Pathogenicity Groups of Bean Common Mosaic Virus in the USDA *Phaseolus* Germ Plasm Collection

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ABSTRACT

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The pathogenicity groups of seedborne bean common mosaic virus (BCMV) strains in the USDA *Phaseolus* germ plasm collection were identified by systemic infection of differential *P. vulgaris* cultivars. All BCMV pathogroups (PG)(sensu Drijfhout) except PG III were detected, and 10 BCMV cultures simulated a new pathogroup, PG VIII. Six of these cultures, however, were resolved into two different BCMV strains, and 14 of 19 PG VI–simulating cultures were similarly resolved. PG V cultures were rare, except in mixed infections with other pathogroups. None of the BCMV cultures simulating PG VI induced temperature-insensitive necrosis on cultivars containing the *I* gene.

Bean common mosaic virus (BCMV) is a ubiquitous seedborne pathogen of common bean (*Phaseolus vulgaris* L.). Many resistant cultivars have been released that were later found to be susceptible to new strains of BCMV. The definitive work of Drijfhout (1) explained the genetic basis of the host–pathogen interaction and described a procedure (2) for differentiating BCMV strains using specific *P. vulgaris* genotypes (cultivars). BCMV strains have been grouped into seven pathogroups (PG) based on systemic infection of differential cultivars. BCMV strains have also been divided into two major serogroups, A and B (9,10). PGs III and VI compose serogroup A and cause temperature-insensitive necrosis in bean cultivars possessing the *I* gene, whereas other pathogroups compose serogroup B and may or may not cause temperature-sensitive necrosis.

The U. S. Department of Agriculture (USDA) *Phaseolus* germ plasm collection is contaminated with BCMV (4). Because the collection contains germ plasm from a variety of sources, there are probably numerous BCMV strains in the collection. However, relatively little information is available regarding the BCMV strains contaminating the collection. Provvidenti (7) described an NY15-like BCMV isolate (PG V) from *P. acutifolius* A. Gray, an isolate described by Silbernagel (8) from *P. vulgaris*, which is now assigned to PG VII. In addition, we recently noted the absence of serogroup A isolates in the collection (4). Consequently, a survey was undertaken to determine the incidence of BCMV pathogenicity groups in the USDA *P. vulgaris* germ plasm collection.

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MATERIALS AND METHODS

Differential bean cultivars. The *P. vulgaris* cultivars used to differentiate the BCMV strains are listed as differential cultivars in Table 1. All cultivars except Topcrop were used in work done at Corvallis, Oregon, whereas only Dubbele Witte, Puregold Wax, Great Northern UI 123, Red Mexican UI 34, Pinto UI 114, Monroe, and Topcrop were used in Pullman.

BCMV cultures, inoculations, and maintenance. BCMV cultures were obtained from plants of Plant Inventory (PI) *P. vulgaris* accessions exhibiting symptoms of infection by seedborne BCMV. Cultures tested at Corvallis originated from PI seed samples planted in glasshouses, whereas those tested at Pullman were recovered from symptomatic seedlings in a glasshouse seed-propagation planting at Pullman. Tissue samples from each infected plant were triturated fresh in 0.02 M phosphate buffer, pH 7.0, with 0.5% diatomaceous earth (Celite), and applied by rub inoculation to the differential cultivars. All differential cultivars were inoculated at the primary leaf stage. At Corvallis, BCMV isolates were simultaneously inoculated onto all differential cultivars (except Topcrop), whereas at Pullman a modified procedure (Fig. 1) was used. An infected differential cultivar was used as inoculum for each succeeding set of differential cultivars under this procedure. Back inoculations were done as needed to separate potentially mixed infections (Fig. 2) or to complete differential host ranges. The inoculation procedure used at Corvallis did not separate mixed infections.

All plants except Topcrop were maintained in aphid-free greenhouses. Solar illumination supplemented with fluorescent lights provided a 14- to 16-hr photoperiod, and temperature was maintained between 15 and 30 C. Topcrop plants were inoculated and maintained in a growth chamber at a constant temperature of 20 or 32 C to determine the temperature sensitivity of the necrotic response upon BCMV infection in this *I*-gene cultivar.

