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Differentiation of *Bean pod mottle virus* isolates based upon host symptoms

Leandro A. Mozzoni · Pengyin Chen · Rose C. Gergerich

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Abstract Accessions from *Glycine*, *Phaseolus*, and *Vigna* genera were screened for their reactions to different subgroups of isolates of *Bean pod mottle virus* (BPMV) in order to establish a differential host system. Screening results indicated that the BPMV isolates differed in pathogenic aggressiveness but not in virulence. No major resistance genes were found in soybean (*Glycine max*) or *G. soja* since all screened accessions showed mosaic or necrotic symptoms to BPMV inoculation. However, these accessions expressed differences in severity of symptoms when challenged by various BPMV isolates. The inoculation of *G. tomentella* accessions did not result in mosaic symptoms, and some accessions did not support systemic infection of some of the isolates. Resistance, presented as a hypersensitive reaction, was observed in some of *Phaseolus* and *Vigna* genotypes, and resistant response or susceptibility was stable to all the isolates used in the screening. In conclusion, the selected *G. soja* genotypes PI 407019, PI 464889A, and PI 464928, and 'Amsoy 71' soybean may help to separate severe (reassortant) from mild isolates of BPMV based upon their phenotypic reactions.

Keywords BPMV · *Glycine* · Necrosis · *Phaseolus* · Resistance · *Vigna* · Virus

L. A. Mozzoni · P. Chen (✉)
Department of Crop, Soil, and Environmental Sciences,
University of Arkansas,
Fayetteville, AR 72701, USA
e-mail: pchen@uark.edu

R. C. Gergerich
Department of Plant Pathology, University of Arkansas,
Fayetteville, AR 72701, USA

Introduction

Disease occurs when host resistance genes and pathogen pathogenicity genes interact in a compatible manner, specific vectors are present, and environmental conditions are conducible (Agrios 2004). The gene-for-gene interaction gives origin to the classification of pathogen phenotypes into physiological races, in which various isolates of the pathogen are separated into different groups based upon their ability to infect host genotypes carrying different resistance genes (Caten 1987). In another situation, no differential interaction is observed when the ability to cause disease by each pathogen isolate is constant across all host genotypes (Johnson 1992); in this case, the classification of the pathogen into races is meaningless because there are no real differences in their ability to initiate disease (Caten 1987).

The concepts of differential systems and gene-for-gene interactions have been successfully applied to many biotrophic pathogens. The *Soybean mosaic virus* (SMV)-soybean pathosystem is a relevant example of different resistance genes in the host interacting with avirulence genes present in the

different strains of the virus. Cho and Goodman (1979) developed a differential system classifying SMV isolates into seven different strain groups (G1–G7) that has been successfully used both by breeders to identify resistance genes and by pathologists to identify new SMV strains. Palmer et al. (2004) provided an updated description of the current differential system and resistance genes available in soybean against SMV. In contrast, limited research has been conducted on other important viruses in soybean, such as *Bean pod mottle virus* (BPMV) and its differential classification. Gu et al. (2002) conducted a study, using molecular approaches, on the genetic variability in diverse isolates of BPMV. They found that the RNA-1 and RNA-2 molecules differed among isolates, allowing the separation of BPMV isolates into two subgroups (I and II) and reassortants between isolates in these subgroups that possessed intermediate characteristics at the RNA level. These reassortant isolates produced the most severe mottling symptoms, whereas subgroup I isolates induced intermediate symptoms, and subgroup II isolates produced mild mottling symptoms when inoculated to soybean cv. Essex (Gu et al. 2002).

