New Source of Resistance to *Cucumber mosaic virus* in *Capsicum frutescens*

Rebecca C. Grube, Graduate Research Assistant, and **Yiping Zhang**, Former Graduate Research Assistant, Department of Plant Breeding, Cornell University, Ithaca, NY 14853; **John F. Murphy**, Associate Professor, Department of Plant Pathology, Auburn University, Auburn, AL 36849; **Fernando Loaiza-Figueroa**, Former Graduate Research Assistant, and **Vincent K. Lackney**, Research Support Specialist, Department of Plant Breeding, Cornell University; **Rosario Provvidenti**, Professor, Department of Plant Pathology, NYSAES-Cornell University, Geneva, NY 14456; and **Molly K. Jahn**, Associate Professor, Department of Plant Breeding, Cornell University

ABSTRACT

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A small-fruited pungent pepper accession, *Capsicum frutescens* 'BG2814-6', is resistant to several isolates of *Cucumber mosaic virus* (CMV). Resistance in BG2814-6 is incompletely penetrant and is controlled by at least two major recessive genes. The accession BG2814-6 and *C. annuum* 'Perennial', the leading source of CMV tolerance, appear to share one or more CMV resistance genes. CMV was detected in uninoculated leaves in a small percentage of both BG2814-6 and Perennial plants, indicating that resistance is not absolute in either genotype. Enzyme-linked immunosorbent assay absorbance values of samples taken from inoculated leaves corresponded well with visible viral symptoms for parental genotypes. While Perennial plants accumulated CMV antigen in inoculated leaves, CMV antigen was not detected in inoculated leaves of 73% of BG2814-6 plants, suggesting that there may be a mechanistic difference in resistance between the two genotypes.

Additional keywords: cucumovirus, disease resistance, pepper, polygenic resistance

Cucumber mosaic virus (CMV) occurs worldwide and is responsible for yield losses in many crops (3,6,8,17,30). Its ubiquity may be attributed to its broad natural host range, nonpersistent transmission by a large number of aphid species, and transmission through seeds in some hosts (3,8,34,35). Moreover, a number of plant species, including many weeds, may serve as reservoirs for CMV between crop seasons (27,28,35). These factors make prediction and management of CMV outbreaks difficult.

Corresponding author: M. K. Jahn
E-mail: mmk9@cornell.edu

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Current address of R. C. Grube: USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905; and current address of Y. Zhang: USDA-ARS, 10300 Baltimore Ave., Beltsville, MD 20705.

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In several crop species, transgenic plants expressing CMV coat protein, replicase, or CMV-associated satellite RNA sequences have been developed, and in some cases, these plants are highly resistant to CMV (10-12,16,18,21,22,33,38-41). In pepper, however, transgenic efforts to obtain CMV resistance are curbed by both the relative inefficiency of transformation in pepper and the limited number of genotypes amenable to transformation (32). Conventional genetic approaches, which may be supplemented by transgenic and other forms of crop protection as they are proven successful, are likely to result in the most complete and stable crop protection in pepper.

There have been many reports of genetic resistance to CMV in *Capsicum* spp., mostly within *C. annuum* L. (13,20). However, classification of inheritance patterns and transfer of resistance from many of these sources have been complicated by isolate specificity or screening difficulties (13). The best characterized source of resistance, *C. annuum* 'Perennial', a pungent Indian chili, has been described as having monogenic recessive, partially dominant, or polygenic recessive inheritance by different researchers (19,25,26,31). Further, it remains unclear whether the response to CMV in Perennial is tolerance or resistance (19,23,25). These inconsistencies may be explained, at least in part, by the use of different viral isolates, environmental conditions, and inoculation tech-

niques by different researchers. Regardless of its source, the variability observed in the performance of Perennial is a significant shortcoming of this genotype as the primary source of genetic resistance to CMV. The characterization of additional or improved sources of resistance to CMV in pepper would facilitate the development of commercially acceptable pepper varieties with adequate levels of CMV resistance.

The main objectives of this study were to evaluate the response of a previously undescribed source of CMV resistance, *C. frutescens* L. accession 'BG2814-6', to inoculation with CMV, and to examine the inheritance of this resistance. We sought to establish whether BG2814-6 represents a new and useful source of CMV resistance or tolerance and to compare BG2814-6 with Perennial, the leading source of CMV resistance available in pepper.

MATERIALS AND METHODS

Germ plasm and genetic populations.

The line BG2814-6 was derived by self-pollinating a single plant from *C. frutescens* BG2814, an accession collected in southern Mexico by J. Laborde, after selection for CMV resistance. The BG2814-6 line was inoculated with CMV, and asymptomatic plants BG2814-6-1 and BG2814-6-2 were selected. Self-pollinated progeny from BG2814-6-1 were inoculated and the asymptomatic plants BG2814-6-1-1 through BG2814-6-1-3 were selected. The symptomatic plant BG2814-6-1-4 was also selected and self-pollinated for progeny testing. Finally, BG2814-6-1-1 was self-pollinated, and two asymptomatic plants, BG2814-6-1-1-7 and BG2814-6-1-1-9, were obtained.

