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Genetics of resistance to the geminivirus, *Bean dwarf mosaic virus*, and the role of the hypersensitive response in common bean

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Abstract *Bean dwarf mosaic virus* (BDMV) is a single-stranded DNA virus (genus: *Begomovirus*, family: *Geminiviridae*) that infects common bean (*Phaseolus vulgaris* L.) and causes stunted plant growth, and mosaic and mottle symptoms in leaves. BDMV shows differential pathogenicity in common bean, infecting germplasm of the Andean gene pool (e.g., the snap bean cultivar Topcrop), but not that of the Middle American gene pool (e.g., the pinto bean cultivar Othello). Resistance to BDMV in Othello is associated with development of a hypersensitive response (HR) in vascular (phloem) tissues. In this study, Middle American germplasm representing the four recognized races (i.e., Durango, Guatemala, Jalisco, and Mesoamerica) and the parents of Othello were inoculated with BDMV and a BDMV-green fluorescent protein (GFP) reporter. All genotypes showed partial or complete resistance to BDMV and BDMV-GFP, indicating the widespread distribution of resistance in the Middle American gene pool. A number of BDMV-resistant germplasm did not show the HR, indicating it is not correlated with resistance. In the F_1, F_2, and F_3 of reciprocal crosses between Othello and Topcrop, a single dominant allele, *Bdm*, conferred BDMV resistance.

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Introduction

Plant viruses in the family *Geminiviridae* have a circular, single-stranded DNA genome encapsidated in twinned icosahedral particles. Geminiviruses are divided into four genera (*Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Begomovirus*) based on the viral genome structure, host range, and type of insect vector. Mastreviruses and curtoviruses have a monopartite genome and are transmitted by various leafhopper species, but infect monocotyledonous and dicotyledonous plants, respectively. The genus *Topocuvirus* includes only *Tomato pseudo-curly top virus*, which has a monopartite genome, is transmitted by treehoppers, and infects dicotyledonous plants. Members of the genus *Begomovirus* have monopartite (one ~2.9-kb DNA) or bipartite genomes (two ~2.6-kb DNAs referred to as "DNA-A" and "DNA-B"), are transmitted by whiteflies (e.g., *Bemisia tabaci* Gennadius), and infect dicotyledonous plants. In tropical and subtropical areas, begomoviruses cause devastating diseases in economically important crops such as common bean (*Phaseolus vulgaris* L.), cotton (*Gossypium hirsutum* L.), pepper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* Mill), and melon (*Cucumis melo* L.) (Bock 1982; Harrison 1985; Morales and Anderson 2001; Polston and Anderson 1997).

Bean dwarf mosaic virus (BDMV) is a bipartite begomovirus that infects common bean, and causes stunting or dwarfing of infected plants and epinasty and mottling in leaves. BDMV was first described from Colombia and has since been identified in other countries in South America (e.g., Argentina and Brazil; Morales et al. 1990). Diseases caused by BDMV are generally of minor importance, as the virus usually occurs at a low incidence. However, BDMV can cause significant yield losses under certain conditions, e.g., plantings of susceptible cultivars in areas having high populations of the whitefly vector, e.g., Argentina (Morales 2001). BDMV is well characterized at the biological (Morales et al. 1990) and molecular levels (Hidayat et al. 1993). The complete sequences of the infectious cloned BDMV DNA-A and

DNA-B components have been determined, and BDMV has been used as a model system to study virus movement in plants (Garrido-Ramirez et al. 2000a; Sudarshana et al. 1998; Wang et al. 1999). BDMV is readily sap transmitted to common bean (Gilbertson et al. 1991b; Morales et al. 1990), and the infectious clones can be introduced by particle bombardment (Gilbertson et al. 1991b) or agroinoculation (Hou et al. 1998).

