Incidence of the Lentil Strain of Pea Seedborne Mosaic Virus as a Contaminant of *Lens culinaris* Germ Plasm

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ABSTRACT

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Seedlot samples of 570 Plant Introduction (PI) accessions of *Lens culinaris* were planted in greenhouses and the resultant seedlings were tested for the presence of seedborne virus by local lesion assays on *Chenopodium amaranticolor* leaves. A seedborne virus, designated the lentil strain of pea seedborne mosaic virus (PSbMV-L), was detected in 38 of 570 accessions. Incidence of PSbMV-L in five selected accessions ranged from < 5 to 10%. Although plants of PI accessions arising from infected seeds usually showed no symptoms, isolates from infected accessions readily induced severe symptoms in plants of commercial lentil cultivars including Chilian, Tekoa, and Precoz. Of 23 other plant species and cultivars tested as possible hosts, PSbMV-L was infectious only to *C. amaranticolor, Vicia faba* var. *minor,* and one (Tempter) of 25 pea cultivars. The virus was not seed transmitted in Tempter peas. PSbMV-L was transmitted from infected to healthy Tekoa lentil plants by sets of three pea aphids (*Acyrthosiphon pisum*) at frequencies ranging from 55 to 88%. PSbMV-L was distinguished from the standard U.S. strain of PSbMV by nonpathogenicity to most pea cultivars, by pathogenicity to *L. culinaris* germ plasm sources independently of PSbMV-immunity-conferring gene *sbv,* by an inoculum reservoir apparently restricted to infected lentil seed, by comparative enzyme-linked immunosorbent assay, and by intrinsic particle instability. Conversely, the two strains produced identical symptoms in Tekoa lentil and on leaves of *C. amaranticolor* and were indistinguishable by SDS-gel immunodiffusion serology or by immunosorbent electron microscopy.

After determination that pea seedborne mosaic virus (PSbMV) was readily seed transmitted in lentil (*Lens culinaris* Medic.) (4), 586 USDA Plant Introduction (PI) accessions were screened for resistance to PSbMV (3). Four PI accessions were found to be immune to PSbMV and immunity was determined to be conferred by a single recessive gene, designated *sbv*. Possible incidence of PSbMV in PI accessions was thereafter investigated and a seedborne potyvirus was indeed discovered in this lentil germ plasm resource. Although the virus closely resembled PSbMV, it was not infectious to selected pea cultivars. The origin of this virus, designated the lentil strain of pea seedborne mosaic virus (PSbMV-L), and its biological and serological characteristics are described in this paper.

MATERIALS AND METHODS

PI accessions of *L. culinaris* were obtained for testing from the Western Regional Plant Introduction Station, Washington State University, Pullman, WA. Twenty to 30 seeds per accession were scarified, treated with captan, and planted in a greenhouse. Resultant seedlings were observed weekly for occurrence of PSbMV-like symptoms and assayed on leaves of *Chenopodium amaranticolor* Coste & Reyn. either individually or by bulked-groups of 10 plants, 4–6 wk after planting. Assay plants were examined for induced local lesions 1 and 2 wk after inoculation. Five PI accessions found to contain seedborne virus were replanted and individual plants were assayed for estimation of virus seed-transmission rates.

After pea cultivars routinely used to propagate PSbMV were found to be immune to the lentil virus, lentil cultivars Tekoa, Chilian, and Precoz and 25 pea cultivars were inoculated. Subsequently, 23 plant species and cultivars (4) were evaluated as possible hosts of this virus.

To further define the relationship between PSbMV and the virus seedborne in lentil, each virus was inoculated into selected PI accessions of *Lens* known to be either immune or susceptible to PSbMV. Symptom development was observed weekly for 3 wk after inoculation, and leaf tissues were sampled from inoculated plants, composited per accession for each virus, and assayed on *C. amaranticolor* plants.

