Seedborne Cucumber Mosaic Virus in Selected *Phaseolus vulgaris* Germ Plasm and Breeding Lines in Idaho, Washington, and Oregon

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ABSTRACT

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In 1978 and 1979, 259 bean plants (*Phaseolus vulgaris*) from principal bean breeding lines in Idaho, Washington, and Oregon were tested for seedborne cucumber mosaic virus. All plants were free of seedborne cucumber mosaic virus as determined by enzyme-linked immunosorbent assay, bioassay, or serology. By the same methods, seedborne cucumber mosaic virus was detected in one of 32 plant introduction bean accessions of international origin. Future monitoring for seedborne cucumber mosaic virus may be desirable in view of current international exchanges of bean germ plasm, which may disseminate this virus.

In 1979, 69,000 ha in the Pacific Northwest was devoted to production of edible beans (*Phaseolus vulgaris*), representing a wholesale value (including production for seed) of about $100 million. Approximately 80% of the snap bean seed produced in the United States is grown in this area, which adds significantly to the regional value of the crop.

Historically, the principal seedborne virus causing economic damage to the bean industry has been bean common mosaic virus (1). This virus is distributed worldwide wherever beans are grown, continues to cause significant crop losses, and receives emphasis in development of resistant cultivars. It is hoped that preventative measures can be developed to preclude new seed-transmitted cucumber mosaic virus (CMV) isolates from posing a parallel disease problem for beans.

CMV infects plants in more than 40 dicotyledonous and monocotyledonous families, is readily transmitted mechanically, and is vectored by more than 60 aphid species in a nonpersistent manner (5,6). CMV strains that cause economic loss and are particularly infectious to legumes have existed in Japan for some time (9). In the past decade, two previously unknown properties of this virus, of particular interest to us, have been described.

First, in 1974 a CMV isolate from eastern Spain was reported to be seed-transmitted in beans at a rate of 7% (2). Despite the extensive host range of this virus, before 1974 CMV had been reported to be seed-transmitted in only 12 hosts, three of which were legumes (17). Since 1974, three isolates of CMV from New York (18), Puerto Rico (15), and France (14) have been reported to be seed-transmitted in certain bean cultivars at frequencies of 0.3, 1.5, and 30%, respectively.

Second, a low molecular weight, CMV-associated satellite RNA (referred to as CARNA 5 by Kaper and Waterworth [11]) has been implicated in altering virulence of isolates naturally infectious to tomato. Diaz-Ruiz and Kaper (4) concluded that this CMV satellite was a helper-dependent RNA capable of inducing severe necrosis in tomato in the presence of CMV. Satellite RNA has been found in several CMV strains and isolates (7,10,12,16,20), and some of these molecules appear to be physically uniform by present techniques (10,16,19). In addition, satellites from different CMV strains can be freely exchanged between their helpers (10,11). Hence, CMV-satellite RNA contributes unique and unknown capabilities to an otherwise well-characterized plant virus.

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Seed transmission of CMV in *P. vulgaris* could result in local and international dissemination of this insidious satellite RNA. Quantifying CMV in the Northwest bean seed industry has allowed us simultaneously to monitor CMV-satellite RNA.

The work reported here was prompted by the strategic role and economic value of this industry, recent reports of CMV transmission in bean seed, extensive international movement of bean germ plasm, and the potential of CMV-satellite RNA to increase CMV virulence.

MATERIALS AND METHODS

In 1978, 28 leaf samples representing diverse germ plasm types were taken from among thousands of bean plants in eight breeding plots of seven seed companies in important bean seed producing areas in eastern Washington and southern Idaho. Sixty-two samples were also taken from USDA (Prosser, WA) and Oregon State University (Corvallis) breeding programs. The plants had mild mosaic, stunting, or yellowing symptoms characteristic of CMV infection but unlike those induced by bean common or bean yellow mosaic viruses, curly top virus, alfalfa mosaic virus, or tobacco streak virus, which are also common to these areas.

Samples from Idaho were ground in buffer and rub-inoculated onto the bean cultivars Bountiful, Black Turtle, and Limelight. Tissue triturates from these hosts were applied 2 wk later to cucumber plants (*Cucumis sativus* 'Improved Long Green') to increase chances of detecting CMV isolates. Samples taken in Oregon were assayed directly onto cucumbers.

Samples from Washington were similarly processed and used to rub-inoculate bean cv. Bountiful and Monroe, cucumber cv. Boston Pickling, pea (*Pisum sativum*) cv. Alaska and Dark Skin Perfection, and cowpea (*Vigna unguiculata*) cv. California No. 5 (Ramshorn). Selected plants were also tested for CMV by leaf-dip or serologically specific electron microscopy and by gel double-diffusion serology. Antiserum used in these studies was produced against isolate CMV-B at Corvallis.