The common bean originated in the Americas, and two major gene pools have been identified. These represent the two major domestication regions of the common bean: Middle America (e.g., Mexico and Central America) and the Andean regions of South America (e.g., Peru, Bolivia, and northern Argentina). Within these two gene pools, at least six races can be identified (Singh et al. 1991a). Middle American and Andean beans can be differentiated based upon morphological (Singh et al. 1991b) and molecular characteristics (Khairallah et al. 1990), as well as partial reproductive isolation (Gepts 1998). Studies of the BDMV-common bean interaction have revealed differential pathogenicity, in which BDMV primarily infects germplasm from the Andean gene pool. Thus, Andean cultivars such as California Dark Red Kidney, Improved Tendergreen, and Topcrop are susceptible to BDMV [i.e., become systemically infected and develop symptoms (Hidayat 1991; Morales et al. 1990; Wang et al. 1999)], whereas Middle American cultivars such as Black Turtle Soup (BTS), Olathe, Othello, and Pinto UI 114 are resistant (i.e., do not develop disease symptoms). In contrast, more extensive screening of common bean germplasm for resistance to *Bean golden yellow mosaic virus* [(BGYMV); previously *Bean golden mosaic virus* (BGMV)] has revealed no genotypes with high levels of resistance to this widespread and economically important begomovirus (Singh et al. 2000).

The mechanism of BDMV resistance in Othello was investigated with a BDMV-green fluorescent protein (GFP) reporter (Sudarshana et al. 1998; Wang et al. 1999). These studies revealed that (1) long-distance movement of the virus was blocked, (2) this blockage occurred at or near the phloem, and (3) resistance was associated with a hypersensitive response (HR) in vascular tissues. This represented one of the first reports of an HR-associated geminivirus resistance response, and the HR provides an easily scored marker of the resistance phenotype (Garrido-Ramirez et al. 2000a; Wang et al. 1999). However, BDMV resistance in BTS also involved a block in long-distance movement, but did not involve an HR (Garrido-Ramirez et al. 2000a; Wang et al. 1999); this raised the question of the role of the HR in the resistance response.

In the present study, we investigated (1) the distribution of BDMV resistance in the Middle American gene pool and the pedigree of Othello, (2) the association of the HR with BDMV resistance, and (3) the genetics of BDMV resistance.

Materials and methods

Germplasm of the Middle American gene pool including the parents of Othello

Germplasm representing the common bean races Durango, Guatemala, Jalisco, and Mesoamerica of the Middle American gene pool were provided by Dr. Steve Beebe [Centro Internacional de Agricultura Tropical (CIAT)] and are listed in Table 1. Othello is an F_7 selection derived from the pedigree shown in Table 2 (Burke et al. 1995). Seed of Othello's parents, namely Aurora, BTS, NW 410, Sutter Pink, UI 35, UI 114, and Victor, were provided by Dr. Phil Miklas (Professor, USDA-ARS, Washington).

Crosses and segregating progeny

Reciprocal crosses between Othello (BDMV-resistant) and Topcrop (BDMV-susceptible) were made by hand pollination. Fourteen F_1 plants of each reciprocal cross were sap inoculated with BDMV, and an additional 10 F_1 plants were bombarded with BDMV-GFP. For each cross, 20 uninoculated F_1 seeds of each cross were planted and allowed to produce F_2 seed, and 100 F_2 plants from each cross were sap inoculated with BDMV. Additional F_2 seed was planted to obtain F_3 families. Seeds of 26 F_3 families derived from each cross were planted, and a total of 15–20 plants per family were sap inoculated with BDMV. It is important to note that direct progeny tests could not be performed because of difficulty of recovering seed from BDMV-infected plants.

Sap inoculation of common bean plants with BDMV

Sap inoculum was prepared as previously described (Gilbertson et al. 1991b). Briefly, young symptomatic leaves were collected from BDMV-infected common bean plants and ground in 0.1 M potassium phosphate buffer (pH 8.0) with a mortar and pestle. Sap was rubbed onto celite-dusted, primary leaves of 10- to 14-day-old common bean plants with a pestle. Inoculated plants were maintained in a growth chamber (250 μmol $m^{-2}s^{-1}$ photosynthetically active radiation; 16 h photoperiod: day, 30°C and night, 25°C), and examined for symptoms 7 and 14 days post-inoculation (dpi).