After preliminary tests had indicated that PSbMV antiserum reacted to the lentil virus in SDS-gel tests (14), this antiserum was evaluated for detection of the new virus by enzyme-linked immunosorbent assay (ELISA) (1) and by immunosorbent electron microscopy (15). Comparative reactivity between the lentil virus and PSbMV (in sap from infected lentil and pea plants, respectively) was assessed by SDS-gel immunodiffusion and quantified as absorbance ($A_{405\ nm}$) of end product *p*-nitrophenol by ELISA serology (1). Parallel titrations of virus preparations were performed by *C. amaranticolor* local lesion assays.

Transmissibility of the lentil virus by pea aphids (*Acyrthosiphon pisum* (Harris)) was evaluated by feeding 7- to 9-day-old apterae for 3 ± 0.5-min acquisition access periods on virus-infected Tekoa lentil plants and transferring them by sets of three to individual test plants of Tekoa lentil or Tempter pea. A standardized virus isolate derived from PI 297772 was used in most tests. Evidence of aphid transmission comprised development of characteristic symptoms in test plants 2–3 wk after inoculation, plus confirmatory assays on *C. amaranticolor*.

Procedures evaluated for purification of the lentil virus included those employed in our laboratory for PSbMV purification (11) and other methods that had proven generally efficacious for potyviruses (2,10,13).

RESULTS

A seedborne virus was detected in 38 of 570 PI accessions of *L. culinaris* by assays on *Chenopodium* (Table 1). On the basis of information herein presented, the virus was designated the lentil

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strain of pea seedborne mosaic virus (PSbMV-L). Seedborne infections were latent in all 38 accessions except PI 176604 and 176988, in which slight plant stunting, leaf roll, and mosaic were observed.

Seedlings of five infected accessions, assayed individually on *C. amaranticolor* leaves, were estimated to contain PSbMV-L at frequencies ranging from <5 to 10%. Incidence of PSbMV-L in seed lots of these accessions were: PI 185602, 1/20; 289077, 2/20; 297772, 1/20; 297746, 1/20; and 374116, 0/20.

Three isolates derived from PI accessions of diverse origin and tested for pathogenicity on several lentil selections and cultivars induced symptoms in plants of lentil cultivars Tekoa, Precoz, and Chilian that were indistinguishable from each other or from those induced by PSbMV. Symptoms consisted of plant stunting, leaf-size reduction, leaf roll, and/or leaf curl. These isolates, however, induced no symptoms in plants of pea cultivars 447, Cascade, Perfected Wales, or Dark Skin Perfection, which are all susceptible to common isolates of PSbMV. Further, PSbMV-L was not detectable in tissue of inoculated pea plants by assay on *Chenopodium.*

TABLE 1. Lentil germ plasm sources infected with the lentil strain of pea seedborne mosaic virus[a]

Accession origin	Introduction year	Infected accession
Turkey	1948	PI 169518
	1949	176604
	1949	176988
	1949	179313
	1949	179319
	1964	298023
	1969	339290
	1969	339292
Egypt	1949	185602
Afghanistan	1953	207492
	1954	212609
Hungary	1963	289077
Greece	1964	297746
	1964	297752
	1964	297753
	1964	297770
	1964	297771
	1964	297772
Argentina	1964	297286
Mexico	1964	299116
	1964	299127
Chile	1964	299183
	1964	299206
	1964	299214
	1964	299215
	1964	299233
	1964	299251
	1964	299265
	1964	299274
	1964	299288
	1964	299295
	1964	299318
	1964	299364
Bulgaria	1967	320945
Iran	1968	329163
	1968	329168
Morocco	1972	374116
	1972	374120

[a]Thirty-eight Plant Introduction accessions of *Lens culinaris* found to be infected, of 570 accessions tested.

