Two Genes from *Phaseolus coccineus* Confer Resistance to Bean Golden Yellow Mosaic Virus in Common Bean

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ABSTRACT. Bean golden yellow mosaic virus (BGYMV), incited by a whitefly (*Bemisia tabaci* Gennadius) transmitted geminivirus, is an important disease that can limit common bean (*Phaseolus vulgaris* L.) production in Central America, the Caribbean, and southern Florida. Only a few genes are currently deployed in BGYMV-resistant common bean cultivars. The identification of novel sources of resistance would help bean breeders broaden the genetic base of resistance to this important virus. *Phaseolus coccineus* L. germplasm accession G35172 was found by International Center for Tropical Agriculture scientists to be resistant to BGYMV. Populations derived from an interspecific cross between *P. vulgaris* and *P. coccineus* were evaluated to study the inheritance of resistance to BGYMV. Segregation ratios of F₂ plants and other populations suggest that BGYMV resistance from *P. coccineus* is controlled by two genes. A recessive gene, with the proposed symbol *bgm-3*, confers resistance to leaf chlorosis and a dominant gene, with the proposed name *Bgp-2*, prevents pod deformation in the presence of BGYMV. Results from allelism tests with previously reported BGYMV resistance genes (*bgm*, *bgm-2*, and *Bgp*) and the absence of the SR-2 sequence-characterized amplified region marker for *bgm* support the hypothesis that *bgm-3* and *Bgp-2* are different genes for BGYMV resistance.

In Central America and the Caribbean, bean golden yellow mosaic virus (BGYMV) can cause significant reductions in seed yield and quality (Morales, 2000). Symptoms incited by this whitefly-transmitted geminivirus include intense leaf chlorosis, pod deformation, and stunted plants (Galvez and Morales, 1994). Chemical control of the vector is only partially effective, and increased production costs preclude the use of insecticides for most small-scale bean producers in the region with limited resources. Plant breeders have developed and released common bean (*Phaseolus vulgaris*) cultivars with resistance to BGYMV (Beaver and Miklas, 1999; Beebe,

1994; Rosas et al., 1997) and researchers have identified specific resistance genes such as *bgm* and *bgm-2*, which confer resistance to leaf chlorosis (Velez et al., 1998), and *Bgp-1*, which confers resistance to pod deformation (Acevedo-Román et al., 2004). The identification of additional genes for BGYMV resistance would permit bean breeders to broaden the genetic base of resistance to this important disease. The scarlet runner bean (*Phaseolus coccineus*) germplasm accession G35172 was identified at the International Center for Tropical Agriculture as a novel source of resistance to BGYMV in Central America and the Caribbean, and bean golden mosaic virus (BGMV) in Brazil (Singh et al., 2000). The objective of this research was to study in common bean the inheritance of BGYMV resistance derived from G35172.

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Materials and Methods

The advance-generation common bean breeding line I9557-9 was derived from the backcross HP8437-95 $\times$ G35172 $\times$ HP8437-95 made at the U.S. Department of Agriculture, Agricultural Research Service Tropical Agriculture Research Station in 1993. HP8437-95 is a BGYMV-susceptible small red line from the cross 15R-148 $\times$ 3M-81. The BC₁F_{2:3:5:7} lines were a single plant selected for BGYMV resistance in the field at the Isabela Substation in the F₂, F₃, and F₅ generations. The F₉ line I9557-9 with resistance derived from G35172 had the susceptible band (530 bp) from the SR-2 sequence-characterized amplified region (SCAR) marker based on a codominant randomly amplified polymorphic DNA diagnostic for presence or absence of the *bgm* gene for resistance to BGYMV (Blair et al., 2007).

A population of F_{2:3} lines from the cross 'Morales' $\times$ I9557-9 was developed to conduct an allelism test to determine whether I9557-9 had the same *bgm* or *Bgp-1* resistance genes present in the cultivar Morales (Beaver and Miklas, 1999). The cross was made in Feb. 1998 at the University of Puerto Rico (UPR), Mayagüez. The F₁ and F₂ nurseries were planted at the Isabela Substation in Nov. 1998 and Nov. 1999. Each generation, individual plants were harvested at random. One hundred ninety-eight F_{2:3} lines were screened for BGYMV resistance in a nursery planted in Feb. 2000 at UPR, Mayagüez. Spreader rows of the BGYMV susceptible cultivar Mayflower (Kelly et al., 1989) were inoculated with viruliferous whiteflies 2 weeks before planting the F_{2:3} nursery. The whiteflies were collected from a greenhouse at UPR, Mayagüez, after feeding for at least 48 h on bean plants infected with BGYMV. Natural populations of whiteflies were used to inoculate the bean plants in the field trials. Susceptible checks used to monitor disease pressure were infected with BGYMV. Individual 1-m rows of each F_{2:3} line were planted. Fifteen seeds were planted in rows that were spaced 0.6 m apart. At 50 d after planting, the F_{2:3} rows were classified as susceptible, segregating, or resistant to leaf chlorosis reaction and pod deformation incited by BGYMV. Chi-square tests were used to compare observed with expected patterns of segregation.