Evaluation of infection. Some differential cultivar–BCMV isolate interactions have been reported to result in systemic infections with questionable or weak symptoms (1). Potential difficulties with evaluation of systemic infection were overcome at Corvallis by inoculation and inspection of more than one differential cultivar in each bean resistance group and at Pullman by indirect enzyme-linked immunosorbent assay (ELISA), using a monoclonal antibody that reacts with all known strains of BCMV (11). Some isolates were also assigned to serogroups on the basis of reactions in indirect ELISA with polyclonal antisera prepared against the NL3 or NY15 strains of BCMV (antibody and antisera provided by G. I. Mink). ELISA conditions were described earlier (4). Reaction of the cultivar Topcrop to BCMV inoculation was visually determined 3–4 wk postinoculation.

RESULTS

A total of 140 BCMV cultures from 41 *P. vulgaris* PI accessions were assigned to pathogroups on the basis of systemic infection of differential bean cultivars (Table 2). The seedborne cultures simulated all known pathogroups except PG III. In addition, several cultures appeared to combine the pathogenicities of PGs VI and VII, hereafter referred to as cultures simulating PG VIII.

Many of the cultures that simulated PGs VI and VIII appeared to be mixed infections (Table 3) on the basis of failures of reciprocal inoculations. For ex-

Table 1. Differentiation and grouping of bean common mosaic virus strains and host resistance groups

Host resistance group	Host resistance genes	Differential cultivar	Virus pathogenicity group[a]						
			I	II	III	IV	V	VI	VII
1	...	Dubbele Witte	+	+	+	+	+	+	+
		Sutter Pink	+	+	+	+	+	+	+
		Stringless Green Refugee	+	+	+	+	+	+	+
2	*bc-1*	Puregold Wax	−	+	−	+	+	+	+
		Redlands Greenleaf C	−	+	−	+	+	+	+
		Imuna	−	+	−	+	+	+	+
3	*bc-1*^{2}	Great Northern UI 123	−	−	−	+	−	+	+
		Redlands Greenleaf B	−	−	−	+	−	+	+
4	*bc-2*	Red Mexican UI 34	−	−	+	−	+	+	−
		Sanilac	−	−	+	−	+	+	−
		Michelite 62	−	−	+	−	+	+	−
5	*bc-1, bc-2*	Pinto UI 114	−	−	−	−	+	+	−
6	*bc-1*^{2}, *bc-2*^{2}	Monroe	−	−	−	−	−	−	+
		Great Northern UI 31	−	−	−	−	−	−	+
9b	*I*	Topcrop	−	−	−	±n	±n	+n	−

[a] − = Resistant, no systemic infection; + = susceptible, systemic; ±n = susceptible or resistant, temperature-dependent necrosis; +n = susceptible, temperature-independent necrosis.