Plants of 21 additional pea cultivars were tested for susceptibility to PSbMV-L. One cultivar, Tempter, was found to be susceptible. Tempter, unlike the other pea cultivars tested, was also susceptible to bean yellow mosaic virus. PSbMV-L-infected Tempter plants showed very slight plant stunting and mild leaf roll. Three weeks after inoculation, the concentration of PSbMV-L in Tempter, determined by assay on *Chenopodium,* was 0.3 to 0.5 the concentration in infected Tekoa lentil plants. Pea cultivars, other than those named, that are susceptible to PSbMV isolates but were not susceptible to PSbMV-L included Alaska, Almota, Alsweet, Aurora, Canner 695, Conway, Early Sweet, Freezer 37, Freezer 47, Freezer 640, Freezer 6650, H312, Little Marvel, Perfection 326, Perfected Freezer 60, Pride, Ranger, Thomas Laxton, WV183F, and Wyola.

Hosts of PSbMV-L other than lentil and Tempter pea included *Vicia faba* var. *minor* and *C. amaranticolor.* Nonhosts were: *Antirrhimum majus* L. 'Mixed Colors'; *Cucumis sativus* L. 'Chicago Pickling'; *Datura stramonium* L., R. Fulton strain; *Glycine max* (L.) Merr. 'Bragg' and 'Davis'; *Gomphrena globosa* L., A. F. Ross strain; *Lycopersicon esculentum* Mill. 'Marglobe'; *Medicago sativa* L. 'DuPuits'; *Nicotiana glutinosa* L., Corvallis strain; *Nicotiana tabacum* L. 'Samsun NN'; *Petunia hybrida* Hort. Vilm.-Andr. 'King Henry'; *Phaseolus vulgaris* L. 'Bountiful,' 'Black Turtle Soup,' and 'Pinto 111'; *Phlox drummondii* Hook. 'Tall Mixed Colors'; *Pisum sativum* L. 'Perfected Wales' and 'Dark Skin Perfection' (previously mentioned); *Spinacea oleracea* L. 'Bloomsdale Long Standing'; *Trifolium pratense* L. 'Kenland'; *T. repens* L. 'New Zealand'; and *Vigna unguiculata* (L.) Walp. 'California Cowpea #5.'

PSbMV-L and PSbMV were clearly distinguished pathogenically on *L. culinaris* PI accessions that were either immune or susceptible to PSbMV. PI 212610 and 368651 were immune to PSbMV-L, PI 251786 and 289071 were immune to PSbMV, and PI 368648 was immune to both strains. Tekoa lentil was susceptible to both strains and responded to infection with identical symptoms. PI 289071 and