A population of F_{2:3} lines from the cross 'Arroyo Loro' $\times$ I9557-9 was developed to study the mode of inheritance of BGYMV resistance derived from G35172. 'Arroyo Loro' is susceptible to BGYMV. Two hundred F_{2:3} lines were screened for BGYMV resistance in a nursery planted in Feb. 2000 at UPR, Mayagüez. Individual 1-m rows of each F_{2:3} line were planted. Fifteen seeds were planted in rows that were spaced 0.6 m apart. The previously described methodology was used to inoculate and to evaluate the BGYMV reaction of the F_{2:3} lines.

In June 2001, PR0157-4-1 (Beaver et al., 2005), a BGYMV-resistant F_{3:5} line from the cross 'Arroyo Loro' $\times$ I9557-9, was crossed with the susceptible cultivar Arroyo Loro in a greenhouse crossing block planted at the Adjuntas Substation in Puerto Rico to generate an additional population for studying the inheritance of the G35172-derived resistance. The F₁ generation was planted in the greenhouse at the Adjuntas Substation in Nov. 2001, and seeds of individual plants were harvested. In addition, the F₁ plants were crossed with each parent to produce BC₁F₁ seeds for the inheritance study.

Two hundred forty-one F₂ plants of the cross PR0157-4-1 $\times$ 'Arroyo Loro' were evaluated for reactions to BGYMV in a nursery planted at Isabela, PR, in June 2002. Spreader rows of

the susceptible cultivar Top Crop were mechanically inoculated (Morales and Niessen, 1988) 6 d after planting. Whitefly populations were used in the field and greenhouse to promote the spread of the virus. Each F₂ plant was evaluated for the absence or presence of leaf chlorosis at 30 d and pod deformation at 55 d after planting. Chi-square tests were used to compare observed with different expected patterns of segregation.

In Dec. 2002, a greenhouse trial was planted at UPR, Mayagüez. The trial included 25 F₁ and 51 F₂ plants from the cross PR0157-4-1 $\times$ 'Arroyo Loro.' In addition, five seeds of each parent and 10 seeds of each backcross were planted in the trial. All the plants were mechanically inoculated 7 d after emergence using the technique described by Morales and Niessen (1988). In addition, aviruliferous whiteflies were introduced into the greenhouse to promote the spread of BGYMV (Adames-Mora et al., 1996). Each plant in the trial was evaluated for the absence or presence of leaf chlorosis and pod deformation at 45 and 55 d after planting respectively. Chi-square tests were used to compare observed with expected patterns of segregation.

Before the F₂ plants in the greenhouse trial were inoculated with BGYMV, samples from young leaf tissue were taken from each plant to screen for the presence of the SR-2 marker for the *bgm* gene for resistance to BGYMV (Blair et al., 2007). DNA extraction was conducted using the procedures for DNA-alkaline extraction by Afanador et al. (1993). DNA amplification was made following the polymerase chain reaction protocols for the SR-2 SCAR marker described by Miklas (2005) using a DNA concentration of 10 ng·L⁻¹. The molecular marker work was conducted in the Biotechnology and Nitrogen Fixation Laboratory at UPR, Mayagüez.

An additional allelism test was planted in Spring 2006 at the University of Florida Tropical Research and Education Center in Homestead in collaboration with Rogers/Syngenta Seeds (Boise, ID) bean researchers and the University of Florida Institute of Food and Agricultural Sciences of Miami-Dade County vegetable extension agent. The field trial included the BGYMV susceptible line XAN 176; 'Morales' (Beaver and Miklas, 1999), which has the *bgm* and *Bgp* genes for BGYMV resistance; PR0003-390, which has the *bgm-2* allele for BGYMV resistance; PR0247-49 (Beaver et al., 2005), which has the putative *bgm-3* and *Bgp-2* genes for resistance to BGYMV; and F₁s from the crosses 'Morales' $\times$ PR0247-49 and PR0247-49 $\times$ PR0003-390. Individual 1-m rows of each line were planted. Twelve seeds of each line and F₁ were planted in single rows that were spaced 0.81 m apart. A randomized, complete block design with two replications was used. The source of inoculum was a bush bean trial used to evaluate the BGYMV resistance of Rogers/Syngenta Seeds breeding lines. The bush bean trial contained several commercial cultivars—Dusky, Lynx, Thoroughbred, Ambra, Caprice, Concesa, Espada, Sahara, Savannah, Cadillac, and Top Crop—susceptible to BGYMV. The source of inoculum was planted 20 d before the allelism tests. At 42 and 62 d after planting, each row in the allelism trial was evaluated for the presence or absence of BGYMV symptoms in the leaves and the pods.