Many researchers have screened various germplasm of soybean and its wild relatives for BPMV resistance. Resistance has been found only in *Glycine* species other than *G. max*, and soybean cultivars have expressed symptoms with varying degrees of severity (Walters 1970; Scott et al. 1974; Schwenk and Nickell 1980; Zheng et al. 2005). In screening the *Glycine* genus for resistance to BPMV, Zheng et al. (2005) found variability in the *G. soja* germplasm for degree of leaf mottling when the accessions were inoculated with one isolate of BPMV. Additionally, Zheng et al. (2005) found resistance to BPMV in several *G. tomentella* accessions. Therefore, they concluded that typical reactions of *Glycine* species to BPMV inoculation include a 'tolerant' response ('York' soybean), stem tip necrosis (*G. soja* PI 407019), and resistance, expressed as no mosaic and no virus accumulation (*G. tomentella* PI 488 91-2) (Zheng et al. 2005). Hypersensitive responses were not observed on *Glycine* spp. when inoculated with BPMV; however, Zaumeyer and Thomas (1948) reported hypersensitive responses to BPMV on *Phaseolus* spp.

Using the differential classification of SMV in soybean as a model, the objective of this research was to screen the germplasm of BPMV hosts, especially the *Glycine* genus, in order to establish a differential classification system based upon the expression of phenotypic symptoms for BPMV infection. In the present study we discuss the reactions observed on *G. max*, *G. soja*, *G. tomentella*, *Phaseolus* spp. and *Vigna* spp. when inoculated with various isolates of BPMV.

Materials and methods

Viral isolates

The BPMV isolates used were K-Ho1, K-U1, K-G7, K-Ha1, K-He2, K-D1, generously provided by Dr. S. Ghabrial (University of Kentucky), and Ar, a mild isolate collected in Arkansas. Isolates K-Ho1 and K-U1 are highly aggressive reassortant isolates. Isolate K-G7 is a member of the subgroup I whereas K-Ha1, K-He2, and K-D1 are members of subgroup II (Gu et al. 2002). The Ar isolate is suspected to be member of subgroup II because it causes mild symptoms on soybean; however, this hypothesis has not been confirmed molecularly.

Each BPMV isolate was maintained separately in the greenhouse by periodic mechanical inoculations on 'Black Valentine' bean from a unique Arkansas seed collection that develops systemic mosaic symptoms when infected with these BPMV isolates.

Plant materials

Thirty-one cultivar/lines of *Glycine max* (Table 1), 36 lines of *G. soja* (Table 2), eight accessions of *G. tomentella* (Table 3), six cultivars of *Phaseolus vulgaris*, one cultivar of *P. lunatus* (Table 4), and six cultivars of *Vigna unguiculata* subsp. *unguiculata* (Table 5) were screened for their reaction to BPMV in the greenhouse. *Glycine soja*, *G. tomentella*, and some *G. max* accessions were obtained from the USDA Soybean Germplasm Collection in Urbana, IL. Other *G. max*, *Vigna*, and *Phaseolus* lines were from the University of Arkansas Germplasm Collection. 'Black Valentine Dry' and 'Black Valentine Stringless' bean were obtained from the Southern Exposure Seed Exchange (Mineral, VA). All plant materials were inoculated with isolates K-Ho1, K-G7, K-Ha1, and Ar; and 17 accessions were selected for further screening with the remaining isolates in each subgroup.

Table 1 Reactions of *G. max* genotypes to inoculation with isolates of *Bean pod mottle virus* (BPMV) representing the reassortant, subgroups I and II, and Ar isolate

Genotype	BPMV isolates			
	Reassortant[a]	Subgroup I[b]	Subgroup II[c]	Ar
CNS	SM[e]	SM	IM	IM
Corsoy	SM	SM	MM	MM
Hutcheson	SM	SM	MM	MM
Kent	SM	IM	IM	IM
L29[d]	SM	IM	IM	MM
York	SM	IM	IM	MM
Mack	SM	IM	IM	MM
V94-3971[d]	SM	IM	MM	IM
Amsoy 71[d]	SM	IM	MM	MM
PI 594922	SM	IM	MM	MM
V94-5152	SM	IM	MM	MM
PI 508267	SM	MM	MM	MM
V-262	SM	SM	IM	–
5002T	SM	IM	IM	–
D437RR	SM	IM	IM	–
HBK4992	SM	IM	IM	–
Prichard	SM	IM	IM	–
5601T	SM	IM	MM	–
94B54	SM	IM	MM	–
AG4403	SM	IM	MM	–
AG4603	SM	IM	MM	–
Benning	SM	IM	MM	–
Boggs	SM	IM	MM	–
Cook	SM	MM	–	—
DP4690RR	SM	MM	–	–
DP4748S	SM	MM	–	–
G112	SM	MM	–	–
DP4331RR	SM	MM	–	–
DP5110S	SM	MM	–	–
Haskell	SM	–	–	–
PI 96983	–	MM	–	–

