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Minimizing the Threat of Seedborne Pathogens in Crop Germ Plasm: Elimination of Pea Seedborne Mosaic Virus from the USDA-ARS Germ Plasm Collection of *Pisum sativum*

The collection, stewardship, and utilization of crop germ plasm is one of the most strategic crop-related activities of the 20th century. Even in the short period of our careers, drastic losses of food-legume land races (19) and wild-plant habitats (3,4) have occurred. By reasons of professional responsibility and natural motivation, we have committed our efforts to enhancing the value and use of food legume germ plasm, in this case pea (*Pisum sativum* L.) germ plasm.

Because of limited funding and other considerations, it has been necessary for Plant Introduction personnel to distribute crop germ plasm seed lots with a disclaimer, passing the risk of possible seedborne pathogens to those requesting germ plasm accessions. This disclaimer, unfortunately, did not protect against repeated introduction of pea seedborne mosaic potyvirus (PSbMV), along with gene sources, into private and public breeding nurseries (10,12). Control of PSbMV in commercial pea production has been appropriately focused on incorporation of well-defined genetic resistance. Such measures are quite irrelevant to germ plasm collections, however. With concerns for seedborne viruses in several U.S. (and world) crop germ plasm collections (8,13) and with the recommendations of the *Pisum* Crop Advisory Committee, we therefore organized our laboratories into a working group to remove this pathogen from the U.S. *P. sativum* germ plasm collection, based on pilot studies begun in 1976 (8,12). Our efforts culminated, in 1991, in the elimination of PSbMV from 2,700 Plant Introduction accessions of *P. sativum*.

An Appropriate Model

It became apparent that the U.S. germ plasm collection of *P. sativum* would be an appropriate experimental model, for several reasons. This collection is regarded by both private and public breeders as a strategic gene resource for numerous research-development objectives. The collection unfortunately was implicated in several PSbMV outbreaks in North America (12), ensuing from breeders requesting *Pisum* germ plasm, then planting PSbMV-infected accessions in breeding nurseries. Once PSbMV was established in nurseries, the viral inoculum was transmitted by aphids to healthy pea breeding materials. Complications from the use of *Pisum* Plant Introduction accessions forced pea breeders to reduce reliance on this collection for genotype development, causing the *Pisum* collection to become an underutilized resource. PSbMV in the collection, therefore, not only was a source of viral inoculum perpetuated and disseminated by seed and aphid transmission but also severely threatened the continuing maintenance of genetic variation for the crop species.

Control of the germ plasm phase of pea seedborne mosaic was also viewed as an opportunity to reduce point sources and subsequent spread of PSbMV inoculum in breeding nurseries (10,12).

Seedborne viruses are particularly widespread in legumes (two of three virus-host combinations resulting in documented viral transmission through seeds have involved legume hosts), further favoring a food legume germ plasm collection as an experimental model. Finally, we anticipated that our efforts with *P. sativum* might encourage similar actions with other crop germ plasm collections.

PSbMV is a classic example of an introduced pathogen (10; see reference 6 for seed, seedling, and plant symptoms in color). Although this virus was traced back to infected U.S. commercial seed lots produced in 1965 (Hampton, *unpublished*), PSbMV was not reported to occur in North America until 1968 (16,20). It had been previously reported in Europe in 1966 (17) and Japan in 1967 (14). Subsequent investigations of PSbMV in U.S. *P. sativum* germ plasm accessions (10) indicated that major introductions of PSbMV-infected germ plasm had occurred after 1961, particularly from *P. sativum* land races originating from northern India (9). PSbMV is transmitted by several aphid vector species (11,15) common to Europe and North America, including the pea aphid (*Acyrthosiphon pisum* (Harris)), the green peach aphid (*Myzus persicae* (Sulzer)), and the potato aphid (*Macrosiphum euphorbiae* (Thomas)). Thus the virus, once established in the field through infected seed lots, may be quickly spread to healthy plants. Unlike many threatening exotic viruses, however, PSbMV in North America is virtually limited to pea seed lots (12) and to lentil (*Lens culinaris* Medik.) germ plasm (5) and is not known to occur naturally in other crop or weed species.

