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Pea seed-borne mosaic virus: occurrence in faba bean (Vicia faba) and lentil (Lens culinaris) in West Asia and North Africa, and further information on host range, transmission characteristics, and purification

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Abstract

In a survey for viruses of cultivated legumes in West Asia and North Africa, pea seed-borne mosaic virus (PSbMV) was found in faba bean, lentil and pea. Using ELISA, it was detected in 107 out of 1554 faba bean samples and 40 out of 496 lentil samples with virus-like symptoms collected in Algeria, Egypt, Ethiopia, Jordan, Lebanon, Libya, Morocco, Sudan, Tunisia and Turkey.

A pea isolate (SP9-88) from Syria was further characterized. Out of 57 plant species tested, 35 were found susceptible, 19 of which are newly reported hosts of the virus. The virus was transmitted efficiently in the non-persistent manner by five aphid species, especially *Myzus persicae*. Purification from systemically infected faba bean plants yielded 10–15 mg of purified virus per kg of infected tissue. Sap-inoculation of the food and forage legume species chickpea, faba bean, lentil, pea, *Vicia narbonensis, V. sativa, Lathyrus ochrus* and *L. sativus* at flowering stage led to 66.0, 40.5, 44.6, 49.2, 31.7, 7.5, 35.7 and 12.0% yield loss, respectively, and to seed-transmission rates of 0.7, 6.0, 10.8, 1.1, 0.3, 0.2 and 0.4%, respectively. No transmission was detected in chickpea seed embryo axes. However, the virus was detected in the seed coat of PSbMV-infected chickpea at an estimated rate of 1.81%.

Additional keywords: aphid transmission, food and forage legumes, seed transmission, yield-loss assessment.

Introduction

Faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Med.) are important and widely cultivated field crops in West Asia and North Africa (WANA). As a source of food they rank next to cereals and constitute an inexpensive source of protein for a large part of the population. Faba bean and lentil plants are susceptible to natural infection by 44 and 9 viruses, respectively (Bos et al., 1988). In WANA, few viruses have been reported to infect faba bean (Kaiser et al., 1968; Fischer and Lockhart, 1976; Allam et al., 1979; Makkouk et al., 1988; Fortass and Bos, 1991) and lentil (Kaiser, 1973; Russo et al., 1981; Fidan and Yorganci, 1990; Makkouk et al., 1992a). During a survey of legume crops for viruses in the region served by ICARDA (WANA) we found isolates resembling pea seed-borne mosaic virus (PSbMV).

The virus was first described in Czechoslovakia (Musil, 1966), and has since been reported in several countries in Europe, Asia, North America and North Africa (Hampton

and Mink, 1975; Khetarpal and Maury, 1987). PSbMV is a potyvirus, has filamentous particles c. 770 × 12 nm, and is transmissible by sap inoculation and by several aphids in the non-persistent manner (Kvičala and Musil, 1967; Aapola and Mink, 1973). It is seed-borne in pea (Mink et al., 1969; Alconero and Hoch, 1989) up to 95% depending on cultivar (Cockbain, 1988), in lentil up to 44% (Hampton and Muehlbauer, 1977), in faba bean up to 3% (Musil, 1980), and at a low percentage in seeds of several forage legume species (Hampton and Mink, 1975; Makkouk et al., 1992b). It may also be spread in pollen of infected plants (Stevenson and Hagedorn, 1973).

This study evaluated the occurrence of PSbMV in a number of countries of WANA and its effect on faba bean, lentil and some other important legume crops in the region, and measured PSbMV seed-transmissibility in several food and forage legumes. In addition, properties of a local PSbMV isolate were investigated.

Materials and methods

Field collections and virus isolates. Samples of faba bean and lentil with symptoms suggestive of virus infection were collected from farmers' fields and from experimental plots of agricultural research stations in Algeria, Egypt, Ethiopia, Jordan, Lebanon, Libya, Morocco, Sudan, Syria, Tunisia and Turkey during 1985–1992. Samples were brought to the ICARDA laboratory in Tel-Hadya, Aleppo, Syria, and each was extracted in 0.2 M phosphate buffer, pH 6.0, using a motorized tissue extractor, and tested for the presence of PSbMV by ELISA. The antiserum used was the one to a Dutch PSbMV isolate from pea (E210: Bos, 1970) and was provided by L. Bos and D.Z. Maat (IPO-DLO, Wageningen, the Netherlands).

The PSbMV isolate (SP9-88) obtained from a naturally infected pea plant at Tel-Hadya, Syria, was maintained and propagated on faba bean and used for further characterization of the virus.

Host-range studies. Leaves of infected faba bean were ground in a mortar and pestle with 0.01 M phosphate buffer, pH 7.2, mixed with celite. A total of 57 plant species belonging to 6 families were inoculated mechanically, and kept in the glasshouse at 20–30 °C. Four weeks after inoculation, symptomless uninoculated leaves from all species were tested by ELISA for the presence of PSbMV.