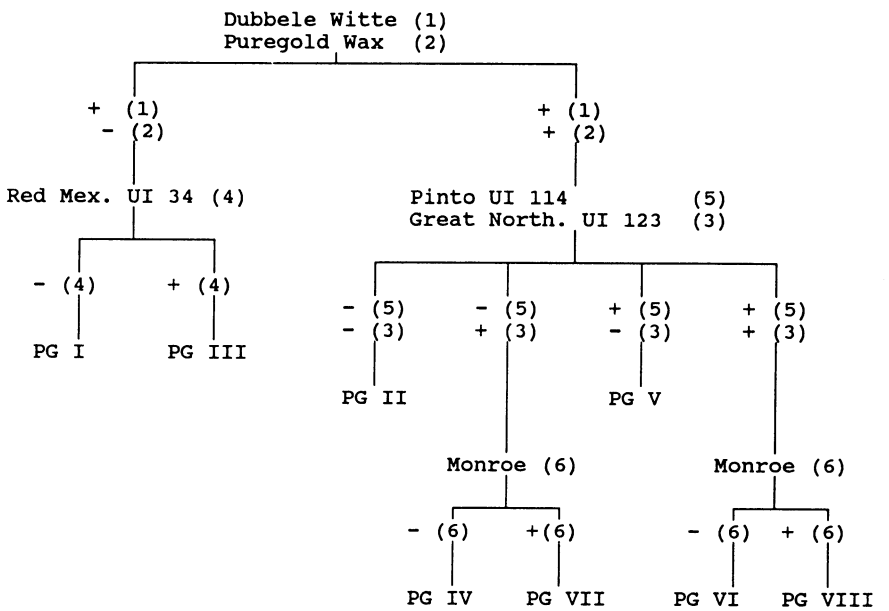

Fig. 1. Stepwise inoculation procedure followed at Pullman, Washington, to determine bean common mosaic virus pathotypes present as seedborne infections in the USDA *Phaseolus vulgaris* germ plasm collection. Leaf tissue from the lowermost systemic infection served as inoculum for the subsequent inoculation step. Parenthetical numbers refer to host resistance group (see Table 1). + = Systemic infection of the host resistance group cultivar, and − = no systemic infection of the host resistance group cultivar. Systemic infections were confirmed by enzyme-linked immunosorbent assay.

ample, inoculum derived from a seedborne infection infected both Pinto UI 114 and Great Northern UI 123, but inoculum derived from the infected Pinto UI 114 failed to infect Great Northern UI 123, and vice versa. It was concluded that these cultures, while simulating PG VI, were a mixture of at least two strains appearing to be PGs IV and V. Of the cultures examined at Pullman, 20 (16% of the total number) contained BCMV strains from two pathogroups. PG V was usually detected in mixed infections and only rarely by itself. Cultures examined at Corvallis were not tested for mixed infections.

Two of eight PG VIII-simulating cultures were not resolvable into mixed infections by reciprocal inoculations of Red Mexican UI 34 and Monroe, suggesting that they may have been distinct new strains. A total of 10 cultures simulating PG VI failed to cause temperature-insensitive necrosis in Topcrop, and only two of the 10 caused temperature-sensitive necrosis. In addition, all 10 isolates were serogroup B.

It was not our intention to characterize cultures by the severity of symptoms induced in specific differential hosts. However, isolates within pathogroups varied widely in symptom severity.

DISCUSSION

The strains of seedborne BCMV were not completely identified as described by Drijfhout et al (2), because of the inability of the procedure to deal efficiently with a large number of cultures. In addition, the high incidence of mixed infections was not anticipated when the procedure was devised. Mixtures of PGs IV and V, V and VI, and VI and VII are the only combinations of strains that can be separated with the current set of differential cultivars, and then only by laborious stepwise inoculation (Fig. 2). Thus, the composition of mixed infections cannot be readily determined (3).

We were interested in viral and host genetic factors that might favor or limit BCMV pathogroups in the germ plasm collection. When BCMV was monitored during a germ plasm propagation, nearly 90% of the accessions became infected; seed harvested from these accessions indicated that only 60% of the accessions contained seed-transmitted BCMV (5). It seems likely that the relative incidence of seedborne BCMV strains does not result from the ability of the strain to infect a broad range of germ plasm accessions, but is it more likely indicative of its ability to survive within the collection as a seedborne infection. Morales and Castaño (6) investigated seed transmissibility of selected BCMV isolates from PGs I, IV, V, VI, and VII in various differential cultivars. The incidence of their seed transmission in Dubbele Witte