Results and Discussion

The presence of BGYMV-susceptible and segregating F_{2:3} rows from the cross I9557-9 $\times$ 'Morales' suggested that the

resistance to chlorosis and pod deformation derived from G35172 differs from the *bgm* and *Bgp-1* resistance genes found in 'Morales' (Table 1). F_{2:3} lines rather than individual F₂ plants were used in the allelism test planted in 2000 to reduce errors incited by plants in the field that escaped BGYMV infection. The level of BGYMV resistance in I9557-9 (absence of chlorosis and pod deformation) is greater than the moderate levels of BGYMV resistance found in bean lines such as DOR 303, which have the *bgm-2* gene for leaf chlorosis (Singh et al., 2000) but lack resistance to pod deformation.

In the field evaluation of the 'Arroyo Loro' × I9557-9 cross, only 8 of 200 F_{2:3} lines were completely resistant in the leaves and pods to BGYMV. These results support a hypothesis that two genes contribute to the BGYMV resistance of the F_{2:3} lines (Table 2).

Phenotypic ratios from the evaluation of F₂ plants from the PR0157-4-1 × 'Arroyo Loro' cross in field and greenhouse experiments suggested that a recessive gene confers resistance to leaf chlorosis and a dominant gene controls resistance to pod deformation incited by BGYMV (Table 3). Chi-square tests found both greenhouse and field data to fit a 9:3:3:1 segregation ratio. The symbol *bgm-3* is proposed for the recessive gene for resistance to leaf chlorosis and *Bgp-2* is proposed for the dominant gene for resistance to pod deformation in the presence of BGYMV.

In Florida, Ferwerda (2001) evaluated BGYMV resistance in the interspecific cross G35172 × ICA Pijao. He reported that a recessive gene conferred resistance to leaf chlorosis and presented evidence of a second gene involved in the expression of BGYMV resistance. In Brazil, Bianchini et al. (1994) studied the inheritance of resistance to BGMV in an interspecific cross between *P. vulgaris* and *P. coccineus* and reported a tendency of dominance for resistance to pod deformation, whereas resistance to chlorosis appeared to be recessive.

When inoculated in the greenhouse with BGYMV, 'Arroyo Loro' plants had both leaf chlorosis and pod deformation,

Table 1. Bean golden yellow mosaic reaction of F_{2:3} lines of the common bean population I9557-9 × 'Morales' in a field trial planted in Feb. 2000 at the University of Puerto Rico, Mayagüez.

F _{2:3} plants	Susceptible or segregating (no. of plants)	Resistant to chlorosis and pod deformation (no. of plants)
Observed	78	120

Table 2. Bean golden yellow mosaic reaction of F_{2:3} lines of the common bean population I9557-9 × 'Arroyo Loro' in a field trial planted in Feb. 2000 at the University of Puerto Rico, Mayagüez.

F _{2:3} plants	Susceptible or segregating (no. of plants)	Resistant to chlorosis and pod deformation ^a (no. of plants)	χ ² 15:1	P
Observed	192.0	8.0	1.73	0.19
Expected ^b	187.5	12.5		

^aOur hypothesis is that only F₂ plants with the proposed genotype *bgm-3 bgm-3* (chlorosis resistant) and *Bgp-2 Bgp-2* (pod deformation resistant) should be symptom free in F₃ progenies.

^bExpected ratio of 15 of 16 susceptible or segregating F_{2:3} lines: 1/16 resistant lines for presence of independent genes, *bgm-3* with recessive and *Bgp-2* with dominant gene action.

whereas PR0157-4-1 had normal leaves and pods (Table 4). Twenty-three of the 25 F₁ plants from the cross PR0157-4-1 × 'Arroyo Loro' had the expected phenotype of chlorotic leaves and normal pods after inoculation with BGYMV. However, two of the plants had normal leaves and pods, which could be explained if these plants escaped infection or were selfs. Four BC₁F₁ (PR0157-4-1 × PR0157-4-1 × 'Arroyo Loro') plants had chlorotic leaves and normal pods, and four BC₁F₁ plants had normal leaves and pods, which is consistent with an expected 1:1 ratio. One BC₁F₁ plant had chlorotic leaves and deformed pods, which could not be explained. Severe root rot caused the premature death of almost all the plants from the backcross with the susceptible parent BC₁F₁ (PR0157-4-1 × 'Arroyo Loro' × 'Arroyo Loro'). Consequently, the results are not presented in Table 4. The SCAR SR2 marker band, which is linked to the *bgm* gene, was not present in PR0157-4-1, 'Arroyo Loro', or any of the resistant F₂ plants from the PR0157-4-1/'Arroyo Loro' population, thus providing further evidence that the recessive resistance to leaf chlorosis derived from G35172 and *bgm* are different.