C. annuum cvs. Jupiter (Rogers Seed Co./Novartis, Naples, FL), CalCompac 648 (CalCompac Foods, King City, CA), Verdel (Rogers/Novartis), and Jalapeno, a potyvirus-resistant jalapeno breeding line (B. Villalon, Texas A&M University) were used as susceptible controls. The cultivar Perennial, provided by A. Palloix (INRA, Montfavet Cedex, France), was included as a resistant control.

Perennial and BG2814-6-1 were each intercrossed with the susceptible genotype Jupiter to produce F₁, F₂, and backcross (BC; to both resistant [R] and susceptible [S] parents) progenies. Crosses between

Perennial and BG2814-6-1 were made in order to assess the relationship of resistance from the two sources to one another. Data from reciprocal crosses (when available and tested) were pooled when χ^2 tests of homogeneity (37) indicated that similar ratios were obtained for both crosses.

CMV isolates and disease screening. Six CMV isolates were used for this study and maintained in the Cornell University squash breeding line *Cucurbita pepo* PMR Caserta. Three of the CMV isolates were obtained from John Hubbard, Asgrow/Seminis Vegetable Seeds: PPRC-V26 (CMV-V26), PPRC-V28 (CMV-V28), and PPRC-V27 (CMV-V27). Three Cornell University CMV isolates were used: CMV-NY, CMV-Fny, and CMV-CA (R. Provvidenti, T. Zitter, and P. Palukaitis). In initial experiments that included the resistant genotype Perennial and the susceptible genotype Jupiter as controls, BG2814-6 was essentially similar to Perennial in its phenotypic response to all six tested isolates of CMV (*data not shown*). Of these, inoculation with CMV-V27 consistently resulted in the most severe symptoms on susceptible genotypes under our conditions, and it was therefore used for all subsequent experiments. Pepper seedlings were germinated under plastic covers in 20-row seeding flats (Griffin Greenhouse and Nursery Supplies, Tewksbury, MA) and were transplanted into 6-by-12 styrofoam Speedling trays (Speedling, Inc., Sun City, FL) when cotyledons were fully expanded. All plants were rub-inoculated with inoculum consisting of 1 g of systemically infected squash leaf tissue ground in 10 ml of 50 mM potassium phosphate buffer, pH 7.0. Plants were rinsed with water within 30 min after inoculation. Three distinct experiments were conducted; inoculation procedures varied slightly for each experiment as described below.

To evaluate the phenotypic response of resistant, susceptible, and F_1 populations, the second- and third-oldest leaves of 5-week-old plants (at the 4 to 6 true leaf stage) were inoculated with CMV. Plants were monitored for symptom development on a daily basis, and accumulation of CMV in inoculated and uninoculated leaves was determined by enzyme-linked immuno-

sorbent assay (ELISA) in one-third of the plants from each genotype (11 to 18 plants per genotype per time period) at 8, 16, and 34 days post inoculation (dpi).

To examine inheritance of resistance, CMV was applied to the third- and fourth-oldest pair of leaves of plants at the 10 to 12 true leaf stage, followed 7 days later by inoculation of the next younger pair of leaves. Plants were monitored for symptom development every 3 to 5 days, and the final phenotypic evaluation was performed 13 weeks post inoculation (wpi).

For the third experiment, conducted to examine the performance of inbred *C. frutescens*-derived lines, a cotyledon stage assay similar to that described by Chainé et al. (5) was used. Seven days after inoculation of fully expanded cotyledons, the first pair of true leaves were inoculated. Plants were monitored for symptom development every 3 to 4 days. Leaf samples were taken for ELISA and the final phenotypic evaluation was performed 8 wpi. Cotyledon stage and older plant inoculations have been shown to give similar results for the genotypes used (14).

Indirect ELISA. Antiserum to CMV was obtained from the American Type Culture Collection (Rockville, MD). Immunoglobulin was precipitated using ammonium sulfate (15) and then passed through a DEAE Sephacel (Sigma Immunochemicals, St. Louis) column to selectively purify immunoglobulin G (IgG). Indirect ELISA was used for detection of CMV coat protein antigen (CMV CP) essentially according to Voller et al. (36). Plants were tested for CMV infection by analysis of 0.1 g of leaf tissue (two leaves) ground in 1.0 ml of 50 mM carbonate buffer, pH 9.6, and subsequently diluted 1:20. Anti-CMV IgG was used at 0.5 μ g/ml and goat antirabbit IgG conjugated to alkaline phosphatase (Sigma Immunochemicals) was used at a dilution of 1:8000. At each step, plates were incubated for at least 8 h at 4°C. Substrate (*p*-nitrophenylphosphate at 1.0 mg/ml in 10% diethanolamine, pH 9.8) reactions were allowed to develop for 30 min at room temperature. A sample was considered positive for CMV infection when the ELISA absorbance value (at 405 nm) was greater than the average absorbance value

of healthy uninoculated control tissue plus three standard deviations.