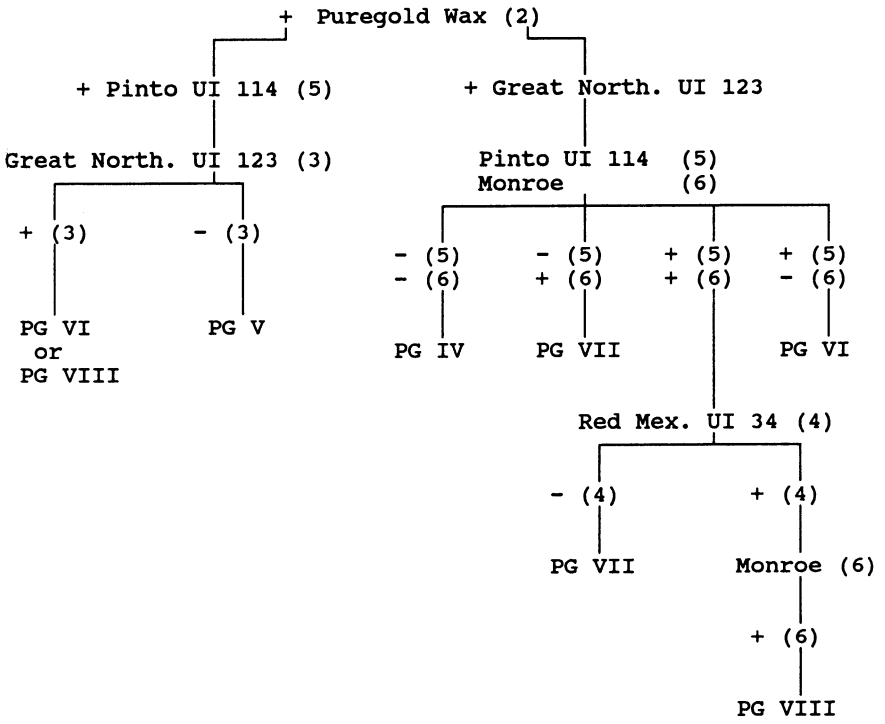

Fig. 2. Stepwise inoculation procedure followed at Pullman, Washington, for the separation of potentially mixed seedborne bean common mosaic virus infections (isolates simulating pathogroups VI and VIII in Figure 1). Leaf tissue from the lowermost systemic infection served as inoculum for the subsequent inoculation step. Parenthetical numbers refer to host resistance group (see Table 1). + = Systemic infection of the host resistance group cultivar, and − = no systemic infection of the host resistance group cultivar. Systemic infections were confirmed by enzyme-linked immunosorbent assay.

Table 2. Bean common mosaic virus strain groups simulated by 140 seedborne isolates from 41 Plant Introduction accessions of *Phaseolus vulgaris*

Strain group simulated	Number of isolates
I	29
II	27
III	0
IV	47
V	2
VI	22
VII	3
VIII[a]	10

[a] Strain group VIII has not been formally described. These isolates simulated a combined pathogenicity of strain groups VI and VII.

Table 3. Bean common mosaic virus (BCMV) strains apparently present with selected seedborne cultures simulating pathogenicity groups VI and VIII

Strain simulated	Number of cultures examined	BCMV presence: Strains	BCMV presence: Number of cultures
VI	19	IV and V	14
		VI	5
VIII[a]	8	V and VII	1
		VI and VII	5
		VIII	2

[a] This strain group has not been formally described. These strains simulated a combined pathogenicity of strain groups V, VI, and VII.

was greatest with a PG IV isolate, followed by isolates from PGs I, VI, VII, and V. With the exception of PG V, this is the same order of incidence observed in seedborne strains (mixed infections included) in the USDA collection. Morales and Castaño (6) also found US2 (NY15), a PG V isolate, to be seed-transmitted at a lower percentage than other BCMV strains. PG V isolates were more common in the USDA germ plasm collection than one would expect from their inherently limited seed transmissibility and, when detected, were almost always in mixed infections. This suggests that some strains of BCMV may "complement" PG V strains and result in a higher rate of seed transmission than would be otherwise possible.

All PG VI isolates prior to this investigation induced temperature-insensitive necrosis on *I*-gene cultivars and were typed as serogroup A. The failure of PG VI–simulating isolates in this study to cause this type of necrosis or to serotype as serogroup A suggests that the viral associated factors conditioning temperature-insensitive necrosis could be associated with the coat protein, and it further emphasizes the association of serogroup A and temperature-insensitive necrosis (9,10).

The USDA *P. vulgaris* germ plasm collection is contaminated with a diverse array of BCMV strains and therefore represents both a *Phaseolus* and a BCMV collection. Unfortunately, the geographical origin of the strains cannot be determined, because of fluctuations in BCMV incidence and spread during seed propagation (5). As BCMV eradication from the *Phaseolus* collection proceeds, some BCMV-infected accessions will be maintained as a separate collection to ensure that this BCMV collection is maintained for use in future research.

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