Incidence of Bean Common Mosaic Virus in USDA *Phaseolus* Germ Plasm Collection

R. E. KLEIN, Postdoctoral Research Associate, and S. D. WYATT, Associate Professor, Department of Plant Pathology, Washington State University, Pullman 99164-6430, and W. J. KAISER, Research Plant Pathologist, USDA-ARS, Western Regional Plant Introduction Station, Washington State University, Pullman 99164-6402

ABSTRACT

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The USDA *Phaseolus* germ plasm collection was surveyed to determine the incidence and serogroups of seedborne bean common mosaic virus (BCMV) in accessions of 10 *Phaseolus* species. BCMV was found in accessions of *P. vulgaris, P. acutifolius, P. aborigineus*, and *P. angustifolius*. This is the first report of seedborne BCMV in the latter two species. BCMV was not found in accessions of *P. anisotrichus, P. coccineus, P. dumosus, P. lunatus, P. microcarpus*, and *P. polystachios*. All 490 BCMV isolates that were serotyped were serogroup B. BCMV appears to be a serious problem in *P. vulgaris* accessions; approximately 60% of the 207 tested were contaminated. Of 8,147 *P. vulgaris* plants tested, 591 (7%) were positive for BCMV.

Although not a new problem, the presence of seedborne viruses in germ plasm collections has become a major concern (1,2,8). The United States Department of Agriculture Regional Plant Introduction Station at Pullman, WA, maintains the USDA *Phaseolus* germ plasm collection, which currently numbers approximately 10,000 accessions in 16 species. The collection is contaminated with at least two seedborne viruses, bean common mosaic virus (BCMV) and cucumber mosaic virus (3); BCMV is by far the more prevalent. The many pathotypes of BCMV have been separated into two broad categories on the basis of symptoms in indicator plants, the mosaic-inducing strains and the more recently described temperature-insensitive necrosis-inducing strains (5). These groups also appear to be serologically distinct and have been described as serogroups B and A, respectively (16). A few reports have been published concerning BCMV in the *Phaseolus* collection at Pullman (9,12), but no comprehensive survey has been made to determine the incidence and serogroups of BCMV in this collection. A survey was needed prior to initiation of a virus eradication program. Results of such a survey of 10 of the 16 *Phaseolus* spp. in the collection are reported herein. Authorities for the *Phaseolus* spp. studied appear in Table 1.

MATERIALS AND METHODS

Approximately 30–50 seeds of each selected Plant Introduction (PI) accession were planted in steam-sterilized potting soil mix in a metal flat 30 cm wide × 50 cm long × 7 cm deep. Flats were maintained under greenhouse conditions of 25–35 C, and natural light was supplemented with fluorescent light to provide a 15-hr photoperiod. Plants were sampled individually at the first trifoliate leaf stage for BCMV with indirect enzyme-linked immunosorbent assay (ELISA) (10) using a broad-spectrum monoclonal antibody capable of detecting all known strains of BCMV (17). Leaf tissue was ground at approximately 1:100 (w/v) in 0.05 M carbonate buffer, pH 9.6, with polyvinylpyrrolidone-40 (2%) and ovalbumin (0.2%). Samples were absorbed to Dynatech (Dynatech Laboratories, Inc., Chantilly, VA) ELISA plates either 1.5–2 hr at room temperature or overnight at 4 C. Incubation of antivirus antibody and antimouse IgG:alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO) was 1.5–2 hr at room temperature. The substrate used was *p*-nitrophenyl phosphate, and plates were read on a Minireader II (Dynatech) at 410 nm when color development was optimal.

Many plants testing positively in these tests, as well as plants showing typical BCMV symptoms in normal seed increases, were tested for inclusion into either serogroup A or serogroup B. Indirect ELISA using rabbit antisera prepared against the NL-3 and NY-15 strains of BCMV were used to establish serogroupings. Antisera prepared against NL-3 reacted with both serogroup A and serogroup B isolates, whereas antisera against NY-15 reacted only with isolates of serogroup B (14,16). ELISA was performed as described above, except that antirabbit IgG:alkaline phosphatase conjugate (Sigma) was used.

Accessions tested for BCMV incidence included 207 of *P. vulgaris*, 26 of *P. acutifolius*, 18 of *P. coccineus*, 29 of *P. lunatus*, and 14 of six other *Phaseolus* species. Attempts were made to test accessions from diverse geographic origins and of differing dates of original collection.

RESULTS

Results of ELISA performed on plants of tested accessions are shown in Table 1. BCMV was detected in accessions of *P. aborigineus, P. acutifolius, P. angustifolius*, and *P. vulgaris*. BCMV was also detected in one accession (PI 358087 Yugoslavia) identified originally as *P. coccineus* but since reidentified as *P. vulgaris*. BCMV was not detected in accessions of *P. anisotrichus, P. dumosus, P. lunatus, P. microcarpus*, and *P. polystachios*.