In Puerto Rico, three common bean lines—PR9771-3-1, PR0247-49, and PR0157-4-1—that have BGYMV resistance derived from G35172 have been released as improved common bean germplasm (Beaver et al., 2005). These lines are highly resistant to BGYMV but do not have the SR-2 marker linked to the recessive resistance gene *bgm*. After several years of use by different breeding programs, there are no reports that the linkage between the SR-2 marker and the *bgm* resistance gene has been broken (P. Miklas, pers. comm.).

A decline in the incidence of BGYMV in Puerto Rico has coincided with the widespread use of the resistant cultivar Morales. As a consequence, it has not been possible during the past few years to conduct additional field screenings for BGYMV.

XAN 176 was susceptible to BGYMV in the trial conducted at Homestead, FL. Several plants of PR0003-390, which has the *bgm-2* resistance gene, also developed typical BGYMV symptoms in the leaves and had severely deformed pods. The *bgm-2* allele has been found to have a lower level of resistance than *bgm* (Velez et al., 1998). 'Morales', which has the *bgm* and *Bgp* resistance genes, and PR027-49, which has the putative *bgm-3* and *Bgp-2* resistance genes, did not develop BGYMV symptoms. Results from this trial suggest that both the *bgm* and *Bgp* resistance genes and the BGYMV resistance derived from G35172 would be useful for developing BGYMV-resistant bean lines for southern Florida and the Caribbean.

A few of the F₁ plants from the cross Morales × PR0247-49 developed attenuated BGYMV symptoms (data not shown), which support the hypothesis that *bgm* and *bgm-3* are not alleles. The lack of intensity of symptom expression in the F₁ plants may have been the result of the presence of the SW12 quantitative trait loci from 'Morales', which has been reported by Miklas et al. (2000) to confer moderate levels of resistance to BGYMV.

Because the F₁ plants derived from the cross PR0247-49 × PR0003-390 were dwarf hybrids (Singh and Gutiérrez, 1984), it was not possible to evaluate the stunted plants in the Homestead, FL, trial for BGYMV reaction. We encountered a similar problem during the previous year in the Dominican Republic when we attempted to screen F₁ plants from the same cross for BGYMV reaction. Given the difference in the degree of BGYMV resistance between PR0003-390, which has *bgm-2*,

Table 3. Leaf and pod reaction to bean golden yellow mosaic of F₂ plants of the common bean population PR0157-4-1 × ‘Arroyo Loro’ planted and inoculated at the Isabela Substation (Summer 2002) and in the greenhouse at the University of Puerto Rico, Mayagüez (Dec. 2002).

F ₂ phenotype F ₂ genotype	Mosaic (+) deformed pods (–) <i>Bgm-3 Bgp-2</i> (no. of plants)	Mosaic (+) deformed pods (+) <i>Bgm-3 bgp-2</i> <i>bgp-2</i> (no. of plants)	Mosaic (–) deformed pods (–) <i>bgm-3 bgm-3</i> <i>Bgp-2</i> (no. of plants)	Mosaic (–) deformed pods (+) <i>bgm-3 bgm-3</i> <i>bgp-2 bgp-2</i> (no. of plants)	χ^2 9:3:3:1	<i>P</i>
Isabela Substation						
Observed	144.0	41.0	49.0	7.0	5.18	0.16
Expected	135.0	45.8	45.8	14.5		
Mayagüez Greenhouse						
Observed	22.0	9.0	14.0	6.0	6.30	0.98
Expected	28.6	9.7	9.7	3.1		

Table 4. Inheritance of resistance to mosaic and pod deformation incited by bean golden yellow mosaic of F₁, backcross and parents of the common bean population PR0157-4-1 × ‘Arroyo Loro’ inoculated in the greenhouse at the University of Puerto Rico, Mayagüez (Dec. 2002).

	Mosaic (+) deformed pods (–) (no. of plants)	Mosaic (+) deformed pods (+) (no. of plants)	Mosaic (–) deformed pods (–) (no. of plants)	Mosaic (–) deformed pods (+) (no. of plants)
F ₁	23	0	2	0
BC ₁ F ₁ (resistant)	4	1	4	0
PR0157-4-1	0	0	4	0
Arroyo Loro	0	4	0	0

and PR0247-49, which has *bgm-3*, it is not likely that the two lines derive their BGYMV resistance from the same allele. In addition, there is no evidence of *P. coccineus* parentage in the pedigree of DOR 303 (‘Porillo Sintetico’ × ‘Cacahuete 72’ × ‘Jamapa’ × ‘Cacahuete 72’ × ‘Red Kloud’), which is the source of the *bgm-2* allele. The two new genes from *P. coccineus* should contribute to more durable resistance to BGYMV in common bean.

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