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.
Materials and Methods

The advance-generation common bean breeding line I9557-9 was derived from the backcross HP8437-95 × G35172 × 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 × 3M-81. The BC1F2;F2:3;5:7 lines were a single plant selected for BGYMV resistance in the field at the Isabela Substation in the F2, F3, and F4 generations. The F0 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 F2;3 lines from the cross ‘Morales’ × 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), Mayaguez. 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 F2;3 lines were screened for BGYMV resistance in a nursery planted in Feb. 2000 at UPR, Mayaguez. Spreader rows of the BGYMV susceptible cultivar Mayflower (Kelly et al., 1989) were inoculated with viruliferous whiteflies 2 weeks before planting the F2;3 nursery. The whiteflies were collected from a greenhouse at UPR, Mayaguez, 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 F2;3 line were planted. Fifteen seeds were planted in rows that were spaced 0.6 m apart. At 50 d after planting, the F2;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 F2;3 lines from the cross ‘Arroyo Loro’ × I9557-9 was developed to study the mode of inheritance of BGYMV resistance derived from G35172. ‘Arroyo Loro’ is susceptible to BGYMV. Two hundred F2;3 lines were screened for BGYMV resistance in a nursery planted in Feb. 2000 at UPR, Mayaguez. Individual 1-m rows of each F2;3 line were planted. Fifty 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 F2;3 lines.

In June 2001, PR0157-4-1 (Beaver et al., 2005), a BGYMV-resistant F3;5 line from the cross ‘Arroyo Loro’ × 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 BC1F2 seeds for the inheritance study.

Two hundred forty-one F2;3 plants of the cross PR0157-4-1 × ‘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, Mayaguez. The trial included 25 F1 and 51 F2 plants from the cross PR0157-4-1 × ‘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 µL⁻¹. The molecular marker work was conducted in the Biotechnology and Nitrogen Fixation Laboratory at UPR, Mayaguez.

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’ × PR0247-49 and PR0247-49 × 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, Cadilac, 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 F2;3 rows from the cross I9557-9 × ‘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). F2:3 lines rather than individual F2 plants were used in the allelism test planted in 2000 to reduce errors incurred 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 F2:3 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 F2:3 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, 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 F_2 plants of the common bean population PR0157-4-1 × ‘Arroyo Loro’ inoculated in the greenhouse at the University of Puerto Rico, Mayaguez (Dec. 2002).

| F_2 phenotype | Mosaic (+) deformed pods (–) Bgm-3_Bgp-2 (no. of plants) | Mosaic (+) deformed pods (–) Bgm-3_Bgp-2 (no. of plants) | Mosaic (-) deformed pods (–) bgm-3 bgm-3_Bgp-2 (no. of plants) | Mosaic (-) deformed pods (+) bgm-3 bgm-3 (no. of plants) |
|---------------|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| F_2 genotype  | Isabela Substation                                    | Mayaguez Greenhouse                                    |                                                        |                                                        |
| Observed      | 144.0                                                  | 22.0                                                   | 28.6                                                   | 135.0                                                  |
| Expected      | 41.0                                                   | 9.0                                                    | 9.7                                                    | 45.8                                                   |

Table 4. Inheritance of resistance to mosaic and pod deformation incited by bean golden yellow mosaic of F_1, backcross and parents of the common bean population PR0157-4-1 × ‘Arroyo Loro’ inoculated in the greenhouse at the University of Puerto Rico, Mayaguez (Dec. 2002).

| F_1 genotype | Mosaic (+) deformed pods (–) (no. of plants) | Mosaic (+) deformed pods (+) (no. of plants) | Mosaic (–) deformed pods (–) (no. of plants) | Mosaic (–) deformed pods (+) (no. of plants) |
|--------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| PR0157-4-1   | 23                                          | 0                                           | 2                                          | 0                                           |
| BC1F1 (resistant) | 4                                          | 1                                           | 4                                          | 0                                           |
| Arroyo Loro  | 0                                           | 0                                           | 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 pedigreed of DOR 303 (‘Porillo Sintetico’ × ‘Cacahuate 72’ × ‘Jamapa’ × ‘Cacahuate 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.

Literature Cited

Acevedo-Román, M., A.M. Molina-Castañeda, J.C. Angel Sánchez, C.G. Muñoz, and J.S. Beaver. 2004. Inheritance of normal pod development in bean golden yellow mosaic resistant common bean. J. Amer. Soc. Hort. Sci. 129:549–552.

Adames-Mora, C., J.S. Beaver, and O. Diaz. 1996. Una metodología de evaluación del virus del mosaico dorado de habichuela en el invernadero. J. Agr. Univ. Puerto Rico 80:65–72.