Inheritance studies. The observed segregation of resistance in populations grown to examine inheritance was evaluated for goodness of fit to the ratios predicted for one, two, or three independently segregating recessive genes with complete or incomplete penetrance. For genetic models involving genes with complete penetrance, expected R:S ratios for the BC R and F_2 data were 1:1 and 1:3 (1 gene), 1:3 and 1:15 (2 genes), and 1:15 and 1:63 (3 genes), respectively. For incomplete penetrance models, expected frequencies of resistant and susceptible individuals were modified to reflect the proportion of the resistant parent plants that show symptoms (Table 1). For all three incomplete penetrance models (p_1), the proportion of symptomatic plants in the resistant parent line was estimated from the results of the 1998 assays ($p_{1[\text{Perennial}]} = 0.057$, $p_{1[\text{BG2814-6}]} = 0.4074$).

RESULTS

***C. frutescens* BG2814-6 is resistant to CMV infection.** Most Jupiter plants (28 of 36 plants) inoculated with CMV-V27 exhibited systemic vein clearing, mild mosaic, and leaf distortion by 8 dpi. By 34 dpi, all surviving CMV-inoculated Jupiter plants had developed systemic symptoms that included severe leaf distortion and mosaic (Table 2). The BG2814-6 plants were heterogeneous in their response to CMV inoculation. By 34 dpi, most BG2814-6 plants remained asymptomatic, but 10 of the 44 plants inoculated had developed severe systemic mosaic and leaf distortion, comparable to the response seen in Jupiter. The cultivar Perennial was also heterogeneous in its response to inoculation with CMV. Although most remained asymptomatic throughout the experiment, 15 plants developed symptoms, including mosaic and leaf distortion, that were milder than those seen on Jupiter or BG2814-6.

At the latter sampling dates (16 and 34 dpi), inoculated leaves had abscised from several plants, preventing analysis of these leaves by ELISA (Table 3). All intact inoculated leaves of Jupiter tested positive for CMV CP at all three testing dates.

Table 1. Expected frequencies of resistance using models of one, two, and three completely recessive genes with incomplete penetrance

Population/genotype ^b	Expected frequencies (resistant/susceptible) ^a		
	1 gene	2 genes	3 genes
Susceptible parent	0/1	0/1	0/1
Resistant parent	$(1 - p_1)/p_1$	$(1 - p_1)/p_1$	$(1 - p_1)/p_1$
F_1	0/1	0/1	0/1
BC R	$0.5(1 - p_1)/1 - 0.5(1 - p_1)$	$0.25(1 - p_1)/1 - 0.25(1 - p_1)$	$0.0625(1 - p_1)/1 - 0.0625(1 - p_1)$
BC S	0/1	0/1	0/1
F_2	$0.25(1 - p_1)/1 - 0.25(1 - p_1)$	$0.0625(1 - p_1)/1 - 0.0625(1 - p_1)$	$0.01653(1 - p_1)/1 - 0.01653(1 - p_1)$

^a p_1 is defined as the probability of infection of a plant homozygous for the resistance gene. For complete penetrance models, $p_1 = 1$. Genes are considered completely recessive.

^b BC = backcross, R = resistant, and S = susceptible.

Similarly, all or nearly all (15 of 15 at 8 dpi, 11 of 12 at 16 dpi, and 14 of 15 at 34 dpi) intact inoculated leaves of Perennial also tested positive for CMV CP. In contrast, very few inoculated leaves of BG2814-6 tested positive for CMV CP throughout the experiment (1 of 14 at 8 dpi, 3 of 14 at 16 dpi, and 0 of 8 at 34 dpi).

CMV CP was detected in uninoculated leaves of all CMV-inoculated Jupiter plants tested at 8 and 34 dpi, and 8 of 11 of those tested at 16 dpi (Table 3). For both BG2814-6 and Perennial, CMV CP was detected in uninoculated leaves of only a

few plants, but this number increased over time. For Perennial, CMV CP was detected in uninoculated leaves of 1 of 15 plants at 8 dpi, 4 of 16 plants at 16 dpi, and 7 of 16 plants tested at 34 dpi. Similarly, for BG2814-6, CMV CP was detected in uninoculated leaves of 0 of 14 plants at 8 dpi, 3 of 15 at 16 dpi, and 3 of 15 plants tested at 34 dpi.