In 1979, 73 plant samples were taken from seed company breeding plots in eastern Washington, southern Idaho, and western Oregon, and 96 samples were collected from USDA and Oregon State University breeding programs. Samples from southern Idaho and western Oregon were assayed for CMV on cowpea, *Chenopodium amaranticolor* (Corvallis strain), and Improved Long Green cucumber. These samples were also tested for CMV by enzyme-linked immunosorbent assay (ELISA) by a modified Clark and Adams method (3). Antiserum to isolate CMV-B (18) was produced from virus purified by the method of Lot et al (13). Coating immunogammaglobulin and alkaline phosphatase-immunogammaglobulin conjugate were each used for ELISA at concentrations of 1 μg/ml.

Samples taken in eastern Washington were processed and assayed as in 1978; samples from Oregon State University breeding plots were only assayed by ELISA.

Seeds of 32 *P. vulgaris* plant introduction lines, selected to represent areas from which seed-transmitted isolates of CMV have been reported or are considered likely to occur, were obtained from the W-6 Regional Plant Introduction Station in Pullman, WA, and tested either by bioassay on *Chenopodium* and cucumber plants or by ELISA.

CMV-infected (positive) and CMV-free (negative) controls were routinely included in CMV assays. Sensitivity of *Chenopodium* assay and ELISA methods was determined by testing dilution series of CMV-infected tissues and purified virus.

Table 1. Seedborne cucumber mosaic virus in *Phaseolus vulgaris* germ plasm and plant introduction lines in the Pacific Northwest

Year	Location	Source	No. of lines	No. of plants sampled	Assay method[a]	Results[b]
1978	S. Idaho	Seed companies	6	26	B	0/26
	E. Washington	Seed companies	2	2	B,M,S	0/2
	Prosser	Breeding program		59	B,M,S	0/59
	Corvallis	Breeding program		3	B	0/3
1979	S. Idaho	Seed companies	4	43	B,E	0/43
	W. Oregon	Seed companies	1	4	B,M,S	0/4
	E. Washington	Seed companies	4	26	B,M,S	0/26
	Prosser	Breeding program		56	B,M,S	0/56
	Corvallis	Breeding program		40	B,E,	0/40
1979–1980	PI collection, Pullman, WA	PI lines, U.S.	6[c]	188	B,E	0/188
		PI lines, Int.	26[c]	1,070	E	2/1,070[d]

[a] B = bioassay, M = electron microscopy, S = gel double-diffusion serology, E = enzyme-linked immunosorbent assay.
[b] Number of samples in which CMV was detected per number of samples tested.
[c] Six *Phaseolus* lines of U.S. origin, 26 of international origin.
[d] Infection found in 2 of 56 PI 271998 plants from Spain.

RESULTS AND DISCUSSION

CMV was not detected in any of the 259 bean plants sampled in Idaho, Washington, and Oregon. However, one of the 32 plant introduction lines of U.S. and international origin contained seedborne virus serologically identified as CMV (Table 1). Two of 56 seeds of PI 271998, from Spain, contained this virus designated CMV-Pg. CMV-infected control plants were detected with 100% accuracy.

Although sampling among Pacific Northwest bean breeding programs was limited and the appearance of CMV-induced symptoms in diverse bean germ plasm types was unknown to us, we took samples judiciously from among thousands of plants on the visual criteria available. It was also our objective to determine whether seedborne CMV had become a significant problem in breeding programs aimed at developing new, improved bean cultivars for the United States. The absence of CMV in the 259 samples taken from three states suggests that CMV has not yet become a problem to the Pacific Northwest bean seed industry. Surveillance for CMV is continuing, with the use of highly susceptible bean cultivars as trap crops.

By ELISA methodology, we readily detected CMV in infected fresh tissue controls diluted 1,000-fold (100-fold by healthy plant tissue, 10-fold by buffer), in desiccated tissue at 100,000-fold dilution with buffer, and in the purified state at 6 ng/ml. The corresponding values for bioassay on *Chenopodium* plants were 10-fold, 100-fold, and 700 ng/ml for fresh and desiccated infected tissue and for purified virus, respectively. Thus, ELISA was roughly 1,000 times more sensitive than bioassay for detection of CMV.

Certain characteristics of the seedborne CMV isolates may have limited our ability to detect them during this survey. For example, these isolates typically induce mild mosaic or vein banding, which can be caused by other factors or may be masked, and these isolates may be seed-transmitted in beans at frequencies that would easily escape attention. However, detection would be aided by the readiness with which the established virus is transmitted by aphids to healthy plants. Even at low concentrations, the highly sensitive ELISA methodology also should have aided detection.

Despite the absence of seedborne isolates in the bean-producing areas of the Pacific Northwest, CMV is likely to be introduced through the international movement of bean germ plasm unless preventive measures are taken. Disease prevention is increasingly strategic for successful bean production. Substantial losses to the pea seed industry might have been avoided if pea seedborne mosaic virus (8) had been detected and prevented at an early stage. Concern about the threat of seedborne CMV to the bean seed industry is justified by the pathogenic implications of CMV-satellite RNA in tomatoes, recent reports of seed trans-

mission of CMV through beans, lack of CMV-resistant *Phaseolus* germ plasm, and the occurrence of CMV in at least one international PI bean accession.

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