[a] The reassortant isolate used in this study was K-Ho1

[b] The subgroup I isolate used in this study was K-G7

[c] The subgroup II isolate used in this study was K-Ha1

[d] In addition to the listed isolates, this accession was also tested with K-U1 reassortant isolate, and with K-D1 and K-He2 subgroup II isolates

[e] *SM* Severe mosaic, *IM* intermediate mosaic, *MM* mild mosaic, – not tested

Table 2 Reaction of *G. soja* accessions to inoculation with diverse *Bean pod mottle virus* (BPMV) isolates representing the reassortant, subgroup I and II, and Ar isolate

Accession	BPMV isolates			
	Reassortant[a]	Subgroup I[b]	Subgroup II[c]	Ar
PI 366123[d]	STN[e]	STN	STN	STN
PI 407019[d]	STN	STN	STN	STN
PI 464928[d]	STN	SM	SM	SM
PI 464889A[d]	STN	SM	SM	IM
PI 578345	SM/STN	SM	MM/STN	MM/STN
PI 447003A	SM/STN	MM	–	MM
PI 549047	IM/STN	IM	IM	MM
PI 507651	IM/STN	MM	MM	NM
PI 597457A[d]	MM/STN	MM/STN	MM/STN	MM/STN
PI 378700[d]	MM/STN	MM/STN	MM/STN	MM
PI 424001[d]	NM/STN	MM	MM	MM
PI 597452A	SM	SM/STN	SM/STN	MM
PI 508060	SM	SM/STN	MM	MM/STN
PI 483461	SM	IM/STN	IM/STN	IM/STN
PI 65549	SM	MM	MM	MM
PI 407175	SM	MM	MM	MM
PI 468399A	–	STN	STN	STN
PI 507623	–	STN	STN	SM
PI 407272	–	STN	STN	IM
PI 532453B	–	STN	STN	MM
PI 366120	–	STN	IM	STN
PI 163453	–	STN	IM	MM
PI 522226	–	STN	MM/STN	MM
PI 483462B	–	IM/STN	IM	–
PI 407046	–	SM	STN	STN
PI 487431	–	SM	IM/STN	MM
PI 339735B	–	SM	IM	IM
PI 578343	–	SM	IM	IM
PI 507641	–	IM	SM	IM
PI 549033	–	IM	IM	MM
PI 407155	–	IM	MM	SM
PI 468400B	–	IM	MM	SM
PI 203246	–	IM	MM	MM
PI 407285	–	MM	MM/STN	NM
PI 507593	–	MM	MM	SM
PI 507654	–	MM	–	MM/STN

[a] The reassortant isolate used in this study was K-Ho1

[b] The subgroup I isolate used in this study was K-G7

[c] The subgroup II isolate used in this study was K-Ha1

[d] In addition to the listed isolates, this accession was also tested with K-U1 reassortant isolate, and with K-D1 and K-He2 subgroup II isolates

[e] *STN* Stem-tip necrosis, *SM* severe mosaic, *IM* intermediate mosaic, *MM* mild mosaic, *NM* no mosaic, *xx/yy* mixed reactions, – not tested

Planting and inoculation procedures

Two pots each of 8–12 plants per genotype per viral isolate were grown in a greenhouse with temperatures at 20–25°C and a 14-h photoperiod. A control pot of 8–12 mock inoculated plants was also included in the

