

# Effect of Diseased Plant Elimination on Genetic Diversity and Bean Common Mosaic Virus Incidence in *Phaseolus vulgaris* Germ Plasm Collections

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## ABSTRACT

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Genetic diversity and incidence of seedborne bean common mosaic virus (BCMV) in Centro Internacional de Agricultura Tropical (CIAT) *Phaseolus vulgaris* germ plasm seed samples was compared to seed samples of the USDA accessions from which the CIAT samples were originally derived. Following approximately three seed-increase cycles at CIAT during which individual plants obviously infected with BCMV were eliminated, overall and within-sample diversity in 120 seed samples had declined by 45 and 66%, respectively. BCMV incidence also declined by 30%, from an average of 6% in 26 USDA samples to 4.2% in the CIAT samples, but tolerant and visually symptomless infections became more common in the CIAT samples. The distribution of BCMV incidences among samples was also altered.

Both the United States Department of Agriculture (USDA) Regional Plant Introduction Station in Pullman, Washington, and the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia, maintain large *Phaseolus* germ plasm collections. Although the CIAT collection is now much larger, many of the USDA Plant Introduction (PI) accessions served as the initial nucleus of the CIAT collection in the early 1970s.

While the collections are affected by a number of seedborne pathogens, bean common mosaic virus (BCMV) is a particular problem. The USDA collection is contaminated at an average incidence of about 7% (6), but incidence fluctuates widely between and within accessions depending on conditions of seed increase

(7). Similar data are unavailable for the CIAT collection.

CIAT and the USDA have differed drastically in their response to BCMV and its control. The USDA did little to eliminate BCMV and attempted to maintain the collection without discarding diseased plants. CIAT discards obviously diseased plants during seed increase (David Wood, *personal communication*).

Accessions are frequently phenotypically heterogeneous. An accession may contain seed types differing in color, size, and shape, and it is not uncommon to detect three or more seed types in a single USDA accession. A comparison of diversity (seed phenotype) and BCMV incidence in selected seed samples between the two collections was appropriate before the initiation of a BCMV eradication program for the USDA collection. The main function of the USDA *Phaseolus* collection is to maintain genetic diversity within the genus. The results of these comparisons are reported herein.

## MATERIALS AND METHODS

Seed samples of 120 *Phaseolus vulgaris* L. accessions were obtained

from CIAT. Each seed sample contained 30 or 31 seeds. These CIAT lines, originally PI accessions, had been increased about three times at Palmira, Colombia, with pesticide protection and with obviously diseased plants discarded (David Wood, *personal communication*). Samples of 30–130 seeds of the corresponding PI accessions were then obtained from the Pullman collection. PI accessions had been increased, on average, less than one time.

Seed types within each sample were separated by seed size, color, shape, and color pattern. The number of different seed types within each sample was summed across all accessions as a measure of overall diversity. In addition, the proportion of each seed type within the sample was determined and the diversity (8) within each sample was calculated as:  $H = - \sum_{i=1}^n P_i \ln P_i$  where  $P$  is the proportion of the  $i$ th seed type within the sample and  $H$  is the measure of diversity. The measures of within-sample diversity were then averaged across the samples.

Seed of 26 paired samples from the CIAT and the PI accessions were planted in flats and grown in an aphid-free greenhouse. Solar illumination supplemented by fluorescent light provided a 16-hr photoperiod and the temperature was maintained between 15 C and 30 C. Each plant was sampled at approximately the first trifoliate leaf stage, 14–21 days after planting, and tested with indirect enzyme-linked immunosorbent assay (ELISA) with a monoclonal antibody capable of detecting all known strains of BCMV (11). ELISA conditions were described previously (6). Between 21 and 106 plants were sampled for each USDA PI accession and 21–31 for each CIAT accession. Because of observed fluctuations in BCMV incidence within USDA PI acces-

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sions as a result of seed increase (7) and an obvious non-normal distribution, a two-sample Kolmogorov-Smirnov test (10) was used to compare BCMV incidences.

## RESULTS AND DISCUSSION

Seed samples from PI accessions were considerably more diverse than those of the corresponding CIAT accessions (Table 1), both in overall number of seed types and in calculated within-sample diversity. The loss of within-sample diversity for CIAT accessions was 66% while overall diversity had declined 45% (Table 1). Although PI accessions are more diverse, it is not known whether these accessions are as diverse as when first inventoried into the germ plasm collection because of incomplete information or a lack of information concerning the variability present in the seed sample establishing the accession. There is also no information on the diversity within an accession while that accession has been maintained. Long-term effects of seed increase of the USDA collection on diversity should be measured under Pullman conditions.