Particle bombardment inoculation of common bean seedlings with infectious cloned BDMV DNA

Seed was germinated on moist paper towels for 2 or 3 days at 28°C, and seedlings were inoculated with infectious cloned BDMV DNA [wild-type BDMV DNA-A in pBDA1.5 or BDMVA-GFP in pBDG1.5 plus wild-type BDMV DNA-B in pBDB1.5 (Gilbertson et al. 1991b; Sudarshana et al. 1998)] by particle bombardment as previously described (Paplomatas et al. 1994; Sudarshana et al. 1998). Inoculated seedlings were planted in soil and maintained in a growth chamber. Plants were examined for symptoms 7 and 14 dpi.

Detection of GFP and HR in common bean hypocotyl tissues

Seedlings bombarded with the BDMV-GFP reporter (BDMVA-GFP plus BDMV DNA-B) were examined for GFP fluorescence and HR development. Transverse sections of bombarded hypocotyl tissues of 3- to 4-day-old seedlings were prepared by hand with a razor blade. Hypocotyl sections were mounted in water and viewed with a Nikon Opitophoto-2 microscope in fluorescence mode (excitation 450–495 nm, dichroic mirror 510 nm, barrier filter 520 nm) to detect GFP fluorescence, and bright-field microscopy for detection of necrosis (HR). Images were recorded as tagged image file formats (.tiff) or JPEG (.jpg) formats at a PC workstation.

Table 1 Infectivity of *Bean dwarf mosaic virus* (BDMV) in germplasm of the Middle American gene pool of common bean (*Phaseolus vulgaris* L.)

Race	Genotype	Cell-to-cell movement[a]	HR[b]	Systemic infection[c]	
				BDMV-GFP	BDMV
Durango	G 2635	12/12[d]	12/12	0/12	0/12
	G 3578	12/12	12/12	1/12*	2/12*
	G 3585	9/9	0/9	0/12	6/9***
	G 11010	6/6	6/6	0/9	1/3
	G 11423	12/12	4/12	0/12	2/12***
	G 22814	9/9	5/9	1/10*	3/12***
Guatemala	G 685	12/12	11/12	0/12	0/12
	G 2601	9/9	9/9	0/10	3/12***
	G 16291	12/12	3/12	0/12	0/12
Jalisco	G 11061	6/6	6/6	0/6	0/6
	G 11071	12/12	1/12	0/12	6/12***
	G 11098	12/12	11/12	0/12	11/12***
	G 13046	6/6	5/6	0/6	0/6
	G 13624	12/12	11/12	0/9	0/9
Mesoamerica	G 801	6/6	0/6	0/7	0/9
	G 3936	9/9	3/9	0/6	0/6
	G 18454	12/12	0/12	0/12	1/12***
Controls	Othello[e]	12/12	12/12	0/12	0/12
	Topcrop[f]	12/12	0/12	12/12**	12/12***

[a] Cell-to-cell movement was determined based on detection of spread of foci of green fluorescent protein (GFP) fluorescence in transverse sections of hypocotyls from bean seedlings 4 days after inoculation with BDMV-GFP by particle bombardment
[b] The hypersensitive response (HR) was determined based upon observation of necrosis in vascular tissues of hypocotyl sections 4 days after inoculation with BDMV-GFP by particle bombardment
[c] Symptoms were evaluated and systemic infection was determined by PCR detection of BDMV-DNA 7–14 days after inoculation with BDMV-GFP or wild-type BDMV by particle bombardment. Symptom severity is indicated as follows: no asterisk = symptomless, * mild, ** moderate, *** severe
[d] Number of plants in which either cell-to-cell movement, HR, viral DNA, or symptoms were detected/total seedlings inoculated
[e] Middle American cultivar
[f] Andean cultivar

Table 2 Infectivity of BDMV in the parents of Othello pinto bean

Cultivars	Race	Cell-to-cell movement[a]	HR[b]	Systemic infection[c]	
				BDMV-GFP	BDMV
Parents					
Aurora	Mesoamerica	12/12[d]	12/12	0/12	4/12
BTS	Mesoamerica	12/12	0/12	0/12	0/12
NW-410	Durango	12/12	12/12	0/12	0/12
Sutter Pink	Durango	12/12	12/12	0/12	0/12
UI 35	Durango	11/12	10/11	0/10	0/11
UI 114	Durango	12/12	12/12	0/12	0/12
Victor	Durango	11/12	10/12	0/12	0/12
Control					
Othello	Durango	12/12	12/12	0/12	0/12
Topcrop	Nueva Granada	12/12	0/12	12/12**	12/12***