Table 3 Phenotypic reaction of *G. tomentella* to inoculation with four different *Bean pod mottle virus* (BPMV) isolates

Accessions	Reaction to BPMV isolates and virus assay							
	Reassortant[a]		Subgroup I[b]		Subgroup II[c]		Ar	
PI 505208	NM[d]	+[e]	NM	+	NM	+	NM	+
PI 483227	NM	−/+	NM	−/+	NM	+	NM	−
PI 424095	NM	+	−	−	NM	+	NM	+
PI 446947	NM	−/+	NM	−	NM	+	NM	+
PI 563876	NM	−/+	NM	−	NM	+	NM	+
PI 573070	NM	+	NM	−	NM	−	NM	+
PI 505215	NM	+	NM	−	NM	−	NM	−
PI 483218	NM	−	NM	−	NM	+	NM	+

[a] The reassortant isolate used in this study was K-Ho1

[b] The subgroup I isolate used in this study was K-G7

[c] The subgroup II isolate used in this study was K-Ha1

[d] *NM* No mosaic, – not tested

[e] + and − Positive or negative tissue blotting results for BPMV accumulation, −/+ variation in serological results among individual plants in the same genotype, – not tested

Table 4 Reaction of *Phaseolus* spp. cultivars to four different isolates of *Bean pod mottle virus* (BPMV) from different subgroups

Bean genotype	Reaction to BPMV isolates and virus assay							
	Reassortant[a]		Subgroup I[b]		Subgroup II[c]		Ar	
Black Valentine Dry[d]	HR[f]	–[g]	HR	−	HR	−	HR	−
Pinto[d]	HR	−	HR	−	HR	−	HR	−
Black Valentine Stringless[d]	MM	+	MM	+	MM	+	MM	+
Gallatin[d]	MM	+	MM	+	MM	+	MM	+
Jackson Wonder lima bean[e]	MM	+	MM	+	MM	+	MM	+
Black Valentine (AR)[d]	SM	+	SM	+	SM	+	SM	+
Cherokee Wax[e]	SM	+	SM	+	SM	+	SM	+

[a] The reassortant isolates used in this study were K-Ho1and K-U1

[b] The subgroup I isolate used in this study was K-G7

[c] The subgroup II isolates used in this study were K-Ha1, K-He2, and K-D1

[d] *Phaseolus vulgaris* cultivar

[e] *Phaseolus lunatus* cultivar

[f] *HR* Hypersensitive response (necrotic lesions on inoculated leaves), *SM* severe mosaic, *MM* mild mosaic

[g] + and − Positive and negative result in tissue imprints for BPMV accumulation

Table 5 Reaction of *Vigna* sp. cultivars (Arkansas collection) to different isolates of *Bean pod mottle virus* (BPMV)

Genotype	Reaction to BPMV isolates and virus assay							
	Reassortant[a]		Subgroup I[b]		Subgroup II[c]		Ar	
Monarch	S[e]	+[f]	S	+	S	+	S	+
Georgia-21[d]	S	+	S	+	S	+	S	+
Crimson	S	+	S	+	S	+	S	+
Chinese Red	S	+	S	+	S	+	S	+
Coronet	NM	−	NM	−	NM	−	NM	−
TVm-612	NM	−	NM	−	NM	−	NM	−

[a] The reassortant isolate used in this study was K-Ho1

[b] The subgroup I isolate used in this study was K-G7

[c] The subgroup II isolate used in this study was K-Ha1

[d] In addition to the listed isolates, this accession was also tested with K-U1 reassortant isolate, and with K-D1 and K-He2 subgroup II isolates

[e] *S* Susceptible response (mosaic, leaf curling), *NM* no mosaic or curling symptoms

[f] + and − Positive and negative results from tissue blotting for BPMV accumulation

screening of reaction to each isolate. Eight to 15 seeds per pot were planted in 15-cm diameter plastic pots containing Scott's Redi-Earth (Marisville, OH) potting soil mixture. Plants were fertilised with a slow release N–P–K (14–14–14) fertiliser together with a dose of granulated imidacloprid insecticide (0.03 g active ingredient per pot) at planting, and watered daily after emergence.

