Influence of Propagation on Incidence of Seedborne Bean Common Mosaic Virus in the USDA *Phaseolus* Germ Plasm Collection

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ABSTRACT

Klein, R. E., Wyatt, S. D., and Kaiser, W. J. 1989. Influence of propagation on incidence of seedborne bean common mosaic virus in the USDA *Phaseolus* germ plasm collection. Plant Disease 73: 759-761.

The influence of propagation on the incidence of seedborne bean common mosaic virus (BCMV) in *Phaseolus vulgaris* accessions of the USDA germ plasm collection was investigated by comparing seedborne BCMV incidence in original seed lots with that in harvested seed. Propagation in the greenhouse during January through June resulted in a 66% reduction in average seedborne BCMV incidence in 26 accessions. In most accessions, seedborne BCMV incidence was reduced to a level that would allow BCMV eradication by elimination of infected plants without affecting genetic diversity. BCMV spread occurred during the July through December greenhouse increase and in the summer field increase, but no significant change in average seedborne BCMV incidence was detected in the 32 and 42 accessions examined, respectively. Virus spread appeared to be caused by noncolonizing aphids. In the absence of effective vector control, average seedborne BCMV incidence in the *P. vulgaris* germ plasm collection has apparently reached an equilibrium at about 7%.

The U.S. Department of Agriculture (USDA) Regional Plant Introduction Station at Pullman, WA, maintains the USDA *Phaseolus* germ plasm collection, which numbers about 10,000 accessions. As seed supplies are depleted through distribution to various investigators or as the germinability of the seed declines, it becomes necessary to grow individual accessions to maturity to replenish seed supplies. This process is referred to as seed increase.

SP 7957, College of Agriculture and Home Economics Research Center, Project 7495, Washington State University, Pullman 99164.

Accepted for publication 23 March 1989 (submitted for electronic processing).

P. vulgaris accessions are currently increased under three conditions: a greenhouse planting in January through June (winter increase); a greenhouse planting in July through December (summer increase); and a summer field planting at a remote location near Central Ferry, WA (field increase). Greenhouse increases are done in a single greenhouse containing up to 162 accessions; field increases vary in size but may include up to 300 accessions. Each accession in an increase is represented by an average of 35 plants.

Many accessions in the germ plasm collection are contaminated with seedborne bean common mosaic virus (BCMV) (6). Accessions of *P. vulgaris* appear to be the most heavily contaminated of the 23 *Phaseolus* species maintained; nearly 60% of the accessions tested contained BCMV. Germ plasm collections, by their very nature, operate under limitations not normally encountered in commercial agriculture. Because germ plasm collections are responsible for maintaining genetic diversity, accessions cannot be discarded because of virus contamination, nor can many individual virus-infected plants be eliminated without eroding genetic diversity (2).

The USDA *Phaseolus* Crop Advisory Committee has determined that up to 10% of an accession may be eliminated without significantly affecting diversity within an accession. Thus it is desirable to determine what practices can be employed to lower BCMV incidence to levels amenable to virus elimination without risking the loss of genetic diversity. Seedborne BCMV incidence is known to be affected by the host, the infecting BCMV strain, and plant age at time of infection (8). We investigated the influence of the three seed increase conditions on the incidence of BCMV in seed.

MATERIALS AND METHODS

Winter greenhouse increase. Samples of seed of 26 Plant Inventory (PI) *P. vulgaris* accessions increased in January 1986 were obtained. We studied two samples of seed from each accession, one representing the seed supply before the increase, the other representative of the seed increase itself. About 50 seeds from each sample were planted and raised in the greenhouse to the first trifoliate leaf stage.

All plants were sampled and tested

individually by indirect enzyme-linked immunosorbent assay (ELISA) (7). A small leaf piece was ground 1:50 (w/v) in 0.05 M carbonate buffer, pH 9.6, with 2% polyvinylpyrollidone-40T (PVP) (Sigma Chemical Co., St. Louis, MO) and 0.2% ovalbumin. Samples were incubated 1.5 hr at room temperature or overnight at 4 C in Immulon II ELISA plates (Dynatech Laboratories, Alexandria, VA). Ascites fluid containing a monoclonal antibody that reacts with all known strains of BCMV (12) and goat antimouse IgG:alkaline phosphatase conjugate (Sigma) were used at 1:75,000 and 1:2,000 (v/v) dilutions, respectively, in phosphate-buffered saline, pH 7.4, to which was added 2% PVP and 0.2% ovalbumin. After appropriate rinses, monoclonal antibody and conjugate solutions each were incubated in the plates for 1.5 hr at room temperature. The substrate used was *p*-nitrophenyl phosphate at 1 mg/ml, and plates were read at 410 nm on a Dynatech Minireader II. BCMV-infected and healthy bean leaf tissue sampled as above were used as positive and negative controls, respectively, within each ELISA plate.

