model. (B) Predicted ZYMV phenotypes if resistance to ZYMV and WMV is controlled by a common gene

Genotype	(A) Three-gene model for WMV resistance				(B) Common gene models for WMV and ZYMV resistance			
	Expected phenotypes		Ratios for unlinked genes		Predicted ZYMV phenotypes			
	3A[a]	3B[a]	F_2	BC	Model 1 *zymv = wmv-2*	Model 2 *zymv = wmv-3*	Model 3A *zymv = wmv-3 + Wmv-4*	Model 3B *zymv = wmv-3 + wmv-4*
W2- W3- W4-[b]	S[c]	S	27	1	S	S	S	S
W2- W3- w4w4	S	S	9	1	S	S	S	S
W2- w3w3 W4-	R	S	9	1	S	R	R	S
W2- w3w3 w4w4	S	R	3	1	S	R[e]	S	R
w2w2 W3- W4-	R	R	9	1	R	S	S	S
w2w2 W3- w4w4	R	R	3	1	R	S	S	S
w2w2 w3w3 W4-	R	R	3	1	R	R	R	S
w2w2 w3w3 w4w4	R	R	1	1	R	R	S	R
Total R:S ratio			3A:25:39 3B[d]:26:38	5:3 5.38:2.62	F_2:1:3 BC:1:1	F_2:1:3 BC:1:1	F_2:3:13 BC:1:3	F_2:2.5:13.5 BC:2.75:5.25

[a] Model 3A: resistance is conferred by either w_2w_2 or $w_3w_3W_4$-. Model 3B: resistance is conferred by w_2w_2 or $w_3w_3w_4w_4$ where w_3 and w_4 are linked at a distance of ca. 20–30 cM

[b] *W2*, *W3*, *W4* = *Wmv-2*, *Wmv-3*, and *Wmv-4*, respectively

[c] S = susceptible, R = resistant

[d] F_2 ratios calculated at ca. 25 cM; BC ratios calculated at ca. 30 cM (assuming no double crossovers)

[e] Progeny class resistant to ZYMV but susceptible to WMV

Table 5 Segregation data for resistances to WMV and ZYMV using clonal pairs of vegetatively propagated F_2 cutting plants. Each member of a pair of vegetatively propagated, genetically identical F_2 plants was inoculated with either WMV or ZYMV. Data were pooled from three independent experiments

Phenotype		Observed	Predicted Ratios (Number of plants)			
ZYMV[a]	WMV[b]		Model 1 *zymv = wmv-2*	Model 2 *zymv = wmv-3*	Model 3A[c] *zymv = wmv-3 + Wmv-4*	Model 4 Four independent genes
S	S[d]	93	39 (108)	36 (100)	39 (108)	117 (81)
S	R	42	9 (25)	12 (33)	13 (36)	75 (52)
R	S	14	0 (0)	3 (8)	0 (0)	39 (27)
R	R	29	16 (44)	13 (36)	12 (33)	25 (17)
			χ^2 nd[e]	4.41 ns	nd	17.59**

** $P < 0.01$; ns = not significant, $P \geq 0.05$. Significant χ^2 value indicates that the observed data do not support the proposed genetic model

[a] The ratios for ZYMV alone were consistent with a single recessive gene (135:43, S:R; $\chi^2 = 0.029$)

[b] The ratios for WMV alone were consistent with the three-gene models (107:71, S:R; $\chi^2_{3A} = 0.023$; $\chi^2_{3B,\ 25\,cM} = 0.006$)

[c] The values are shown for Model 3A, but both 3A and 3B can be eliminated because these models predict that there would be no individuals susceptible to WMV but resistant to ZYMV.

[d] S = susceptible, R = resistant

[e] nd = not determined. These models were rejected due to the presence of individuals in the ZR/WS class. χ^2 cannot be determined

Table 6 Sequential virus inoculation data: inoculation of ZYMV-resistant plants with WMV. Plants classified as resistant to ZYMV did not exhibit ZYMV symptoms and the upper leaves were free of virus as determined by ELISA immediately preceding the WMV inoculation

Genotype	Observed response to WMV		Expected ratios in response to WMV inoculation							
			Model 1 *zymv* = *wmv-2*		Model 2 *zymv* = *wmv-3*		Model 3A or B *zymv* = *wmv-3* + *Wmv-4* or *wmv-3* + *wmv-4*		Model 4 Four independent genes	
	R[a]	S	R:S	χ^2	R:S	χ^2	R:S	χ^2	R:S	χ^2
F_2[b]	62	15	1:0	nd[d]	13:3	0.0003 ns	1:0	nd	34:30	21.1**
F_1 × TMG-1[c]	41	12	1:0	nd	3:1	0.056 ns	1:0	nd	5:3	4.4*

* $P < 0.05$; ** $P < 0.01$; ns, $P \geq 0.05$. Significant χ^2 values indicate that the observed data do not support the proposed genetic model

[a] R = resistant, S = susceptible

[b] Data pooled from two independent experiments. Each experiment fits the expected ratios for Model 2: $\chi^2_{exp1} = 0.13$ and $\chi^2_{exp2} = 0.044$. χ^2 homogeneity = 0.53

[c] Data pooled from two independent experiments. Each experiment fits the expected ratios for Model 2: $\chi^2_{exp1} = 0.009$ and $\chi^2_{exp2} = 0.31$. χ^2 homogeneity = 0.00

[d] nd = not determined. These models were rejected due to the presence of individuals in the ZR/WS class. χ^2 cannot be determined

there were individuals that were resistant to ZYMV susceptible to WMV. These findings suggest that it is unlikely that *zym* is *wmv-2* (Model 1), or that it is equivalent to the epistatic interaction of *wmv-3* and *Wmv-4* (Model 3A) or *wmv-4*(Model 3B). Again, a significant χ^2 value was obtained for the model that the ZYMV resistance is independent of the WMV resistances (Model 4). Finally, the hypothesis that *zym* is the same as, or very tightly linked to *wmv-3* (Model 2) is supported by these observations. Both experimental approaches led to acceptance of the same hypothesis.