Plants were inoculated using the standard mechanical technique of sap inoculation (Ketharpal et al. 1998). Virus inoculum was prepared as follows: one trifoliolate leaf of mosaic-expressing Black Valentine bean, collected from plants about 7 days after inoculation (DAI), was ground in 5 ml of 0.05 M potassium phosphate buffer (pH 7.0), and kept chilled on ice. Fully expanded unifoliolate leaves of young seedlings (growth stage V_1) were dusted with 600-mesh silicon carbide and inoculated by rubbing both unifoliolate leaves with a pestle or with a cheesecloth pad that had been dipped in the inoculum. Plants were maintained and observed in the greenhouse with moderate temperatures (20 to 25°C) for 4 weeks until viral symptoms were fully expressed.

Disease rating

Ratings were performed on a weekly basis, using a four-step scale for mosaic/mottling symptoms (no

symptoms, mild, intermediate, and severe), and a separate class for stem-tip necrosis. In the rating scale used, 'no symptoms' was considered when the reaction of the inoculated plants was similar to that of the non-inoculated control. The mild symptoms presented by Amsoy 71 soybean when inoculated with isolate K-Ha1 were used as a reference for the 'mild mosaic'. 'Intermediate mosaic' symptoms were expressed by Amsoy 71 when inoculated with K-G7 isolate, whereas the 'severe mosaic' was observed in Amsoy 71 when inoculated with K-Ho1. 'Stem-tip necrosis' was characterised by necrotic spots on leaves, veinal necrosis, stem-browning, and death of the tip of the plant. Stem-tip necrosis could occur as early as 5 DAI for some plants within a genotype, while others plants from the same accession exhibited necrotic symptoms at the V3–V4 stages.

Data from three replicated experiments (planted in December 2003, May and December 2004) were combined in order to establish the reaction type of each line. Since a differential system is based on compatible/incompatible interactions which are qualitative reactions, no statistical analysis was performed on the phenotypic data. Plants expressing resistance exhibited no mosaic symptoms, had no systemic viral accumulation, and were easily distinguishable from susceptible and necrotic plants.

Viral detection

Tissue Blotting Four weeks after inoculation, three young leaflets, one set from each of three different plants per pot, were rolled together and blotted onto the blotting paper. Virus presence was then detected through the tissue imprint methodology described by Holt (1992) with some modifications, including the use of regular-grade copy paper to replace the nitrocellulose membrane, non-fat dry milk to replace bovine serum albumin as the blocking agent, and a shorter wash-time (1 min instead of 10 min) and incubation time (20 min instead of 30 min). A rabbit polyclonal antibody to BPMV described previously (Wang et al. 1992) was used to detect BPMV.

ELISA Virus detection was also performed using a semi-quantitative protein-A double-antibody sandwich ELISA as described by Edwards and Cooper (1985). Four weeks after inoculation, leaflets of the uppermost, expanded trifoliolate leaf of the test plant were ground with a mortar and pestle and diluted 1:10 with PBS-Tween buffer (Phosphate Buffered Saline containing 10.9 g l^{-1} Na_2HPO_4, 3.2 g l^{-1} NaH_2PO_4, 90 g l^{-1} NaCl, and 0.05% Tween 20). Viral antigen from infected plants was detected using the rabbit polyclonal antibody to BPMV described above. Absorbance at 405 nm was recorded 30 min after reaction initiation using a plate reader (Model 7520, Cambridge Technology Inc, Cambridge, MA). Positive and negative readings were separated using the threshold of three times the mean of the healthy control wells (Sutula et al. 1986).