[a] Cell-to-cell movement was determined based on detection of spread of foci of GFP fluorescence in transverse sections of hypocotyls from bean seedlings 4 days after inoculation with BDMV-GFP by particle bombardment
[b] HR was determined based upon observation of necrosis in vascular tissues of hypocotyl sections 4 days after inoculation with BDMV-GFP by particle bombardment
[c] Symptoms were evaluated and systemic infection was determined by PCR detection of BDMV-DNA 7–14 days after inoculation with BDMV-GFP or wild-type BDMV by particle bombardment. Symptom severity is indicated as follows: no asterisk = symptomless, * mild, ** moderate, *** severe
[d] Number of plants in which either cell-to-cell movement, HR, viral DNA, or symptoms were detected/total seedlings inoculated

PCR detection of geminivirus DNA in leaf tissues

DNA was extracted from newly emerged trifoliolate leaves collected from plants at 14 dpi. PCR was performed as previously described with the degenerate begomovirus primer pairs: PAL1v1978 and PAR1c496 (directs the amplification of a ~1.1-kb DNA-A fragment), and PBL1v2040 and PBV1c970 (directs the amplification of a ~1.6-kb DNA-B fragment) (Gilbertson et al. 1991a; Rojas et al. 1993). PCR parameters were 25 cycles of 94°C, 1 min; 50°C, 2 min; and 72°C, 3 min. PCR products were examined by agarose gel electrophoresis in 1.0% Tris-borate EDTA buffer.

PCR analysis to identify hybrids

PCR with the SCJ1d1/SCJ1d2 primer pair was used to identify hybrids. This primer pair directs the amplification of a ~1.6-kb DNA fragment from genomic DNA of Andean common beans, a ~1.2-kb DNA fragment from Middle American common beans, and both fragments from hybrids (Adam-Blondon, 1994). PCR parameters were 40 cycles of 94°C, 1 min; 58°C, 1 min; and 72°C, 2 min. PCR products were examined by agarose gel electrophoresis.

Results

Evaluation of Middle American common beans for BDMV resistance

In control experiments, Topcrop seedlings inoculated with sap from BDMV-infected plants or bombarded with cloned DNAs of wild-type BDMV or BDMV-GFP developed mosaic and dwarfing symptoms 7–10 dpi, whereas Othello plants inoculated in the same manner did not develop symptoms (Table 1). As previously reported by Sudarshana et al. (1998), wild-type BDMV induced more severe symptoms in Topcrop than did BDMV-GFP (Table 1). Examination of hypocotyl sections of seedlings bombarded with BDMV-GFP revealed GFP fluorescence in epidermal and cortical cells of Topcrop and Othello by 24–36 h post-bombardment (hpb). By 48–60 hpb, fluorescence had reached the vascular tissues of both cultivars and, by 96 hpb, HR was observed in vascular tissues of Othello, but not Topcrop. These results are in agreement with previous reports (Sudarshana et al. 1998; Wang et al. 1999) indicating equivalent cell-to-cell movement of BDMV-GFP in hypocotyl tissues of Othello and Topcrop and development of a vascular HR in hypocotyls of Othello.

The extent to which BDMV resistance occurs in the Middle American gene pool was assessed by inoculating common beans representing the four Middle American races with wild-type BDMV and BDMV-GFP by particle bombardment and determining infectivity and symptom development, cell-to-cell movement (BDMV-GFP), and HR development (BDMV-GFP). Most Middle American beans were resistant to BDMV based upon the failure of symptoms to develop in inoculated plants (Table 1), whereas Topcrop plants consistently developed symptoms of BDMV infection. Genotypes of each Middle American race were highly resistant to BDMV and BDMV-GFP (e.g., G 685, G 801, G 2635, G 13046, G 3936, G 11061, G 13624, and G 16291), whereas other genotypes (9/17) showed partial resistance (i.e., resistant to BDMV-GFP, but some plants susceptible to wild-type BDMV). In most of the partially resistant genotypes, a small proportion of plants (i.e., <20%) were susceptible to BDMV. However, three genotypes, G 3585 (race Durango), G 11071 (race Jalisco), and G 11098 (race Jalisco) were more susceptible to wild-type BDMV with 6/9, 6/12, and 11/12 plants infected, respectively (Table 1).