The incidence of seedborne BCMV in CIAT seed samples was significantly lower than that observed in the USDA PI accessions (Table 2). The number of contaminated accessions was also fewer in the CIAT samples, but the highest observed BCMV incidence, 33%, was present in a CIAT sample. Seedborne BCMV infections were rarely visually detectable in CIAT samples, although the few examined caused easily detectable mosaic symptoms on *P. vulgaris* 'Bountiful.' All infections in PI samples were visually detectable, although some did not produce symptoms until 30 or more days after seeding. Drijfhout (5) noted that some BCMV strains possessing particular pathogenicity genes failed to cause clear symptoms when inoculated on hosts possessing particular resistance genes, although BCMV was readily re-

covered from the inoculated plants. It appears that the selective pressure exerted by the frequent discarding of symptomatic plants at CIAT has largely eliminated BCMV strains incapable of non-symptomatic infections in hosts susceptible to seedborne BCMV infections.

Selection in the CIAT collection appears to be primarily for tolerance in the seedborne infection and perhaps only secondarily for BCMV tolerance to a wide variety of strains or weakly virulent BCMV strains. Seedborne BCMV, present as latent infections, presumably results in virulent infections when spread during seed increase. These latent infections thus act as an unseen and uncontrolled inoculum source for further BCMV spread.

Selection is ubiquitous, continuous, and occurs simultaneously for many factors. Factors other than the elimination of BCMV-infected individuals may have contributed to the diversity loss in the CIAT samples. Viruses other than BCMV occur in tropical areas, but these viruses are virtually unknown at higher latitudes. These include bean golden mosaic, southern bean mosaic, and bean mild mottle viruses. A seedborne fungal pathogen, *Macrophomina phaseolina* (Tassi) Goidanich (= *Macrophomina phaseoli* (Maubl.) S. Ashby) was detected in CIAT samples but has not been detected in the USDA germ plasm collection. In addition, it is not unlikely that the USDA collection is somewhat adapted to the higher latitudes by virtue of being maintained at Pullman and that increase by CIAT could select for other adaptations. The elimination of BCMV-infected plants in our opinion, however, appears to be the primary cause of the diversity loss in CIAT samples, because the other problems are relatively minor and selection pressure is relatively weak (David Wood, *personal communication*). The elimination of diseased indi-

viduals has also resulted in a loss of diversity among samples from the USDA *Pisum* collection (1), although the loss detected was not as severe as noted in the CIAT *Phaseolus* collection.

Regardless of the cause, more consideration must be given to diversity loss considering how uncommon some genes are within the germ plasm collections. Virtually all resistance to barley yellow dwarf virus in barley is derived from a single geographic region (9) and nearly all resistance to *Fusarium* root rot in beans is derived from a single accession (2). Different seed phenotypes with bean germ plasm accessions are known to differ in their resistance to a number of diseases (2-4) and it is not difficult to conceive how selection for one factor can eliminate useful germ plasm.

Unfortunately, there currently is no practical alternative to the elimination of BCMV-infected individuals if BCMV is to be eradicated from the USDA *Phaseolus* germ plasm collection. Reliance upon visual symptoms for disease detection tends only to eliminate obvious seedborne inoculum sources, and the use of direct virus detection methods (such as ELISA) instead of visual inspections will reduce selection for tolerance and masking of BCMV contamination. It is clear that the elimination of large numbers of plants in successive generations from individual accessions will adversely affect genetic diversity. However, limiting diseased plant elimination to 10% or less for a single generation is unlikely to result in significant germ plasm loss because of the population parameters of the accessions. This appears to be the most practical means of BCMV elimination and should serve to eliminate BCMV from up to 75% of the USDA collection. The loss of diversity noted in the CIAT collection will occur within the USDA collection as BCMV is eliminated unless sound techniques are employed.

**Table 1.** Comparison of number of seed types and phenotypic diversity in seed samples of 120 *Phaseolus vulgaris* accessions from the Centro Internacional de Agricultura Tropical (CIAT) and the United States Department of Agriculture (USDA) *Phaseolus* germ plasm collections

Seed source	Seed types <sup>a</sup>	Diversity <sup>b</sup>
USDA	281	0.499
CIAT	156	0.172
Percent reduction	44.5%	65.5%

<sup>a</sup> Seed types within each sample were separated by seed size, color, shape, and color pattern. The number of different seed types within each sample was summed across samples.

<sup>b</sup> Within samples diversity calculated as  $H = -\sum_{i=1}^n P_i \ln P_i$  where  $P_i$  equals the proportion of each seed type averaged across samples.

**Table 2.** Incidence of bean common mosaic virus (BCMV) in 26 *Phaseolus vulgaris* seed samples from the Centro Internacional de Agricultura Tropical (CIAT) and the United States Department of Agriculture (USDA) germ plasm collection from which the CIAT samples were originally derived

BCMV incidence (%)	Number of samples	
	CIAT	USDA
0	20	7
1-5	1	7
6-10	0	5
11-15	1	6
16-20	1	1
>21	3	0
Average <sup>a</sup>	4.2	6.0
	(28/702) <sup>b</sup>	(97/1,591) <sup>b</sup>

<sup>a</sup> Average incidence across accessions. Values were significantly different at  $P \leq 0.05$ .

<sup>b</sup> Number of ELISA-positive plants/total number of plants tested.

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