The frequency with which PSbMV is seed-transmitted varies significantly

The authors either are or have been president of the National Pea Improvement Association and are members of the *Pisum* Genetics Association and the *Pisum* and Special Food Legumes Crop Advisory committees. Dr. Kraft exercised particular leadership in obtaining USDA-ARS funding for the special project reported herein.

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among cultivars and, for a variety of reasons, among seed lots of the same cultivar. Seed lots, whether of genotypes or of genetic mixtures (e.g., many germ plasm accessions), are rarely infected at frequencies exceeding 30%. This phenomenon facilitates selection of virus-free seed sources, while preserving extant genetic diversity.

The USDA-ARS *Pisum* germ plasm collection has been an inoculum source from which North American breeders may have inadvertently introduced PSbMV into their breeding stocks, notwithstanding exposure also to PSbMV-infected seed lots from other sources. Further, since many breeders have depended on the collection as a *Pisum*-gene resource, most breeding programs have been repeatedly exposed to PSbMV infection. Our remedial efforts have hopefully interrupted a principal component of the PSbMV disease cycle in the United States and reduced breeding program exposure to this virus. Continued precautions against PSbMV-infected seed lots, i.e., admission into breeding nurseries, are mandatory.

Knowing the PSbMV threat and disadvantages of wild-plant characters associated with northern India land races, most breeders have utilized *Pisum* gene *sbm-1* (conferring resistance to PSbMV pathotype P1 [1]) originating in only a few germ plasm accessions introduced before 1970, particularly PI 193586 and PI 195835 (7). The resource of gene *sbm-1* and other traits among the diverse northern India land races (9) therefore has been neglected. In retrospect, support by plant pathologists could have enabled breeders to utilize this abundant resource, thus diversifying the germ plasm base associated with *sbm-1* in European and North American pea cultivars resistant to PSbMV.

The Project

By 1987, the *Pisum* Crop Advisory Committee considered the presence of PSbMV a major impediment to the use of the *Pisum* collection for germ plasm enhancement and unanimously supported the objective of establishing virus-free nuclear seed sources of 2,700 *P. sativum* germ plasm accessions. "Nuclear seed source" is herein defined as an elite, greenhouse-produced, pathogen-free seed lot derived from a minimum of 24 mother plants representing, so far as possible, the seed or plant phenotype(s) of the antecedent Plant Introduction accession. Project initiation to fulfill the objective consisted of three preliminary measures: 1) securing USDA-ARS funding to support the effort at Corvallis, Oregon, and Prosser and Pullman, Washington (author locations); 2) subdividing accessions among the cooperators according to pea use type (horticultural or agronomic) and growth habit (determinant or indeterminant); and 3) allocating laboratory and greenhouse space at each location for the project and assigning standardized tasks to location personnel. The work was initiated in September 1988 and completed 32 months later, in May 1991.

We sought optimal sources of *P. sativum* Plant Introduction accessions that were minimally contaminated by PSbMV (e.g., obtained from Geneva, New York, prior to 1970). A lower incidence of seedborne virus enabled us to minimize loss of within-line genotype diversity, i.e., minimize rejection of seed and plant phenotypes during selection of virus-free mother plants.

The available seed lots of germ plasm accessions consisted of 50–100 seeds each. Wherever accessions consisted of more than one seed phenotype, seed types were partitioned and planted separately in Styrofoam block planters filled with vermiculite. Emerging plants were categorized into plant phenotypes, and those with PSbM-like symptoms were discarded. From the remaining seedlings, 24 candidate mother plants were selected to represent both seed and plant phenotype diversity. They were transplanted to 8-in. plastic pots containing pH-neutralized soil amended with complete fertilizer. During early plant growth, climbing-vine genotypes were trained onto strings suspended from overhead wires or on bamboo stakes. Candidate PSbMV-free mother plants were assayed one or more times, typically between the fourth and the 10th node stage, by DAS-ELISA. ELISA procedures conducted at Corvallis or Prosser were standardized to detect PSbMV at 1/1,000th the virus concentrations typically encountered in commercial pea cultivar seed lots. Selected mother plants were sometimes assayed once more as they approached maturity. Plants containing ELISA-detectable PSbMV were removed. To estimate the infection frequency of accessions being processed by our laboratories, we sometimes assayed randomly selected seedlings in Styrofoam planters or seedlings suspected of being infected with PSbMV.