Results

Responses of soybean genotypes to BPMV isolates

Thirty-one soybean genotypes were screened for their reaction to diverse isolates of BPMV representing subgroups I and II, reassortant, and a local Arkansas isolate. All the cultivars/lines were found to be susceptible to the virus, expressing various degrees of mosaic/mottling symptoms (Table 1). For instance, Amsoy 71 showed severe mosaic symptoms to inoculation with the reassortant isolates of BPMV, intermediate mosaic symptoms to the subgroup I isolate, and mild mosaic symptoms to other isolates of BPMV. We observed that the reassortant isolates K-Ho1 and K-U1 were more aggressive and induced more severe mosaic symptoms than other subgroups of isolates, and that the subgroup I isolate was less aggressive than the reassortants but more aggressive than the Ar and subgroup II isolates (Table 1). Additionally, tissue imprints revealed that BPMV was able to accumulate and systemically spread in plants of all the soybean germplasm screened (data not shown).

Reaction of *G. soja* accessions to BPMV isolates

Thirty-six accessions of *G. soja* were selected from the literature for their stem-tip necrotic reaction to the reassortant isolate K-Ho1 (Zheng et al. 2005) in an attempt to find resistance to milder isolates of BPMV. Our assumption was based on the report by Cho and Goodman (1979) that inoculation of a resistant soybean genotype with a more severe isolate of

SMV induced a stem-tip necrosis reaction. The 36 *G. soja* accessions were screened with at least one representative isolate from each of the subgroup I, subgroup II, reassortant isolates, and the Ar isolate. All the genotypes were susceptible to BPMV, as demonstrated by their positive reaction in serological tests and the development of either mosaic/mottling or stem-tip necrosis symptoms, indicating no complete resistance for BPMV in the screened accessions of *G. soja* (Table 2).

The screened accessions of *G. soja* exhibited different patterns of symptom expression to the BPMV isolates used. Two accessions (PI 407019, PI 366123) showed stem-tip necrosis to all the BPMV isolates (Table 2). PI 464889A and PI 464928 showed a stem-tip necrosis reaction when inoculated with the reassortant isolates of BPMV, but mosaic symptoms when challenged with other BPMV isolates. PI 407046 showed severe mosaic symptoms when inoculated with the BPMV subgroup I isolate, and stem-tip necrosis when inoculated with an isolate from subgroup II. PI 507623 exhibited stem-tip necrosis when inoculated with the BPMV isolate from the subgroup I, and severe mosaic symptoms when inoculated with the Ar isolate. Also, PI 366120 expressed stem-tip necrosis to the Ar BPMV isolate, but intermediate necrosis when inoculated with an isolate from the subgroup II.

Reactions of *G. tomentella* to BPMV isolates

Eight *G. tomentella* accessions were screened for their reaction to isolates K-Ho1, K-G7, K-Ha1, and Ar, representing the reassortant, subgroup I, subgroup II, and local AR isolate, respectively (Table 3). These accessions were resistant (no systemic infection) to some isolates of BPMV but became systemically infected with others, as determined by tissue imprint methodology; however, all accessions tested were symptomless.

Reaction of *Phaseolus vulgaris, P. lunatus*, and *Vigna unguiculata* subsp. *unguiculata* to BPMV isolates

The *P. lunatus* genotype and the six *P. vulgaris* cultivars screened with BPMV isolates exhibited two types of reactions: resistance or mosaic symptoms. Resistance was observed as a hypersensitive reaction (necrotic spots on inoculated leaves with no symptoms on the upper leaves) (Table 4), and these plants showed no systemic virus accumulation when inspected by the tissue blotting technique. Pinto bean and 'Black Valentine Dry' bean were resistant to all the isolates of BPMV, as first reported by Zaumeyer and Thomas (1948). Two types of susceptibility symptoms were observed in *Phaseolus* spp. when inoculated with BPMV isolates: severe mosaic and mild mosaic. Black Valentine (AR) and 'Cherokee Wax' beans both showed severe mosaic to all BPMV isolates, whereas 'Black Valentine Stringless' bean (SESE), 'Gallatin' bean, and 'Jackson Wonder' lima bean exhibited mild mosaic symptoms. All susceptible genotypes had systemic virus accumulation, as indicated by positive tissue blotting reactions.