BDMV-GFP moved cell-to-cell in hypocotyl tissues of all inoculated Middle American genotypes, but did not move long distance. The HR was observed in the vascular tissues of some, but not all, BDMV-resistant genotypes by 4 dpb, indicating that HR development was not strongly correlated with resistance (Table 1). Thus, some highly resistant germplasm developed HR (e.g., G 2635, G 3578, G 685, G 13046, and G 13624), whereas others did not (e.g., G 801, G 16291, and G 18454). Additionally, G 11098 developed the HR, but was susceptible to BDMV.

BDMV resistance in genotypes comprising the pedigree of cv. Othello

None of the Othello parents developed disease symptoms after inoculation with wild-type BDMV or BDMV-GFP (Table 2). These results are consistent with all of these genotypes belonging to the Middle American gene pool. BDMV DNA-A was detected in newly emerged leaves of 33% (4/12) of Aurora plants inoculated with wild-type BDMV (Table 2), indicating a symptomless infection in these plants. Cell-to-cell movement of BDMV-GFP was detected in hypocotyl tissues of all parents (Table 2); and the HR was observed in the vascular tissues of all parents (4–5 dpb), except BTS (Fig. 1 and Table 2). Thus, all Othello's parents were resistant to BDMV and most developed the HR.

Genetics of BDMV resistance

Because no highly susceptible Middle American genotype was identified, crosses were made between the BDMV-resistant Othello and the BDMV-susceptible Topcrop for the genetic analysis of BDMV resistance. F_1 seed was obtained for the reciprocal crosses (i.e., Topcrop × Othello and Othello × Topcrop), and hybridity was confirmed based on morphological characters (seed color and growth habit) and PCR analysis with the SCJ1d1/SCJ1d2 primer pair. Othello has a cream-spotted seed and an indeterminate, prostrate, semi-climbing growth habit (type III), whereas Topcrop has a light brown-red seed and a determinate bush growth habit (type I). Seeds harvested from the F_1 hybrids had an intermediate color (i.e., light brown-red spotted and parents were of growth habit type III). PCR analysis of DNA extracted from F_1 plants confirmed their hybrid origin (data not shown).