To examine the relationship between CMV CP levels detected and CMV symptoms, the mean absorbance value for inoculated and uninoculated leaves of asymptomatic and symptomatic plants of

each genotype are shown in Figure 1. CMV CP levels detected in both inoculated and uninoculated leaves of BG2814-6 were much lower than those detected in Jupiter. Symptomatic BG2814-6 plants, in contrast, showed levels of CMV CP comparable to those detected in Jupiter. A different trend was apparent for Perennial. As with BG2814-6, symptomatic Perennial plants accumulated levels of CMV CP similar to those in Jupiter. However, although CMV CP levels were quite low in uninoculated leaves of asymptomatic Perennial plants, high levels of CMV CP were detected in the inoculated leaves.

In order to determine whether the observed heterogeneity of resistance apparent phenotypically and by ELISA reflected genotypic variability, BG2814-6-derived inbred lines were inoculated with CMV-V27. No BG2814-6-derived line consistently gave rise to 100% resistant progeny using standard inoculation procedures, although susceptible controls were always 100% symptomatic (Table 4). Further, asymptomatic and highly symptomatic siblings (BG2814-6-1-1, -2, -3, and -4) gave rise to similar percentages of resistant progeny.

Inheritance of CMV resistance in BG2814-6: F₁ populations. For F₁ genotypes, symptoms were similar in severity to those of Jupiter and consisted of systemic mosaic and leaf distortion. At 8 dpi, almost all (44 of 49) Perennial × Jupiter F₁ plants were asymptomatic, whereas only 14 of 40 Jupiter × BG2814-6 F₁ and 8 of 36 Jupiter plants were asymptomatic (Table 2). By 34 dpi, all Jupiter and Jupiter × BG2814-6 F₁ plants had developed symptoms, while five of the Perennial × Jupiter F₁ plants remained asymptomatic.

Table 2. Phenotypes determined 8, 16 and 34 days post inoculation (dpi) with *Cucumber mosaic virus* isolate V27 for parent genotypes and F₁ populations

Genotype ^a	dpi	Symptomatic ^b	Asymptomatic	Total ^c
Jupiter	8	28	8	36
	16	33	3	36
	34	35	0	35
Perennial	8	1	46	47
	16	2	44	46
	34	15	31	46
BG2814-6	8	4	40	44
	16	10	32	42
	34	10	32	42
Perennial × 14-6 F ₁	8	0	44	44
	16	1	43	44
	34	11	33	44
Jupiter × 14-6 F ₁	8	26	14	40
	16	35	2	37
	34	40	0	40
Jupiter × Perennial F ₁	8	5	44	49
	16	27	20	47
	34	43	5	48

^a BG2814-6 = 14-6.

^b For Perennial, symptomatic plants showed a mild mosaic, mild leaf distortion, or both. For all other genotypes, symptomatic plants showed severe leaf distortion and severe mosaic symptoms. For all genotypes, asymptomatic plants showed no viral symptoms.

^c Discrepancies in total numbers of plants at different sampling times resulted from either death of plants or slow regrowth after earlier sampling for enzyme-linked immunosorbent assay, preventing determination of phenotype.

Table 3. Detection of *Cucumber mosaic virus* (CMV) coat protein (CP) by indirect enzyme-linked immunosorbent assay (ELISA) in several pepper genotypes after inoculation

Genotype ^b	Sampled (dpi)	ELISA results for inoculated/uninoculated leaves ^a					Total
		-/-	A/-	+/-	+/+	A/+	
Jupiter	8	...	...	...	12	...	12
	16	...	2	1	5	3	11
	34	...	...	...	10	3	13
Perennial	8	...	...	14	1	...	15
	16	1	...	11	4	...	16
	34	1	1	7	7	...	16
BG2814-6-1-2	8	13	...	1	...	...	14
	16	11	...	1	2	1	15
	34	8	4	...	...	3	15
(Per × BG2814-6-1) F ₁	8	5	...	10	1	...	16
	16	6	...	5	2	...	13
	34	...	...	4	10	...	14
(Jup × BG2814-6-1) F ₁	8	1	...	6	6	...	13
	16	...	1	...	5	7	13
	34	...	5	2	1	6	14
(Per × Jup) F ₁	8	...	...	11	4	...	15
	16	...	6	1	5	4	16
	34	...	7	5	4	2	18

^a Symbols to the left of the slash designate ELISA results for inoculated leaf samples, and symbols to the right designate ELISA results for uninoculated leaves; - = no CMV CP detected by ELISA, A = abscised, + = positive ELISA reaction for the presence of CMV CP. Threshold values for a positive ELISA reaction were determined as described above, and for 8, 16 and 34 days post inoculation (dpi) the thresholds were 0.188, 0.159, and 0.444, respectively.

^b Jupiter = Jup and Perennial = Per.

Further, while Jupiter $\times$ BG2814-6 F₁ plants developed symptoms at roughly the same rate as observed for Jupiter plants, those Perennial $\times$ Jupiter F₁ plants that developed symptoms did so more slowly.