The screening of cowpea cultivars showed that 'Monarch', 'Georgia-21', 'Crimson', and 'Chinese Red' allowed BPMV multiplication and exhibited leaf curling and mosaic symptoms when inoculated with all four BPMV isolates (Table 5). However, the cowpea cvs 'Coronet' and 'TVm-612' did not develop symptoms of infection when inoculated with any of the four BPMV isolates used, and no virus was detected by tissue blotting in the inoculated plants.

Discussion

The results from our screening of soybean germplasm agree with those of Wang et al. (2005) who reported that 52 soybean ancestral lines were susceptible, having positive serological results with various degrees of mosaic and leaf distortion, to the inoculation with a subgroup I BPMV isolate. In another study, Zheng et al. (2005) did not find resistance in 18 soybean genotypes screened but observed that the severity of mosaic symptoms varied among genotypes and BPMV isolates. We also observed that the reassortant isolates K-Ho1 and K-U1 were more aggressive and induced more severe mosaic symptoms than other subgroups of isolates (Table 1). Gu and Ghabrial (2005) attributed the increased aggressiveness of the reassortant isolates to mutations in the Co-pro and He1 proteins. Also, our results are in agreement with those of Zheng et al. (2005) that the subgroup I isolate was less aggressive than the reassortants but more aggressive than the Ar and subgroup II isolates. The subgroup I isolate induced moderate symptoms

while subgroup II and AR isolate induced mild mosaic symptoms.

Based upon the presented results, the *G. max* genotypes cannot be used to differentially classify BPMV isolates due to the complete lack of resistance genes or expression of necrotic reactions. Additionally, the differences in mosaic symptom severity among isolates in the subgroup I, II, and Ar isolate was not large enough to be a reliable tool to differentiate those isolates; nevertheless, reassortant isolates can be readily separated phenotypically from isolates in the subgroup I, II, and Ar because reassortant isolates consistently induced more severe symptoms across all genotypes.

Similar to *G. max* reaction to BPMV, all screened *G. soja* genotypes showed more severe symptoms when inoculated with reassortant isolates than with subgroup I and II isolates or the Ar isolate of BPMV (Table 2). Based on the pattern of mosaic and necrotic symptoms, the four groups of BPMV isolates can be differentiated by the phenotypic reactions of *G. soja* PI 464889A, PI 507623, PI 366120, and PI 407046. PI 464889A can be used for differentiating the reassortant (stem-tip necrosis) and subgroup I (severe mosaic); PI 407046 for subgroup I (severe mosaic) and subgroup II (stem-tip necrosis); PI 507623 for subgroup I (stem-tip necrosis) and Ar (severe mosaic), and PI 366120 for subgroup II (intermediate mosaic) and Ar isolate (stem-tip necrosis). However, these reactions may not be a reliable tool to separate the four groups of BPMV isolates because they are highly influenced by environmental and inoculation conditions.

The reactions presented by *G. tomentella* accessions to BPMV (Table 3) partially agree with those reported by Zheng et al. (2005). Under their experimental conditions, PI 483218, PI 573070, and PI 563876 were resistant to Ar and subgroup I, but susceptible (positive serological results for virus accumulation) to subgroup II and reassortant. Additionally, PI 483227 and PI 446947 were susceptible to the reassortant isolate only, whereas PI 505208 and PI 505215 were susceptible to all four isolates. However, under our experimental conditions, only PI 505208 was susceptible to all four isolates of BPMV, and PI 505215 expressed resistance to subgroup I and II, and Ar, but susceptibility to the reassortant. The accessions PI 483218, PI 573070, and PI 563876 were resistant to subgroup I isolate which agrees with Zheng et al. (2005); however, they responded differently to the other isolates of BPMV as compared to the results by Zheng et al. (2005). Similarly, PI 446947 and PI 483227 were susceptible to the reassortant isolate as previously reported, but also accumulated BPMV when inoculated with subgroup I and II, and Ar. The discrepancies in responses to BPMV inoculation between the two studies could be the result of small sample size of the tested genotypes because a limited number of seeds were available from the USDA Soybean Germplasm Collection, or the result of different environmental conditions for the two experiments (e.g. 12-h photoperiod for the previous study, but 14-h photoperiod in our test).