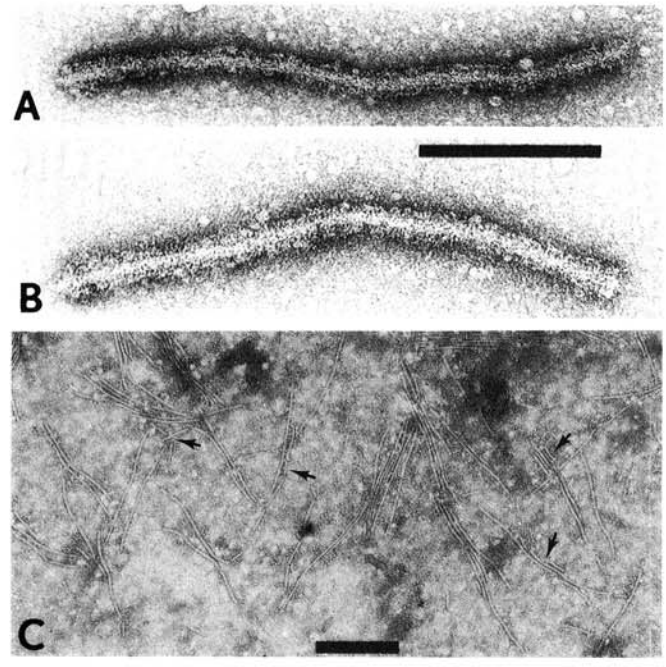

Fig. 1. Appearance of particles of the lentil strain of pea seedborne mosaic virus (PSbMV-L) in crude lentil extracts; **A,** PSbMV-L particle coated with immunogammaglobulin (IgG) to a standard strain of pea seedborne mosaic virus (PSbMV); **B,** PSbMV particle coated with homologous IgG. Virus particles coated with 20-fold dilution of PSbMV antiserum. Note slightly thinner IgG coating on PSbMV-L than on PSbMV particle; coated particles of both viruses stained equivalently with 2% uranyl acetate; **C,** PSbMV-L particles pretreated with 2% glutaraldehyde and negatively stained with phosphotungstic acid, pH 5.0. Particle fragments indicated by arrows. Scale bars a, b = 250 nm, and c = 500 nm.

368651 were found, incidentally, to be genetically heterogeneous, relative to virus immunity. PI 289071 contained 5–10% PSbMV-susceptible plants and PI 368651 contained 5–10% PSbMV-L-susceptible plants.

When PSbMV-L and PSbMV were compared serologically, using antiserum prepared against PSbMV, they were indistinguishable by SDS-gel immunodiffusion; ie, precipitin bands at several concentrations of antiserum and crude-antigen preparations were precisely conterminous for the two viruses in gels containing 0.25, 0.40, or 0.55% Ionagar. Likewise, particles of both viruses had equal affinities to electron microscope grids coated with 100-fold dilutions of PSbMV immunogammaglobulin (IgG) and decorated equivalently when reacted with 20-fold dilutions of this IgG (Fig. 1A and B). In typical leaf dip preparations PSbMV-L particles were characteristically fragmented (Fig. 1C), compared to those of PSbMV. Pretreatment of PSbMV-L preparations with glutaraldehyde (6) reduced, but did not prevent, particle fragmentation. Ostensibly intact particles ranged from 750 to 810 nm in length (50 particles measured), based on TMV particles (modal length, 300 nm) as an internal calibration standard.

By comparative ELISA serology with PSbMV antiserum, PSbMV-L reactions ($A_{405\ nm}$) were consistently fractional to those of PSbMV, illustrated in Table 2. Although concentrations of infective particles, estimated by assay on *Chenopodium,* were approximately equivalent at each dilution of PSbMV-L- and PSbMV-infected-plant extracts, the $A_{405\ nm}$ values for PSbMV-L ranged from 40 to 50% of those for PSbMV. Nevertheless, PSbMV antiserum proved effective in detecting PSbMV-L in lentil plants with latent infections; its sensitivity ranged from fivefold to 25-fold that of assays on *Chenopodium.*

PSbMV-L was found to be highly transmissible by the pea aphid. In three sequential tests PSbMV-L was transmitted to Tekoa pea test plants by aphid triplets at frequencies of 55, 80, and 88%. PSbMV-L was as readily transmitted to Tempter pea test plants by aphids as by mechanical inoculation.

Preliminary attempts to purify PSbMV-L by methods entirely satisfactory for PSbMV were unsuccessful. Particles of PSbMV-L were generally much less stable in initial phases of clarification than PSbMV in agreement with electron microscopic observations, and tended to be lost during centrifugation at relatively low gravity force; ie, less than 10,000 *g*. Irreversible aggregation frequently occurred during selective precipitation with polyethylene glycol 6,000, and density gradient banding was unsuccessful in sucrose density gradients and marginally successful in gradients of cesium chloride. All of these characteristics suggest gross-structural features that are distinct from those of PSbMV. The requisites for PSbMV-L purification are being reported separately.