BCMV incidence in each seed sample was calculated as the percentage of positive plants from that sample. These percentages were converted to arcsine values, and the incidence of seedborne BCMV in the seed sample representative of the preexisting seed supply (preincrease seed) was compared to that in the seed increase itself (postincrease seed) by a paired comparisons test (11). The Kolmogorov-Smirnoff two-sample test (11) was also used to compare the distribution of BCMV incidence before and after seed increase.

Summer greenhouse increase. All plants of 32 PI accessions increased in the 1986 summer increase were sampled and tested for BCMV as described above within 2 wk of plant emergence. When it became apparent from visual symptoms that BCMV was spreading within the greenhouse, plants were sampled and tested a second time, about 6 wk after the initial test. Seed samples of the seed increase itself were subsequently obtained for each accession, and these seed were planted and tested as described above. BCMV incidence in the first plant test for each accession was used as a measure of preincrease seedborne BCMV incidence, and this was compared to seedborne BCMV incidence in the postincrease seed sample as described above.

Summer field increase. Paired seed samples were obtained for each of 42 PI accessions increased at Central Ferry during 1985. As above, one sample was representative of the seed supply before increase, while its pair was representative of the seed harvested from the increase itself. Sampling, testing, and comparisons were performed as described above. In addition, linear regression analysis was used to determine whether preincrease and postincrease seedborne BCMV incidences were related. To determine whether BCMV was spreading within the planting, sequential sets of *P. vulgaris* 'Bountiful' bait plants were placed within the planting. After 1 wk of exposure, bait plants were returned to Pullman, sprayed with insecticide, and tested for BCMV by ELISA after incubation in the greenhouse for about 4 wk.

Table 1. Influence of propagation on the incidence of seedborne bean common mosaic virus (BCMV) in Plant Inventory accessions of *Phaseolus vulgaris*

Sample source	BCMV incidence[a]	Average incidence[b] (%)	Range of incidence (%)	Number of contaminated accessions[c]
Greenhouse propagation, January–June 1986 (26 accessions)				
Preincrease seed	96/986	10.7	0–46.2	16 (10)
Postincrease seed	28/1,187	3.6	0–13.5	11 (2)
Greenhouse propagation, July–December 1986 (32 accessions)				
First plant test	71/1,053	7.4	0–55.6	16 (8)
Second plant test	286/998	30.4	0–90.6	28 (24)
Postincrease seed	92/1,561	6.1	0–32.4	19 (6)
Field propagation at Central Ferry, 1985 (42 accessions)				
Preincrease seed	131/1,765	7.7	0–42.6	25 (12)
Postincrease seed	133/2,300	6.4	0–45.1	30 (10)

[a] Number of BCMV infections detected by enzyme-linked immunosorbent assay/total number of plants tested.

[b] Incidence averaged across accessions. In the winter greenhouse test, values differ significantly at $P < 0.01$. In the summer greenhouse test, the values for the first and second plant tests differ significantly at $P < 0.01$; the values for the first plant test and the postincrease seed test do not differ at $P \leq 0.05$. In the summer field test, values do not differ significantly at $P \leq 0.05$.

[c] Values in parentheses indicate the number of accessions with BCMV incidence of 10% or greater.

RESULTS

BCMV incidence among the 26 *P. vulgaris* accessions increased in the 1986 winter increase test was 10.7%. Even though diseased plants were not rogued, spread of BCMV was not observed. As a result of winter greenhouse increase, seedborne BCMV incidence declined by over 66%, and the distribution of BCMV incidences, as reflected in the range of incidence, was different (Table 1). The number of accessions in which seedborne BCMV could be detected declined, as did the number of accessions with greater than 10% seedborne BCMV incidence.

In contrast, BCMV incidence increased more than fourfold during the approximately 6 wk between plant sampling dates in the 1986 summer greenhouse increase test (Table 1). However, BCMV incidence in seed harvested from the increase itself did not differ significantly from average seedborne BCMV incidence before the increase (measured by the first plant test), nor did the range of BCMV incidences across accessions change. Although the number of accessions in which seedborne BCMV could be detected increased, the number with BCMV incidence above 10% declined; this is most likely a reflection of the small sample size.