Glycine tomentella (Zheng et al. 2005)*, G. falcate, G. javanica,* and *G. wightii* (Scott et al. 1974) are *Glycine* species with reported resistance to BPMV, and therefore valuable germplasm sources in breeding soybeans for genetic resistance to BPMV. It should be noted, however, that some *G. tomentella* accessions allowed certain isolates of BPMV to replicate, move, and accumulate, but in every case the inoculated plants remained symptomless. This may indicate that the mechanism of resistance is disrupting symptom expression, but not viral accumulation. It is also worth noting that there is heterogeneity among plants of the same accession. Some plants showed virus accumulation and others did not. This may indicate that *G. tomentella* accessions tested were not genetically homogeneous. The scarce seed availability, together with the potential genetic variation within accessions of *G. tomentella* may discourage the use of this species for the separation of BPMV isolates.

The screening of *Phaseolus* spp. genotypes (Table 4) resulted in unique reactions to BPMV (hypersensitive reaction, severe mosaic, and mild mosaic); however, the selected *Phaseolus* genotypes are not appropriate to be used as differential candidates for BPMV classification because of the lack of differential reactions among isolates. Similarly, the cowpea cultivars did not present any variation in symptom severity among the BPMV isolates used (Table 5). Cultivars resistant to BPMV expressed the resistant reaction to all the isolates used, indicating no differential interaction of resistance genes and BPMV isolates. The susceptible genotypes exhibited mosaic symptoms when inoculated with any of the four BPMV isolates. Therefore, although potentially valuable as resistance gene sources, none of the screened

lines of cowpea may serve as differential hosts in the classification of BPMV isolates.

Han and Murayama (1970) postulated that a differential system for isolate classification relies on the existence of major resistance genes in the host, the presence of different avirulence (*Avr*) genes in the pathogen (variation in pathogenicity), and a clear-cut differential interaction of different host genotypes and pathogen isolates. The first of these requirements was not fulfilled in our experiments because none of the tested genotypes of *G. max* or *G. soja* was resistant to BPMV; and although resistance was observed in some *Phaseolus* and *Vigna* genotypes, no differential reaction was manifested to the inoculation with different subgroups of isolates. Excluding the reactions of *Phaseolus*, *Vigna*, and *G. tomentella* accessions, the BPMV-soybean system is not the only pathosystem for which no resistance genes are known; Fraser (1992) reported that in the UK at least 25 viruses affecting 22 horticultural crops lack major resistance genes.

The absence of major resistance genes, the lack of easily identifiable isolate by genotype interactions, and the variation in aggressiveness of this viral pathogen led to the conclusion that BPMV cannot be classified into strain groups using *G. max* host genotypes tested in this study. On the contrary, BPMV isolates may vary in aggressiveness, with differences in symptom severity possibly determined by the RNA-1 molecule of the virus (Gu and Ghabrial 2005). However, this variation in symptom severity is not reliable enough to be used for isolate differentiation or classification, although variation in aggressiveness between reassortant and other isolates can be easily differentiated using *Glycine* accessions Amsoy 71, L29, PI 507623, PI 366120, PI 407046, PI 464889A, and PI 464928. These genotypes could be a useful tool for pathologists to determine the subgroups, and therefore potential severity, of BPMV isolates from a particular geographical area.

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