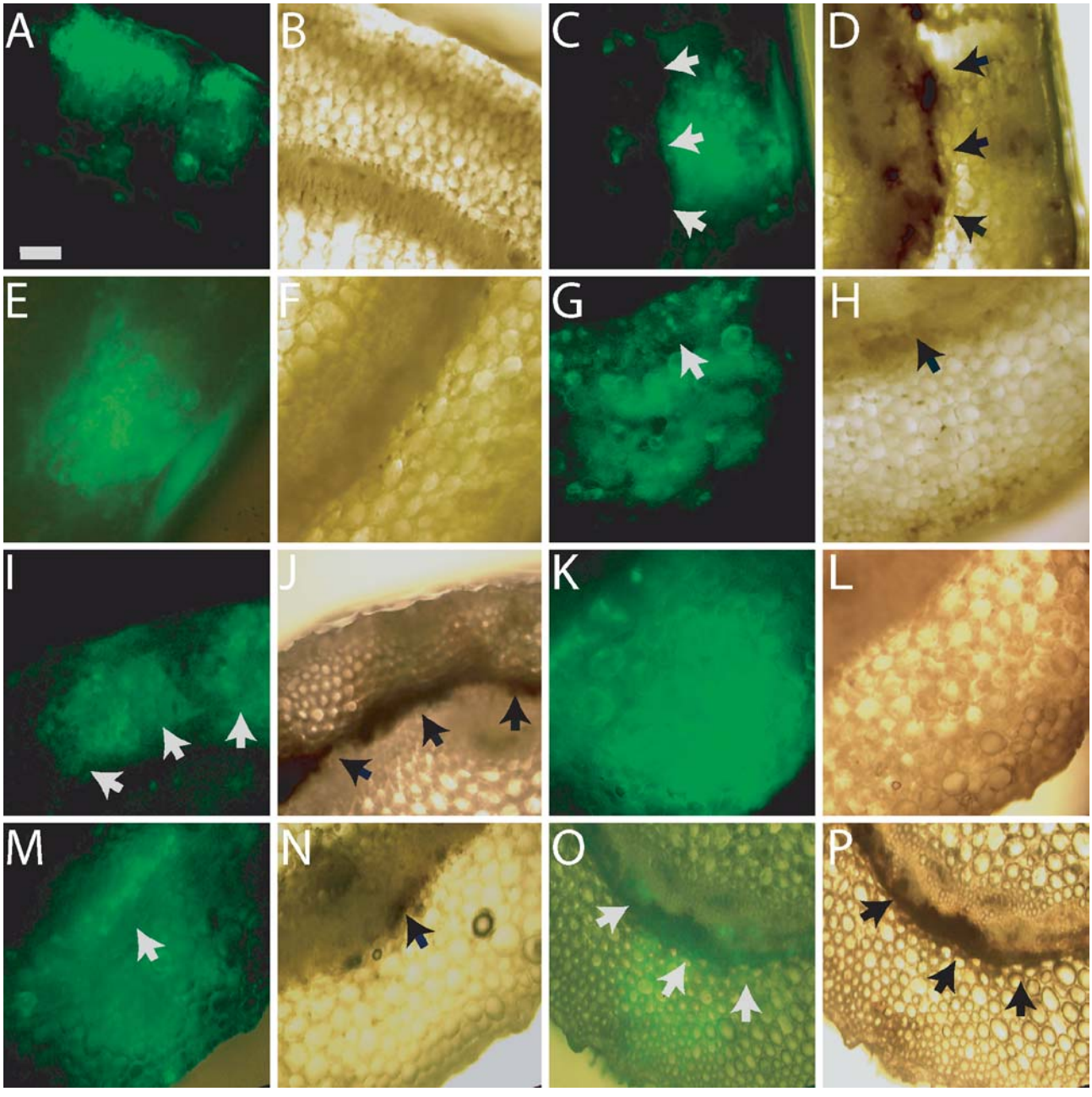

Fig. 1 Cell-to-cell movement and hypersensitive response (HR) development in selected genotypes of *Phaseolus vulgaris* L. after inoculation with a *Bean dwarf mosaic virus*-green fluorescent protein (BDMV-GFP) reporter by particle bombardment. Transverse sections depicting GFP fluorescence in BDMV-GFP-infected hypocotyl tissues 4 days post-inoculation (dpi): **A** Topcrop, **C** Othello, **E** F_1 plant of Othello/Topcrop, **G** F_2 plant of Topcrop/Othello, **I** Aurora, **K** G 11071, **M** G 11423, and **O** G 685. **B**, **D**, **F**, **H**, **J**, **L**, **N**, and **P** bright-field images of sections presented in **A**, **C**, **E**, **G**, **I**, **K**, **M**, and **O**, respectively. All images were collected with a Nikon fluorescence microscope. *Darts* in **C** and **D**, **G** and **H**, **I** and **J**, **M** and **N**, and **O** and **P** indicate necrosis (HR) in the vascular tissues. *Scale bar* in **A** =100 μm and is common to all images presented

None of the F_1 plants, inoculated with wild-type BDMV or BDMV-GFP, developed symptoms by 4 weeks post-inoculation, whereas all control Topcrop plants developed BDMV symptoms. These results suggested that the resistance was dominant. The F_2 segregation data were consistent with a 3:1 ratio of resistant (symptomless):susceptible (symptomatic) plants (Table 3). For both the direct and reciprocal crosses, a good fit to a 1:2:1 ratio of homozygous resistant:segregating:homozygous susceptible F_3 families was obtained (Table 3). Together, these results are consistent with BDMV resistance being conferred by a single dominant allele, *Bdm*.