Seed yields from 20–24 plants of climbing-vine genotypes initially ranged up to 2,000 seeds. However, to shorten the processing cycle from seed planting to seed harvest, we adopted the practice of withdrawing water from the plant sets after some 500 seeds had been produced. A concerted effort was made to maintain aphid-free greenhouses by use of aphid-proof screens, systematic fumigations, sticky yellow monitoring strips, etc. Seeds were harvested only from mother plants determined by ELISA to be free of PSbMV.

Special precautions were taken against number errors in maintaining greenhouse records, harvesting seeds, and labeling seed packets with six-digit accession numbers. Harvested seeds were packed and transported to assigned individuals in the germ plasm system, either at Geneva (Region NE-9) or Pullman (Region W-6). In June 1992, the *Pisum* germ plasm collection was officially transferred from NE-9 to W-6.

Assessment

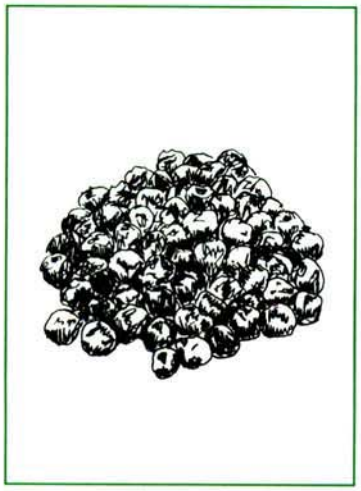
In a 1980 100-accession pilot study (8) and in the current project, germ plasm personnel at Geneva were provided standardized ELISA protocols and anti-PSbMV globulin or antiserum to objectively assess the presence or absence of PSbMV in candidate nuclear seed sources. In the 1980 study, no PSbMV was detected by ELISA in any of the 100 accessions (5). In the cooperative effort described here, PSbMV was thought to have been detected (Geneva) in the first plant of the first nuclear seed lot tested. The ELISA results, however, could not be retested or verified, and procedural error could not be ruled out (J. R. McFerson, *personal communication*). All other seeds or plants tested were unequivocally free of ELISA-detectable PSbMV.

Fifty seeds from each of 200 nuclear seed sources developed by the Corvallis laboratory were planted in uniform plots for observations at the Malheur Experiment Station, Ontario, Oregon, in March 1989. The threefold purpose was to assess: 1) freedom of seed lots from PSbMV, 2) the feasibility of maintaining PSbMV-free seed lots during field seed increases, and 3) the degree to which plant phenotypic diversity had been preserved during the eradication of PSbMV. Every accession was evaluated at or near bloom stage by the first author. The diversity of these nuclear sources in field plots approximated the plant phenotype diversity of their greenhouse-grown antecedent PI accessions.

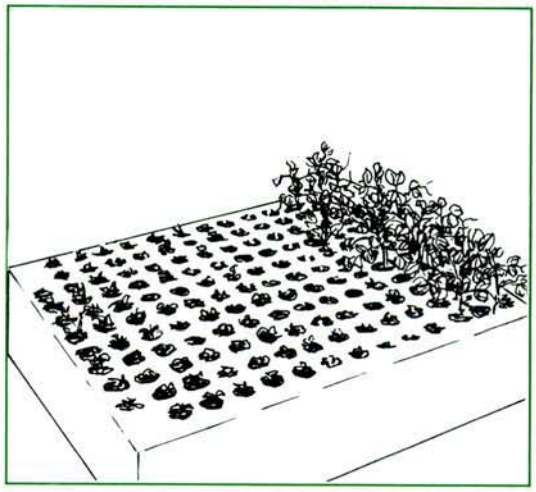
A PSbMV-free radius of 1 mile was established around the field observation plots to minimize risk of seed lot reinfection. Residents within a 1-mile radius of the plots were contacted during the previous winter and offered PSbMV-free pea seed lots. They agreed to use them for 1989 garden planting in lieu of any other pea plantings.