At 8 dpi, inoculated leaves of all Perennial $\times$ Jupiter F₁ plants and 12 of 13 Jupiter $\times$ BG2814-6 F₁ plants tested positive for CMV CP (Table 3). At 16 dpi, inoculated leaves of over half of Perennial $\times$ Jupiter F₁ plants (10 of 16) and Jupiter $\times$ BG2814-6 F₁ plants (8 of 13) had abscised, and the remainder contained CMV CP. At 34 dpi, similar results were obtained; inoculated leaves of 9 of 17 Perennial $\times$ Jupiter F₁ plants and 11 of 14 Jupiter $\times$ BG2814-6 F₁ plants had abscised, and the remainder contained CMV CP. At all time points, sampled inoculated leaves of asymptomatic as well as symptomatic plants from the two F₁ populations contained levels of CMV CP comparable to those detected in Jupiter (Fig. 1).

Uninoculated leaves of symptomatic Jupiter $\times$ BG2814-6 and Perennial $\times$ Jupiter F₁ plants analyzed at 8 and 16 dpi contained amounts of CMV CP comparable to those detected in Jupiter plants (Fig. 1). At 34 dpi, however, uninoculated leaves of both populations contained lower levels of CMV CP than were detected in Jupiter plants. Uninoculated leaves of asymptomatic Jupiter $\times$ BG2814-6 F₁ and Perennial $\times$ Jupiter F₁ plants did not contain detectable amounts of CMV CP at any time.

Variability in amount of time required to develop systemic symptoms was also observed for both F₁ populations in the inheritance studies. For the inheritance experiments, all Jupiter plants were symptomatic by 4 wpi (*data not shown*). In contrast, only 11 of 36 Jupiter $\times$ BG2814-6 F₁ plants were symptomatic by 4 wpi, 22 of 36 were symptomatic by 8 wpi, and all plants were symptomatic by 13 wpi. In the Perennial $\times$ Jupiter F₁ population, only 2 of 35 plants were symptomatic by 4 wpi, but by 8 wpi, all 35 plants had developed systemic symptoms. When the experiment was concluded at 13 wpi, all plants of both F₁ populations were symptomatic (Table 5).

Inheritance of CMV resistance in BG2814-6: F₂ populations. BC R and F₂ populations were inoculated in 1998 for inheritance studies as described, and were examined for symptoms at 8 wpi (Table 5). Goodness of fit χ^2 tests were performed for each genetic model and *P* values for the observed data determined (Table 5). For BG2814-6, the F₂ data (susceptible classes pooled) best support a two-gene model (*P* = 0.295), and do not fit (*P* $\leq$ 0.015) the incomplete penetrance models tested. The BC R data best support the three-gene models (*P* = 0.197 and 0.327 for complete and incomplete penetrance, respectively). The action of two incompletely penetrant genes could also explain these data, although this model fits the data less well

Fig. 1. Detection of *Cucumber mosaic virus* (CMV) coat protein (CP) by indirect enzyme-linked immunosorbent assay (ELISA) in several pepper genotypes. Plants inoculated with CMV-V27 were sampled at either (A) 8, (B) 16, or (C) 34 days post inoculation. Average absorbance values are presented for inoculated (i) and uninoculated (u) leaves of symptomatic (s) and asymptomatic (a) plants of all genotypes tested. Genotypes are: Perennial = Per, BG2814-6 = 14-6, Jupiter = Jup, and uninoculated leaves from plants of all genotypes = CTRL. Number of samples in each category is indicated above the bar. A single standard deviation is represented by the line above the bar. For each time point, the cutoff for determining a positive reaction for CMV CP is shown by a dotted line.

($P = 0.037$). Consistent recovery of 2 to 7% of asymptomatic individuals in other $R \times S$ F_2 populations derived from *C. frutescens* and several susceptible genotypes (1994 and 1995 experiments, Table 5) suggests that two or more loci are involved in the resistance in BG2814-6, any number of which may be incompletely penetrant. The data from the Jupiter $\times$ BG2814-6 F_2 and the Jalapeno $\times$ CMRF4 F_2 populations best supported the model for two recessive genes, although, in both cases, the data could be considered consistent with the model for two genes with incomplete penetrance ($P = 0.024$ and 0.029 , respectively). The CalCompack 648 $\times$ CMRF4 F_2 and Verdel $\times$ CMRF4 F_2 data were best

explained by the action of two recessive genes with incomplete penetrance. For populations involving Perennial, both the F_2 and BC R data best supported a single recessive gene model ($P = 0.889$, F_2 ; $P = 0.099$, BC R), although a single gene with incomplete penetrance model also fit the data ($P = 0.583$, F_2 ; $P = 0.043$, BC R).

Genetic relationship between resistances from *C. frutescens* BG2814-6 and *C. annuum* Perennial. Both BG2814-6 and Perennial give rise to a small percentage of susceptible plants; therefore, a simple evaluation of complementation of resistance alleles from the two sources is not possible. BG2814-6 and Perennial were intercrossed, however, to examine whether

the resulting F_1 population responded to inoculation differently than the inbred parents with respect to symptom severity or frequency of asymptomatic plants. The Perennial $\times$ BG2814-6-1 F_1 contained 51 of 70 (73%) asymptomatic individuals (Table 5). The performance of the $R \times R$ F_1 was inferior to Perennial, but superior to BG2814-6, and was much more resistant than tested $R \times S$ F_1 populations using either resistant genotype alone.