Table 3 Genetic analysis of resistance in common bean to BDMV in Othello/Topcrop populations

Population	Number of plants		χ^2 (3:1)	*P* value
	Resistant	Susceptible		
(Othello/Topcrop) F_1 [a]	24	0	–	–
(Topcrop/Othello) F_1	24	0	–	–
(Othello/Topcrop) F_2 [b]	70	29	0.97	*P*>0.2
(Topcrop/Othello) F_2	74	26	0.05	*P*>0.2
	Number of F_3 families			χ^2 (1:2:1)	*P* value
	Homozygous resistant	Segregating	Homozygous susceptible		
(Othello/Topcrop) F_3 [b]	8	14	4	1.38	*P*>0.2
(Topcrop/Othello) F_3	3	16	7	2.62	*P*>0.2

[a] Seedlings (48-h-old) of F_1 parents were inoculated with BDMV-GFP by particle bombardment and plants (7- to 10-days-old) were sap inoculated with wild-type BDMV. Plants were evaluated for disease symptoms 7–14 days post-inoculation

[b] F_2 and F_3 plants (7- to 10-days-old) were evaluated 7–14 days after sap inoculation of wild-type BDMV

Table 4 Development of the HR in hypocotyls of common bean seedlings inoculated with BDMV

Cultivar/population	Cell-to-cell movement[a]	HR[b]
Othello	12/12[c]	12/12
Topcrop	12/12	0/12
(Othello/Topcrop) F_1	10/10	4/10
(Topcrop/Othello) F_1	10/10	4/10
(Othello/Topcrop) F_2	20/20	8/20
(Topcrop/Othello) F_2	18/18	6/18

[a] Cell-to-cell movement was determined based on detection of spread of foci of GFP fluorescence in transverse sections of hypocotyls from bean seedlings 4 days after inoculation with BDMV-GFP by particle bombardment

[b] HR was determined based upon observation of necrosis in vascular tissues of hypocotyl sections 4 days after inoculation with BDMV-GFP by particle bombardment

[c] Number of plants in which either cell-to-cell movement or HR were detected/total seedlings inoculated

The vascular HR is not correlated with BDMV resistance in segregating progeny

The development of the HR in BDMV-GFP-infected hypocotyl tissues of F_1 and F_2 plants was determined to further assess the role of this response in BDMV resistance. Whereas all F_1 plants were resistant to BDMV and BDMV-GFP, the vascular HR was not observed in hypocotyls of most F_1 plants infected with BDMV-GFP. For example, by 4 dpb with BDMV-GFP, vascular HR was detected in 100% (12/12) of Othello hypocotyls, but only in 33% (4/12) of hypocotyls of BDMV-resistant F_1 seedlings (Fig. 1 and Table 4). Similar results were obtained with F_2 plants (segregating 3:1 for BDMV resistance): vascular HR was observed in 40% (8/20) and 33% (6/18) of hypocotyls of F_2 seedlings of the reciprocal crosses (Table 4). Furthermore, the HR that was observed in most F_2 seedlings was weak; only two plants showed a strong HR, similar to that observed in Othello. These results are consistent with the conclusion that the HR is not correlated with BDMV resistance.

Discussion

Begomovirus diseases, particularly bean golden mosaic, are a serious constraint on common bean production in tropical and subtropical regions (Morales and Anderson 2001). Because resistance is the most desirable disease-management strategy, it is important to identify and characterize the begomovirus resistance in common bean germplasm. To date, the main sources of resistance to begomoviruses (e.g., BGYMV, BGMV, and BDMV) have been from Middle American germplasm (Morales 2001; Morales et al. 1990; Singh et al. 2000). Furthermore, the effectiveness of this resistance is dependent on the virus; thus, moderate resistance (i.e., mild symptoms) is conferred for BGYMV, whereas strong resistance (no symptoms) is conferred for BDMV (Morales et al. 1990; Singh 2001).

The objectives of this study were to determine the extent and genetics of BDMV resistance in Middle American germplasm. Our finding that most Middle American beans, including representatives of all four races and parents of Othello, were highly resistant to BDMV demonstrates that the gene(s) involved in this resistance response is (are) widely distributed within the gene pool. These results are consistent with and extend those of previous reports (Hidayat et al. 1993; Morales et al. 1990; Wang et al. 1999). The finding that some plants, in a number of genotypes, were BDMV-susceptible suggests that either they were a mixture of genotypes or segregating for resistance. Alternatively, this may reflect a capacity of BDMV to overcome the resistance, which may be facilitated by the particle bombardment technique in which very young (48-h-old) seedlings are inoculated with relatively large amounts of cloned viral DNA. Indeed, a comparative study of BGYMV inoculation methods indicated that particle bombardment resulted in higher levels of infection and more severe symptoms than either sap inoculation or agroinoculation (Garrido-Ramirez et al. 2000b).