All plants within the plots were observed for viruslike symptoms, and selected plants were tested for PSbMV by DAS-ELISA. The population of native aphids, particularly the pea aphid, was monitored in both the nearest alfalfa fields and the pea seed increase plots from April through August. Despite lower than normal aphid populations in 1989, due to −32 C temperatures the previous winter, low frequencies of virus-infected plants occurred in the plots. Infected plants were determined by DAS-ELISA to contain either pea enation mosaic virus or bean (pea) leaf roll luteovirus. PSbMV was not detected in any plant tested or in any of the resulting 200 field-harvested seed lots. This zero

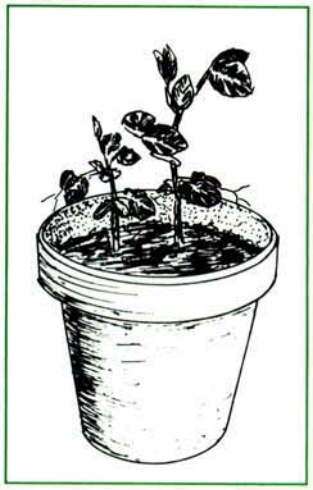
Steps in elimination of pea seedborne mosaic virus (PSbMV) from *Pisum sativum* germ plasm accessions



Step 1
Fifty to 100 seeds of each available germ plasm accession were collected.

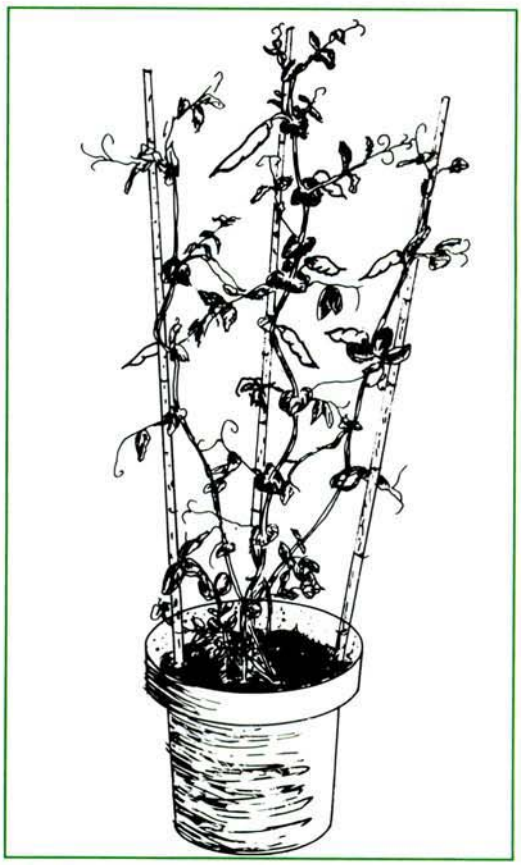


Step 2
When accessions consisted of more than one seed phenotype, seed types were partitioned and planted, one seed per cup, in Styrofoam block planters filled with vermiculite. Emerging plants were categorized into phenotypes, and those with PSbM-like symptoms were discarded.



Step 3
Twenty-four candidate mother plants selected to represent both seed and plant phenotype diversity were transplanted to 8-in. plastic pots.

Step 4
During early growth, vine-type plants were trained on bamboo stakes or on strings suspended from overhead wires. All plants were assayed one or more times by DAS-ELISA, usually between the fourth and the 10th node stage, and some were assayed again as they approached maturity.



Step 5
By maturity, the virus-free mother plants had produced seedpods, and the seeds from all such plants were bulked to become the germ plasm-derived nuclear seed source. A few mother plants were found to be infected after transplanting and were discarded.

rate of PSbMV reintroduction indicates that moderate precautions against "proximal PSbMV inoculum sources" provided effective protection against PSbMV field spread and seed lot reinfection.