The pattern of symptom development and viral accumulation observed for the Perennial $\times$ BG2814-6 F_1 at 8, 16, and 34 dpi was more similar to that observed for Perennial than the to that of BG2814-6 (Table 3, Fig. 1). CMV CP was detected in the inoculated leaves of 11 of 16 plants sampled at 8 dpi, in 7 of 13 plants sampled at 16 dpi, and in all plants sampled at 34 dpi. This is similar to results obtained for Perennial, and contrasts with results obtained for BG2814-6, where CMV CP was detected in only 3 of 15 plants tested at 34 dpi. The number of Perennial $\times$ BG2814-6 F_1 plants for which CMV CP was detected in uninoculated tissue also increased dramatically over time: 1 of 16 plants tested at 8 dpi, 2 of 13 tested at 16 dpi, and 10 of 14 tested at 34 dpi. ELISA values for Perennial $\times$ BG2814-6 F_1 plants did not correlate perfectly with phenotype; 2 of 11 F_1 plants containing CMV CP were asymptomatic, and 3 plants that appeared to have mild symptoms contained no detectable amounts of CMV CP in uninoculated leaves based on ELISA.

DISCUSSION

C. frutescens BG2814-6 represents a previously undescribed and potentially

Table 4. Response of progenies of *Capsicum frutescens* 'BG2814-6' to selection for *Cucumber mosaic virus* resistance followed by inbreeding

Experiment, line	Generations		
	Phenotype ^a	Inbred	% R ^b
Fall 1997			
BG2814-6	R	1	87.5 (16)
BG2814-6-1	R	2	51.4 (35)
BG2814-6-2	R	2	47.2 (36)
BG2814-6-1-1	R	3	72.7 (33)
BG2814-6-1-2	R	3	61.1 (36)
BG2814-6-1-3	R	3	86.8 (33)
BG2814-6-1-4	S	3	83.3 (36)
CalCompack 648	S	...	0 (40)
Fall 1998			
BG2814-6-1	R	2	59.3 (27)
BG2814-6-1-1-7	R	4	41.4 (29)
BG2814-6-1-1-9	R	4	22.5 (40)
CalCompack 648	S	...	0 (40)

^a R = resistant, completely asymptomatic; S = susceptible, includes a wide range and varying levels of symptoms including mosaic, mottle, and leaf distortion

^b Percent resistant offspring, with the total number of progeny individuals inoculated per line given in parentheses.

Table 5. Response of resistant and susceptible *Capsicum* genotypes and their progenies inoculated with *Cucumber mosaic virus* (CMV) isolate V27

Experiment, line/population ^a	NS ^b	S	P values for χ^2 tests					
			1-gene	2-gene	3-gene	1-gene ip ^c	2-gene ip	3-gene ip
Fall, 1998								
<i>C. annuum</i> Jupiter	0 (0%)	54	...	...	...	...	...	...
<i>C. frutescens</i> BG2814-6-1	16 (59%)	11	...	...	...	...	...	...
<i>C. annuum</i> Perennial	33 (94%)	2	...	...	...	...	...	...
F_1 (J $\times$ 14-6-1) ^d	0 (0%)	3	...	...	...	...	...	...
BC S (J $\times$ 14-6-1) $\times$ J ^d	0 (0%)	91	...	...	...	...	...	...
BC R (J $\times$ 14-6-1) $\times$ 14-6-1-1-7	0 (0%)	25	0.000	0.004	0.197	0.001	0.037	0.327
F_2 (J $\times$ 14-6-1) ^d	14 (6.9%)	189	0.000	0.295	0.000	0.015	0.002	0.000
F_1 (Per $\times$ J)	0 (0%)	35	...	...	...	...	...	...
BC R Per $\times$ (Per $\times$ J) F_1	26 (51%)	25	0.889	0.000	0.000	0.583	0.000	0.000
F_2 (Per $\times$ J)	33 (32%)	70	0.099	0.000	0.000	0.043	0.000	0.000
F_1 (Per $\times$ 14-6-1) ^d	51 (73%)	19	...	...	...	...	...	...
Fall, 1994								
F_2 (J $\times$ 14-6)	19 (6%)	291	0.000	0.930	0.000	0.000	0.024	0.000
Fall, 1995								
F_2 (CC648 $\times$ CMRF4)	35 (2.8%)	1,195	0.000	0.000	0.000	0.000	0.111	0.000
F_2 (Jalapeno $\times$ CMRF4)	23 (5.7%)	376	0.000	0.689	0.000	0.000	0.029	0.000
F_2 (Verdel $\times$ CMRF4)	27 (3.8%)	695	0.000	0.005	0.000	0.000	0.959	0.000

^a In all cases, susceptible controls were uniformly infected, and data were taken at approximately 8 weeks post inoculation. Jupiter = J, BG2814-6-1 = 14-6-1, Perennial = Per, BC = backcross, S = susceptible, R = resistant, CMRF4 is a CMV-resistant BG2814-6 $\times$ *C. annuum* NuMex RNaky F_4 , and CC648 = CalCompack 648.