TABLE 2. Comparative ELISA determinations for extracts from plant tissues infected with the lentil (PSbMV-L) and standard (PSbMV) strains of pea seedborne mosaic virus, in relation to parallel assays on *Chenopodium amaranticolor*

Strain	Dilutions of infected plant sap[a]	ELISA results[b] ($A_{405\ nm}$ of reactions) $\bar{x}$	s	Local lesions per half-leaf[c] $\bar{x}$	s
PSbMV-L	10	0.490	0.050	10.5	0.71
	100	0.266	0.017	2.7	1.53
	500	0.166	0.013	0.3	0.58
	2,500	0.114	0.008	0	···
	6,250	0.069	0.006	0	···
	Healthy Tekoa	0.058	0.007	0	···
PSbMV	10	1.14	0.010	16.0	1.41
	100	0.534	0.055	2.3	0.58
	500	0.415	0.008	0.3	0.58
	2,500	0.238	0.008	0.3	0.58
	6,250	0.084	0.008	0	···
	Healthy 447	0.041	0.007	0	···

[a] From PSbMV-L-infected cultivar Tekoa lentil plants; or PSbMV-infected cultivar 447 pea plants.

[b] Absorbance of ($A_{405\ nm}$) by *p*-nitrophenol of final ELISA reactions; average $A_{405\ nm}$, two replicate tests; $\bar{x}$ = average, s = standard deviation.

[c] Numbers of local lesions, three half leaves per treatment.

DISCUSSION

Designations of virus strains and separate viral identities are frequently based on subjective factors and individualistic interpretations. Naming the virus herein reported as the lentil strain of pea seedborne mosaic virus, rather than a PSbMV-like virus, illustrates this generalization. When the virus was discovered in the USDA germ plasm collection of *L. culinaris,* it was presumed to be typical PSbMV, based on SDS-gel serology and leads from prior research. With preliminary evidence of their distinction, it was concluded that the new virus was a deviant strain of PSbMV. Three distinctions were most notable. First, of 25 pea cultivars tested, 24 were immune to the new isolates; one was marginally susceptible. Secondly, the inoculum reservoirs of the two viruses are completely separate: infected pea seeds for PSbMV (5,8), and infected lentil seeds for PSbMV-L. Although PSbMV in the laboratory was more readily seed-transmitted in lentil than in most pea cultivars (9), previous strains of this virus have not been isolated from naturally infected lentil and were not seed-transmitted in 570 PI accessions of *L. culinaris.* By natural host relationships, therefore, the strains are readily distinguishable. Lastly, immunity to the two types in lentil is governed by independent genes; ie, accessions immune to PSbMV by virtue of gene *sbv* (3) are susceptible to PSbMV-L and other accessions that are immune to PSbMV-L are susceptible to PSbMV.

PSbMV-L was not distinguishable from PSbMV by SDS-gel serology or by immunosorbent electron microscopy, but was consistently differentiated by a quantitative application of ELISA serology. The advantage of ELISA in this application is presumably attributable to precision control of system components and photometric quantification of test reactions inherent in the method and lacking in the others.

A recently published attempt to discern distinct strains among the premier PSbMV isolates reported from 1966 to 1970 (7) identified only relatively minor differences among seven isolates from four countries. During that information development, a "*Pisum* latent strain" was reported in Yugoslavia (12), and still more recently personal communications have indicated that at least two other aberrant strains of PSbMV have been discovered. The extent to which new strains are able to circumvent immunity conferred by the *sbm* gene in *Pisum* is presently unknown; such circumvention of *Lens* gene *sbv* by strain PSbMV-L in the present study is clear.

Finally, it is hoped that the demonstrated presence of PSbMV-L in *L. culinaris* germ plasm might stimulate concern for the general phenomenon of seedborne viruses in U.S. crop germ plasm resources. Remedial action on a national scale seems warranted, to protect users of germ plasm from viruses that would be established and perpetuated in genetic materials and breeding programs through seed transmission. Particularly, viruses such as PSbMV-L that are readily vector transmissible would tend to spread to virus-free germ plasm sources in seed-increase plantings.

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