Discussion

We have examined the inheritance of multiple potyvirus resistance in the cucumber line TMG-1. In this study we sought to determine the genetics of resistances to WMV and the relationship between the resistance to WMV and ZYMV. The resistance to WMV from TMG-1 appear to be different from the dominant resistance that has been described in 'Kyoto 3 feet long' (Cohen et al. 1971). Segregation data from the progeny of TMG-1 and WI-2757 indicated that resistance to WMV was due to two independent factors controlled by a total of three separate genes (*wmv-2*, *wmv-3*, *Wmv-4*, or *wmv-4*). Comparisons of experiments where either cotyledons or true leaves were inoculated indicated that the two factors were under different developmental control. The first resistance factor, which was expressed in the cotyledon and throughout the plant, was conferred by a single recessive gene (*wmv-2*). In contrast, the second factor was expressed only in true leaf tissue and appeared to be the result of an epistatic interaction between two genes, either a recessive gene from TMG-1 and a dominant gene from WI-2757 (*wmv-3*, *Wmv-4*) or two recessive genes from TMG-1 linked at distance of 20–30 cM (*wmv-3*, *wmv-4*). At this time we cannot distinguish between these two possibilities. If it is true that the second resistance involves a dominant gene from WI-2757, it will be of interest to determine whether that factor is unique to the WI-12757 gneotype. At this time we also do not know whether it is *wmv-3*, *Wmv-4* (or *wmv-4*), or both that are not expressed until the true leaf stage.

The finding that TMG-1 has two independently assorting resistance factors to a single virus (WMV) is not unprecedented. Two separate resistances were reported for WMV in *Phaseolus vulgaris* (Kyle and Provvidenti 1987) and for peanut mottle virus and soybean mosaic virus in soybean (Buss et al. 1985; Chen et al. 1993; Bowers et al. 1992). Similarly, two pairs of genes located on separate linkage groups have been found to confer resistance to clover yellow vein virus and pea seed-borne mosaic virus in pea (Provvidenti 1987b; Provvidenti and Alconero 1988). In most of the above examples, the separate loci came from different lines and were differentiated by complementation tests. For WMV resistance in TMG-1, the two genes were already in the same line. It is known if this was a result of breeding efforts that led to the combination of the two resistance loci, or to gene duplication and rearrangement, as has been suggested as a possibility in the pea system where are clusters of virus resistance genes located on linkage groups 2 and 6 (Provvidenti 1987b; Kyle and Provvidenti 1993). In *Phaseolus vulgaris*, similar to what was observed in TMG-1, there are independent, unlinked systems, each of which is capable of conferring resistance to bean common mosaic virus (Kyle and Provvidenti, 1993). One resistance is conferred by the dominant *I* allele, the second by recessive *bc* loci. The second resistance is due to an epistatic interaction between two factors, the *bc-u* gene and one of the *bc-1*, *-2* or *-3* alleles (Drijfhout 1991). An epistatic interaction between genes at two loci was also found to be responsible for resistance to cowpea chlorotic mottle virus in soybean (Goodrick et al. 1991).

The resistance to WMV from TMG-1 was also found to be closely related to the resistance to ZYMV. Data from experiments using clonal pairs of F_2 cuttings or sequential inoculations were consistent with a model indicating that the recessive gene for resistance to

ZYMV (*zym*) is the same as, or tightly linked with the recessive gene *wmv-3* that acts epistatically with an additional gene (*Wmv-4* or *wmv-4*) to confer resistance to WMV. Perhaps the additional factor encoded by *Wmv-4/wmv-4* is necessary to confer specificity to WMV versus ZYMV, or alternatively, *zym* and *wmv-3* may be separate, but tightly linked genes with different virus specificities.

The observation that a gene at an apparently non-segregating locus confer resistance to more than one virus has been reported in other species, including *Phaseolus vulgaris* L. (Kyle and Dickson 1988), *Pisum sativum* (Schroeder and Provvidenti 1971), and *Solanum stoloniferum* (Cockerham 1970), and more recently for ZYMV and WMV in *Curcurbita moschata* (Gilbert-Albertini et al. 1993). It is interesting to note though, that multiple virus resistance conferred by a single locus has thus far been reported only for potyviruses (Kyle and Provvidenti 1993). Whether the occurrence of genes that can confer resistance to more than one virus is unique to the potyvirus virus group or is just more readily observed because so many species are infected by more than one potyvirus is unclear. There are also cases of multiple virus resistances that were initially thought to be due to a single locus but later were resolved into independent loci as the appropriate differentially resistant genetic materials became available for study. Examples include clover yellow vein virus resistance and bean yellow mosaic virus resistance in pea (Provvidenti 1987b) and the resistances to potato virus Y and tobacco etch virus in *Capsicum annum* (Cook 1960, 1961). Studies with other susceptible genotypes may help to clarify further the role of the epistatic factors involved in the true-leaf expressed WMV resistance and the genetic relationship of the WMV and ZYMV resistances to each other.

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