Afanador, L.K., S.D. Haley, and J.D. Kelly. 1993. Adoption of a “mini-prep” DNA extraction method for RAPD marker analysis in common bean (Phaseolus vulgaris L.). Annu. Rpt. Bean Improv. Coop. 36:10–11.

Beaver, J.S. and P.N. Miklas. 1999. Registration of ‘Morales’ small white bean. Crop Sci. 39:1257.

Beaver, J.S., C.G. Muñoz Perea, J.M. Osomo, F.H. Ferwerda, and P.N. Miklas. 2005. Registration of bean golden yellow mosaic virus resistant dry bean germplasm lines PR9771-3-2, PR0247-49, and PR0157-4-1. Crop Sci. 45:2126–2127.

Beebe, S.E. 1994. Breeding for resistance to bean golden mosaic virus: History and perspective. p. 148–150. In: F. Morales (ed.). Bean golden mosaic: Research advances. International Center for Tropical Agriculture, Cali, Colombia.

Bianchini, A., V. Moda-Cirino, N.S. Fonseca, and F.F. Toledo. 1994. Genética de resistência ao virus do mosaico dourado do feijoeiro. Fitopotol. Bras. 19:329 (abstr.).

Blair, M.W., L.M. Rodriguez, F. Pedraza, F. Morales, and S. Beebe. 2007. Genetic mapping of the bean golden yellow mosaic geminivirus resistance gene bgm-1 and linkage with potyvirus resistance in common bean (Phaseolus vulgaris L.). Theor. Appl. Genet. 114:261–271.

Ferwerda, F.H. 2001. The investigation of genetic barriers to interspecific crosses between Phaseolus acutifolius A. Gray, Phaseolus coccineus L. and Phaseolus vulgaris L. and the inheritance of resistance to bean golden mosaic virus from P. coccineus L. University of Florida, Gainesville, FL, PhD Diss.

Galvez, G.E. and F.J. Morales. 1994. Virus transmitidos por la mosca blanca, p. 435–464. In: M. Pastor-Corrales and H. Schwartz (eds.). Problemas de producción del frijol en los tropicos. 2nd ed. International Center for Tropical Agriculture, Cali, Colombia.

Kelly, J.D., M.W. Adams, A.W. Saettler, G.L. Hosfield, G.V. Varner, J.S. Beaver, M.A. Uebersax, and J. Taylor. 1989. Registration of ‘Mayflower’ navy bean. Crop Sci. 29:1571–1572.

Miklas, P.N. 2005. DNA markers (SCARS) linked with disease resistance traits in bean (Phaseolus vulgaris L.). 2 Nov. 2006. <www.ars.usda.gov/sp2UserFiles/Place/53540000/Miklas/SCARTable.pdf>.

Miklas, P.N., V. Stone, M.J. Daley, J.B. Stavel, J.R. Steadman, M.J. Bassett, R. Delorme, and J.S. Beaver. 2000. Bacterial, fungal, and viral disease resistance loci mapped in a recombinant inbred common bean population (‘Dorado’/XANI176). J. Amer. Soc. Hort. Sci. 125:476–481.

Morales, F.J. 2000. Methods of control of begomovirus del frijol, p. 133–154. In: F.J. Morales (ed.). El mosaico dorado y otras enfermedades del frijol común causadas por geminivirus transmitidos por mosca blanca en la América Latina. International Center for Tropical Agriculture, Cali, Colombia.

Moraes, F.J. and N.I. Niessen. 1988. Comparative responses of selected Phaseolus vulgaris L. germplasm inoculated artificially and naturally with bean golden mosaic virus. Plant Dis. 72:1020–1023.

Rosas, J.C., O.I. Varela, and J.S. Beaver. 1997. Registration of ‘Tio Canela 75‘ small red bean. Crop Sci. 37:1391.

Singh, S.P. and J.A. Gutiérrez. 1984. Geographical distribution of the DL1 and DL2 genes causing hybrid dwarfism in Phaseolus vulgaris L., their association with seed size, and their significance to breeding. Euphytica 33:337–345.

Singh, S.P., F.J. Morales, P.N. Miklas, and H. Teran. 2000. Selection for bean golden mosaic resistance in intra- and interracial bean populations. Crop Sci. 40:1565–1572.

Velez, J., M.J. Bassett, J.S. Beaver, and A. Molina–Castañeda. 1998. Inheritance of resistance to bean golden mosaic virus in common bean. J. Amer. Soc. Hort. Sci. 123:628–631.