Results with the BDMV-GFP reporter revealed cell-to-cell movement in inoculated hypocotyls of all Middle American germplasm. This is consistent with the mechanism of resistance involving a blockage of long-distance movement, as was previously reported for Middle American Othello and BTS (Wang et al. 1999). The other interesting finding from these experiments was that higher levels of resistance were found for BDMV-GFP compared with wild-type BDMV. Only two plants of all the Middle American germplasm tested became systemically infected by BDMV-GFP, and these had mild symptoms. In contrast, wild-type BDMV infected and caused symptoms in plants of a number of the Middle American genotypes. It had previously been noted that BDMV-GFP induced less severe symptoms than wild-type BDMV (Sudarshana et al. 1998), but our results revealed a much more significant difference between infectivity of BDMV-GFP versus BDMV in the resistant Middle American germplasm. This indicates a more significant role for the capsid protein (CP, replaced by the GFP gene in BDMV-GFP; Sudarshana et al. 1998) in infection of resistant (Middle American germplasm) versus susceptible (Andean germplasm) beans. A role for the CP in begomovirus long-distance movement has been previously demonstrated, where it is required for infection of hosts to which the virus is not well adapted (Pooma et al. 1996; Wang et al. 1999). However, the precise mechanism by which the CP mediates long-distance movement is unknown. Alternatively, these results may indicate a deleterious effect of GFP on viral fitness in the resistant genotypes, e.g., slower cell-to-cell movement, thereby allowing host defense responses to more effectively block viral spread.

Previous studies of the genetics of host plant resistance to begomovirus infection have revealed different inheritance patterns. The *Ty-1* gene, which confers resistance to *Tomato yellow leaf curl virus* (TYLCV), was partially dominant (Zamir et al. 1994). In contrast, inheritance of resistance to BGYMV in two breeding lines of common bean, 9236-6 and 9245-94, was conferred by the recessive resistance genes *bgm-1* and *bgm-2*, respectively (Velez and Bassett 1998). This latter result suggests that resistance to BDMV and BGYMV is under different genetic control. This is further supported by the fact that unlike resistance to BDMV, a relatively higher proportion of Middle American germplasm is susceptible to BGYMV (Garrido-Ramirez et al. 2000b).

The HR is generally considered to be involved in disease resistance through blockage or limitation of pathogen spread, particularly in the case of resistance conferred by a single dominant gene. However, there is some evidence that the HR is not the primary determinant of resistance. For example, *Tobacco mosaic virus* (TMV) particles can be detected outside of the necrotic area of TMV local lesions, and some tobacco species such as *Nicotiana glauca* can localize TMV without the HR (reviewed by Richael and Gilchrist 1999). Previous studies conducted with the BDMV-GFP reporter revealed that (1) HR was not required for resistance in Othello, and (2) HR was not correlated with BDMV resistance in BTS (Garrido-Ramirez et al. 2000a; Wang et al. 1999). Our results showing no HR in some BDMV-resistant genotypes and HR in some BDMV-susceptible genotypes were in agreement with these previous studies. Results with the Othello/Topcrop hybrids also supported the notion that HR is not correlated with BDMV resistance. For example, although all F_1 plants were resistant to BDMV, only 33% developed HR, and the intensity of HR was usually weak in these plants. Together, these results indicate that the HR is not correlated with BDMV resistance. The HR may be a secondary defense response that develops subsequent to the initial defense response. This is consistent with the fact that HR appears 4–5 dpi, well after BDMV infection has reached the vascular system and initiated long-distance transport (~2 dpi: Sudarshana et al. 1998; Wang et al. 1999).

In summary, BDMV resistance was detected in all the genotypes from the Middle American gene pool that were tested. The BDMV-GFP reporter indicated that resistance involved a block in viral long-distance movement and did not require the HR. Genetic analyses revealed that this resistance was conferred by a single dominant allele, *Bdm*. Thus, this resistance should be relatively easy to introgress in susceptible cultivars. This would be particularly useful where Andean cultivars are grown in areas known to have high incidences of BDMV (e.g., Argentina).

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