Discussion and Conclusions

Our purposes in undertaking this project were to prevent spread of PSbMV from infected germ plasm accessions to healthy plants of private and public breeding nurseries and to facilitate increased use of valuable *P. sativum* germ plasm that had become lost to agriculture through disuse, i.e., avoided because of PSbMV contamination. In accomplishing these goals, we believe we have also controlled an important pathogen at the most elementary and economical level (by eliminating it from small germ plasm seed lots) and that we have preserved genetic diversity and guarded against further damage to pea germ plasm by PSbMV.

When selecting PSbMV-free mother plants, we endeavored to preserve the genetic diversity of the 1,200 most heterogeneous pea germ plasm accessions of the total 2,700. It was thus our commitment to produce from the combined mother plants the same proportions of seed and plant phenotypes that were represented in the seed lots initially received.

Two publications (2,18) have emphasized the risk of losing genetic diversity during production of nuclear seed sources. These authors, however, failed to consider the loss of diversity during routine seed increases (without intentional selection) or the loss of resources through disuse because of viral contamination. The net loss attributable to our PSbMV eradication measures is yet to be determined. These authors also did not address the threat of genetic loss from infection or damage caused by seedborne PSbMV in germ plasm accessions. Nevertheless, occasionally divergent motivations of pathologists and germ plasm stewards serve the purpose of prompting assiduous cooperation with mutual accountability among scientists in establishing pathogen-free, user-accessible germ plasm.

The methods followed in selecting PSbMV-free mother plants either eliminated or markedly reduced the presence

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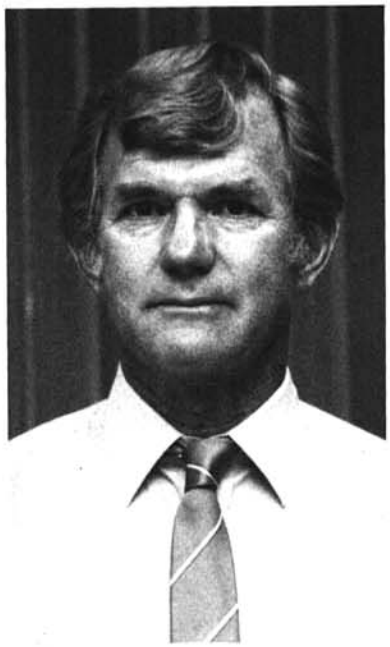

John M. Kraft

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of other seedborne pathogens, fungal and bacterial. The approach included: 1) selection of seed or mother-plant candidates that were free of observable disease symptoms; 2) critical observations of each selected mother plant from seed or seedling to plant maturity; 3) production of seed under carefully controlled greenhouse conditions, including precise irrigation and control of foliar pathogens; and 4) scrutiny of harvested seeds for any traces of disease symptomatology. We believe that the established germ plasm accessions are free of all seedborne pathogens to a greater extent than these genotypes or perhaps other crop germ plasm collections have ever been. With skillful management of seed increases from these nuclear seed sources, abundant high-quality *P. sativum* germ plasm should be available for future needs.

We hope the success of our efforts will encourage similar efforts with germ plasm collections of other economic crop species.

Acknowledgments

We hereby express appreciation for financial support by national and USDA-ARS area administrators, the cooperation of ARS germ plasm system personnel, the helpful support of fellow scientists (state, private, and federal), and especially the research-support personnel of our respective laboratories. Special appreciation is expressed to T. Reiling and associates for deriving virus-free seed sources of 100 PI accessions in facilities of the Green Giant Company (Pillsbury), LeSueur, Minnesota. We dedicate this article to the U.S. *Pisum* Crop Advisory Committee, who strongly supported this pioneering effort, and to the Collections and Germplasm Committee of the American Phytopathological Society, who were excited about our project and the results and who urged the sharing of this information by means of a PLANT DISEASE feature article.

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