^b NS = no symptoms, S = symptoms, including mild or severe mosaic and leaf distortion.

^c For incomplete penetrance (ip) models, p_1 was estimated for 14-6 and Perennial from results in screen I; $p_{14-6} = 0.407$, $p_{\text{Perennial}} = 0.057$.

^d For the crosses indicated, data from reciprocal crosses were combined. For the Jupiter $\times$ 14-6-1 F_1 and (Jupiter $\times$ 14-6-1) $\times$ Jupiter BC S populations, only one phenotypic class existed. For the Jupiter $\times$ 14-6-1 F_2 and the Perennial $\times$ 14-6-1 F_1 , χ^2 tests of genetic homogeneity (37) were performed and revealed no evidence to suggest that the reciprocal populations exhibited different patterns of segregation ($P = 0.8783$ and 0.9732 , respectively).

useful source of CMV resistance. As many as 87% of plants in BG2814-6 populations inoculated with a highly virulent isolate, CMV-27, remained asymptomatic, whereas susceptible controls uniformly developed severe symptoms. Concurrent evaluation of Perennial, another source of CMV resistance (4,19), revealed that these plants also remained asymptomatic. Both resistant genotypes had a small percentage of plants that developed systemic symptoms. The symptoms of BG2814-6, when present, tended to be severe, whereas symptoms of Perennial plants were much milder and often occurred at a lower frequency in the population. Based on ELISA results, however, the frequency of individuals that accumulated CMV CP in uninoculated tissues and the relative amounts of CMV that accumulated in both Perennial and BG2814-6 populations were similar.

Despite several generations of inbreeding, BG2814-6-derived lines remained heterogeneous in their response to CMV. Of these plants, 10 to 70% developed severe mosaic, leaf distortion, and stunting. Symptomatic and asymptomatic inbred plants both gave rise to similar levels of resistant progeny, suggesting that these plants do not differ genetically at CMV resistance loci. This rules out the possibility that heterozygosity at certain loci is required for resistance. Further, these results demonstrate that CMV resistance in BG2814-6 plants exhibits incomplete penetrance. Penetrance describes the degree to which a trait is expressed within a genetically homogeneous population that contains the alleles required for the trait. The level of penetrance of resistance can vary between experiments, depending upon the viral isolate used, light and temperature conditions, and the age and condition of the plant when inoculated and as symptoms develop (M. K. Jahn, *unpublished data*; 7). Incomplete penetrance has been documented for other plant virus resistance loci, including dominant resistance genes for *Tomato spotted wilt virus* in tomato (29) and potyviruses in pepper (2), and may result from the complexity of the plant-pathogen interactions taking place during infection.

In contrast to the results presented by Lapidot et al. (19), we found that ELISA values corresponded directly with CMV symptoms in Perennial, BG2814-6, and Jupiter. Further, an absence of detectable CMV CP in uninoculated tissues through 34 dpi indicates that both BG2814-6 and Perennial possess resistance rather than simply an asymptomatic infection (e.g., tolerance). In BG2814-6, CMV CP was not detected in the inoculated or uninoculated leaves of asymptomatic individuals, suggesting that replication or cell-to-cell movement of CMV was slowed dramatically or inhibited within the inoculated leaf. In contrast, asymptomatic Perennial plants frequently contained high CMV CP

titer within the inoculated leaf as soon as 8 dpi. These results suggest that the mechanisms of resistance to CMV in Perennial and BG2814-6 differ. Previous reports have indicated that Perennial possesses multiple different mechanisms of resistance, including reduced viral replication and reduced specific infectivity of CMV virions (24,25). It is unclear whether BG2814-6 displays a similar array of resistance mechanisms. We occasionally observed hypersensitive-like lesions on inoculated leaves of BG2814-6 and systemic necrosis and tip death in some but not all populations, which may suggest that BG2814-6 also has multiple mechanisms of resistance that may or may not be separable.

Analysis of $R \times S$ F_1 populations for both Perennial and BG2814-6 revealed complete susceptibility of all plants in both populations by 13 wpi, consistent with recessive inheritance for resistance in both sources. Individual F_1 plants, however, developed symptoms at different rates. ELISA values varied considerably for different F_1 plants, and did not relate precisely to F_1 phenotypes. Incomplete dominance could lead to a decrease in viral replication, accumulation, or both, in the $R \times S$ F_1 plants compared with that seen in the susceptible control genotype, and incomplete penetrance of resistance alleles could partially account for the variability seen within these presumably genetically uniform populations. Remaining genetic heterogeneity in both resistant parental genotypes could also contribute to these results. Reciprocal $R \times S$ F_1 populations performed similarly, providing no indication of maternal inheritance of resistance from BG2814-6.