The distribution of BCMV in *P.*

Table 1. Incidence of bean common mosaic virus (BCMV) in accessions of 10 *Phaseolus* species from the USDA germ plasm collection at Pullman, WA

Species	Number of accessions in collection	Incidence	
		Accessions	Plants
P. aborigineus Burk.	2	1/2[a]	5/74[b]
P. acutifolius A. Gray	72	5/26	10/987
P. angustifolius Roxb.	2	1/2	1/96
P. anisotrichus Schlecht.	3	0/3	0/64
P. coccineus L.	362	0/18	0/821
P. dumosus Macfad.	1	0/1	0/64
P. lunatus L.	913	0/29	0/786
P. microcarpus Mart.	2	0/2	0/58
P. polystachios (L.) B.S.P.	4	0/4	0/106
P. vulgaris L.	8,477	116/207	591/8,147

[a] Number of accessions testing positively in ELISA for BCMV/number of accessions tested.
[b] Number of plants testing positively in ELISA for BCMV/number of plants tested (includes plants from accessions testing negatively for BCMV).

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vulgaris accessions is shown in Table 2. No BCMV was detected in 44% of the accessions, and the incidence was 10% or less in about 30%; the highest incidence was 63%. The average incidence across all tested accessions was 7%. BCMV incidence appeared to be randomly distributed throughout the *P. vulgaris* collection, and the average incidence was essentially the same in the older accessions (ca. 1935) as in the more recently collected accessions (ca. 1980).

A total of 490 BCMV isolates from 108 different accessions were serogrouped. All isolates were in serogroup B (mosaic-inducing strains).

Table 2. Incidence of seedborne bean common mosaic virus (BCMV) in Plant Introduction *Phaseolus vulgaris* accessions based on grow-out and ELISA tests

BCMV incidence (%)	Accessions	
	No.	%
0	91	44.0
1–10	63	30.4
11–20	30	14.5
21–30	13	6.3
31–40	3	1.4
41–50	4	1.9
51–60	2	1.0
61–70	1	0.5
Total	207	100.0

DISCUSSION

BCMV was detected in only a few accessions of species other than *P. vulgaris* and usually in relatively low incidences, with the exception of *P. aborigineus*. Accessions of the non-*P. vulgaris* species compose less than 5% of the *Phaseolus* germ plasm collection. Consequently, BCMV in these non-*P. vulgaris* accessions is of comparatively minor concern. In any germ plasm virus eradication program, however, the presence of incorrectly identified accessions within a presumably immune species must be considered. *P. coccineus* may be immune to BCMV (15), but the presence of *P. vulgaris* accessions incorrectly identified as *P. coccineus* means that, in a practical sense, the *P. coccineus* is also contaminated with BCMV. Little information is available on BCMV seed transmission in *Phaseolus* species other than *P. vulgaris* and *P. acutifolius*. Evidence of seedborne BCMV in accessions of *P. aborigineus* and *P. angustifolius* is herein reported for the first time, to our knowledge.

The presence of BCMV in *P. vulgaris* germ plasm is a major problem. Over one-half of the accessions are now known to be contaminated with BCMV. The distribution of such accessions raises questions about the danger to some potential users of the germ plasm collection (seed shipments currently leaving the Regional Plant Introduction Station at Pullman carry a warning to users). The high frequency of BCMV incidence also makes it difficult to evaluate these accessions for useful agronomic characteristics.

Many of the BCMV isolates assigned to serogroup B were detected in accessions of African origin. Although Africa is the presumed origin of serogroup A BCMV strains (14), none of these was detected. The presence of serogroup A strains in untested accessions cannot be excluded, but this appears statistically unlikely. Morales and Castaño (11) speculated that only small white-seeded beans were capable of high incidence of seedborne transmission of the necrotic strains of BCMV. Approximately 11% of the tested accessions and the germ plasm collection are of this seed type. It is possible that conditions of germ plasm increase may mitigate against the perpetuation of serogroup A strains. These conditions include a germ plasm collection in which only a small portion is susceptible to seed transmission, limited inoculum compared with serogroup B strains, cross-protection by other viruses (6), and competition between BCMV strains. Little is currently known of BCMV pathotypes present in the Pullman, WA, germ plasm collection (12,13) and their interaction.

Seed increase in the field is thought to have resulted in the contamination of the USDA *Pisum* and *Arachis* germ plasm collections by pea seedborne (7) and peanut stripe (4) viruses, respectively. The parameters of BCMV spread in the *P. vulgaris* collection during seed increase are not known, and it is not clear whether the collection is at risk of still greater BCMV contamination. Uncontrolled spread of BCMV may also result in the loss of susceptible germ plasm from genetically heterogeneous accessions because of decreased reproduction. Investigations are in progress to determine what effect BCMV infection has on genetic diversity and how BCMV is spread under seed increase conditions.

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