Field increase had no significant effect on either average seedborne BCMV incidence or the range of BCMV incidences (Table 1). The number of accessions in which seedborne BCMV could be detected increased but, again, this probably is because of sample size. The linear regression analysis showed no correlation between BCMV incidence within an accession before increase and the incidence observed in seed from the increase itself. Indeed, in one accession seedborne BCMV incidence rose from 0 to 45%, while in another it dropped from 43 to 12%.

ELISA analyses of bait plants exposed for 1-wk periods within the field seed increase indicated that BCMV spread occurred by the fourth week after planting. The percentage of bait plants infected with BCMV gradually rose from week 4 (2%) to a peak at week 10 (52%); it declined somewhat in subsequent weeks but varied between 10 and 20% for the remaining 6 wk of the seed increase. Bait plants placed on the periphery of the increase plots became infected at a much lower rate, suggesting that the inoculum source was within the *P. vulgaris* collection, not external to it.

DISCUSSION

The winter greenhouse seed increase was the only increase associated with a significant decline in seedborne BCMV incidence, presumably because of the absence of aphid vectors. The decline in BCMV incidence was sufficient in most accessions that BCMV could be eliminated from these accessions by

eliminating BCMV-infected plants without compromising the genetic integrity of the accession.

The decline in BCMV incidence that accompanied the winter greenhouse increase is likely the result of decreased seed yield from BCMV-infected plants and less than 100% seed transmission in seed from infected plants. Data presented by Morales and Castaño (8) indicate that, depending on the bean cultivar and the BCMV strain, the interaction of yield loss and seed transmission rate could result in a decrease in BCMV incidence of 40–100% in seed samples of 30–50 seeds. The decreased seed yield from infected plants could be a cause for concern if it resulted in the loss of BCMV-susceptible germ plasm from genetically heterogeneous accessions. However, when seed samples before and after seed increase have been compared for seed heterogeneity, no loss of phenotypic genetic diversity has been detected.

Seedborne BCMV infections serve as the primary inoculum source for subsequent virus spread through nonpersistent, styletborne aphid transmission. In general, however, aphids are reluctant to colonize beans (9,13); aphid infestations are rarely observed, and in an unrelated experiment, no live aphids were observed on over 7,000 field-grown leaves, although the incidence of BCMV-infected plants rose from less than 20% to over 80% (R. E. Klein, *unpublished*). Zaumeyer and Kearns (13) noted that most aphid species tested were capable of transmitting BCMV, and noncolonizing aphids have been implicated in the spread of other potyviruses in several crops (1,3,5), including beans (10,13). Vector specificity among the potyviruses appears to be the exception.

Alate *Rhopalosiphum padi* (L.) were trapped within the greenhouse during the summer increase, and alate *Acyrthosiphon pisum* (Harris) have been frequently observed trapped by leaf hairs on beans at Central Ferry (R. E. Klein, *personal observation*). Based on aphid trap counts (K. Pike, *personal communication*), it appears that *A. pisum, R. padi,* and *Sitobion avenae* (Fab.) are potentially involved in BCMV spread in the germ plasm collection.

Because BCMV appears to be spread by migrant or transient aphids, pesticidal vector control appears to be of little value in reducing BCMV spread. Therefore, vector avoidance (winter greenhouse increase) offers more effective control of virus spread within the germ plasm collection. Several germ plasm collections are contaminated with seedborne potyviruses (4), and if seedborne viruses are to be eliminated from germ plasm collections and the collections maintained virus-free, field seed increases are likely no longer appropriate.

Seedborne BCMV incidence was not correlated with accession age (6), and a seed increase associated with an increase in average seedborne BCMV incidence could not be identified. Because no trend toward increasing incidence of seedborne BCMV was detected within the germ plasm collection, it appears that the average incidence of seedborne BCMV within the accessions has reached a maximum value of about 7%. This average incidence is unlikely to increase unless BCMV strains more amenable to seed transmission appear or infection occurs at earlier dates. However, wide fluctuations in incidence may be observed within individual accessions as a result of seed increase. Unless one is working with originally collected seed, one cannot assume that the geographic origin of a BCMV isolate is synonymous with the origin of the accession from which it was isolated. This is presumably also true of other pathogens in germ plasm collections of other plant species.

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