Segregating F_2 populations with BG2814-6 used as the resistant parent routinely yielded 2 to 7% asymptomatic plants, which suggests that resistance is conferred by two to three major recessive genes, any number of which may be incompletely penetrant. The biological evidence for incomplete penetrance is strong, and a lack of fit of genetic models accounting for incomplete penetrance is likely due to inaccurate estimation of p_1 , which describes the degree of penetrance. The frequency of symptomatic individuals in BG2814-6-derived populations varied from experiment to experiment; therefore, more reliable estimates of p_1 could be best obtained by including very large numbers of the resistant parent in each experiment. Testing the genetic data (Table 5) using a wide range of theoretical values of p_1 BG2814-6 revealed that no single genetic model is consistent with the data for all populations for any value of p_1 . The best fit was obtained for $p_1 = 0.2$, for which two-gene models with incomplete penetrance give $P > 0.01$ (χ^2 goodness-of-fit test) for all populations tested except the CalCompack 648 $\times$ CMRF4 F_2 . There is some indication from an ongoing CMV resis-

tance breeding program that CalCompack 648 may possess more or different susceptibility alleles than are found in other susceptible genotypes, which may complicate resistance evaluation in this genetic background. We therefore conclude that resistance in BG2814-6 is most likely conferred by two recessive genes, one or both of which may be incompletely penetrant. Resistance in Perennial has been attributed previously to two or three incompletely dominant genes (19). Under our conditions, Perennial exhibited a higher frequency of asymptomatic and mildly symptomatic individuals than BG2814-6 in segregating populations using the same susceptible parent, and fits genetic models for a single resistance gene. The discrepancy between our data and those of Lapidot et al. (19) may be explained by environmental conditions, the CMV isolates used, or the susceptible genetic background used. It is also possible that there are two mechanisms by which Perennial minimizes the impact of CMV: first, a tolerance which only reduces symptom severity as observed in the advanced breeding lines of Lapidot et al. (19) and, second, true resistance which reduces viral accumulation in uninoculated tissue, as we have shown. Perennial may contain alleles conferring tolerance at one or more loci as well as alleles conferring resistance at a different locus. Alleles that confer CMV tolerance result in mild symptoms; therefore, they may be easily selected in place of alleles at true resistance loci, such that advanced breeding lines may contain tolerance rather than the resistance also found in Perennial.

The combination of distinct sets of resistance alleles at multiple loci in a single genotype may increase the durability and level of resistance, as well as the frequency of asymptomatic individuals in offspring, over that afforded by each set alone (9). Perennial and BG2814-6 each have CMV resistance with similar complex inheritance and incomplete penetrance. The frequency of resistant individuals in the Perennial $\times$ BG2814-6 F_1 was nearly as high as in both resistance parents, and was much higher than in the completely susceptible $R \times S$ F_1 populations. Although we cannot rule out the possibility that heterosis or complex interactions between nonallelic CMV resistance genes contributed to improved performance, these results suggest that Perennial and BG2814-6-1 share at least one CMV resistance gene. It is possible that the differences observed in the responses of Perennial and BG2814-6 may be entirely due to allelic differences rather than the involvement of different resistance loci.

Incomplete penetrance of the resistance phenotype and a variable viral assay combine to make phenotypic selection for CMV resistance very difficult. Polygenic recessive inheritance adds another level of complexity when using these sources of resistance in a breeding program, although

this type of resistance may be more stable in the field than that afforded by single genes (1). Linked markers for marker-assisted selection would greatly benefit such efforts. The development of effective markers always relies upon accurate genotypic determination, which in this case will require screening relatively large populations over multiple generations.

The most resistant commercial varieties, such as cvs. Nicklow's Emerald Bell (Nicklow's Vegetables, Inc., Ashland, MA) and Gadir (Clause Seeds, France), have resistance derived from Perennial or *C. baccatum* accessions. Under our assay conditions, both Gadir and Nicklow's Emerald Bell displayed severe symptoms (R. Grube, J. Jantz, and M. K. Jahn, unpublished data). The ongoing need for improved cultivars, in conjunction with our results indicating that BG2814-6 possesses alleles conferring more complete resistance than Perennial, suggest that BG2814-6 may provide very useful resistance when naturally infected in commercial fields. Advanced bell, jalapeno, and Anaheim-type lines with resistance derived from BG2814-6 are currently under development using conventional breeding strategies. Evaluation of this material under commercial growing conditions and field-level CMV inoculum pressure alongside Perennial-derived lines and commercially available material will allow better assessment of the potential utility of this source of CMV resistance.

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