Aphid-transmission tests. *Aphis craccivora* was originally collected from lentil, *A. fabae* from faba bean, *Acyrthosiphon pisum* from pea, *Diuraphis noxia* from wheat, *Myzus persicae* from cabbage and *Rhopalosiphum padi* from barley. These six species all originated from Syria, and were reared in screencages in a glasshouse. In transmission tests the adult insects were fasted for 1 h and then placed in petridishes for acquisition feeding on infected faba bean leaves for 5–10 min. The aphids were then transferred to plants of faba bean 'Syrian Local' (five aphids each) for inoculation feeding, and were left on the plants for 24 h. Plants were thereafter sprayed with 0.5 g/l Pirimor to kill the aphids. Four weeks after inoculation, uninoculated leaves were harvested from all tested plants and assayed for virus by ELISA.

Yield-loss assessment and seed-transmission tests. Two field experiments were conducted during the growing season 1990–1991 at Tel-Hadya, Syria. The first experiment was with food legumes (the 'Syrian Local' cultivars of chickpea, faba bean, lentil and pea). The second experiment was with forage legumes (*Vicia narbonensis* 'IFIV 2933', *V. sativa* 'IFVI 594', *Lathyrus ochrus* 'IFLA 585' and *L. sativus* 'IFLA 513'). The experi-

ments were carried out in a randomized complete block design with four replicates. Each replicate plot consisted of four rows (3.5 m long for food legumes and 2 m long for forage legumes), 30 cm apart, with 10, 17.5, 7, 12, 8, 8, 8 and 8 cm between plants within rows for chickpea, faba bean, lentil, pea, *V. narbonensis*, *V. sativa*, *L. ochrus* and *L. sativus*, respectively. Plants were inoculated mechanically at the flowering stage. After maturation, dry seeds were collected and weighed. The dry seeds were planted in sterilized sand in germination boxes and incubated at 20–25 °C for 1–2 weeks. Each emerging seedling was divided into four portions: root, shoot, seed-coat and cotyledon. The seedling parts were extracted in 0.2 M phosphate buffer, pH 6, and tested by ELISA in groups of ten. Extracts from healthy seedling parts were placed in eight wells of each ELISA plate. Seed infection rates were estimates calculated by the formula $P = [1-(H/N)^{1/n}] \times 100$, where: P = percentage of seed infection, H = number of virus-free groups, N = total number of groups tested, and n = number of seeds per group (Maury et al., 1985).

Virus purification. The virus was purified from infected faba bean harvested 15–20 days after inoculation. The purification procedure was similar to the one used for two potyviruses from *Phaseolus vulgaris* (Azzam and Makkouk, 1986), but the extract was clarified with 25% chloroform. The virus was concentrated by precipitation with 6% polyethylene glycol plus 2% NaCl followed by high-speed centrifugation (60 000 rpm for 1.5 h) over a 20%-sucrose pad, rate-zonal sucrose density-gradient centrifugation, and final differential centrifugation.

Electron microscopy. Small pieces of infected leaf, either fresh or desiccated over calcium chloride, were chopped in sodium phosphotungstate (PTA, pH 6.5) for negative staining and viewing in the electron microscope. Immunospecific decoration was also done using PSbMV antiserum.

Antiserum production and serology. Antiserum against the purified isolate SP9-88 was prepared by giving a rabbit five weekly intramuscular injections with 1–2 mg virus each. Partially purified virus was emulsified with an equal volume of Freund's complete adjuvant for the first injection, and with Freund's incomplete adjuvant for subsequent injections. The rabbit was bled five times at weekly intervals starting 1 week after the fifth injection.

Gammaglobulins were fractionated from the antiserum using the caprylic acid method (Steinbuch and Audran, 1969). Conjugation of gammaglobulin with alkaline phosphatase and DAS-ELISA were according to Clark and Adams (1977). Plates were IgG-coated with 2 µg/ml for SP9-88 and 1 µg/ml for E210, and the conjugate dilution used was 1/500 for SP9-88 and 1/1000 for E210. Absorbance was measured 2 h after addition of the substrate at 405 nm (A405) by an ELISA plate reader (Titertek Multiscan Mark II, Flow Laboratories). Field samples were considered PSbMV infected when the A405 values exceeded the mean of the healthy controls by three standard deviations.

Results

Field survey. During the 1985–1992 survey, 1554 faba bean and 496 lentil samples with virus-like symptoms were collected. When tested by ELISA, 107 faba bean and 40 lentil samples were found to be infected with PSbMV. Infected faba bean samples were 0/2 (0 out of 2 samples tested) from Algeria, 15/150 from Egypt, 7/41 from Ethiopia, 0/10 from Jordan, 1/61 from Lebanon, 1/47 from Libya, 2/23 from Morocco, 16/494 from Sudan, 50/565 from Syria and 15/161 from Tunisia. Infected lentil samples were 1/43 from

Algeria, 2/5 from Egypt, 0/44 from Lebanon, 0/6 from Libya, 5/6 from Morocco, 28/315 from Syria, 1/1 from Tunisia and 3/76 from Turkey.

Host range and symptoms. Symptoms of SP9-88 on faba bean 'Syrian Local' consisted of rolling, mild mosaic and reduction in size of tip leaves. Several forage and wild legumes showed leaf rolling and reduction of leaf size, followed by chlorosis of the plant tip. Plants of all systemically infected species were significantly stunted when compared with healthy controls. Responses of the different species are summarized in four categories:

(1) Local reaction (chlorotic or necrotic lesions) without systemic invasion: *Chenopodium album, C. amaranticolor, C. quinoa.*

(2) Systemic symptoms (leaf rolling and yellowing, plant stunting) without local reaction:
   (a) Food-legume species: *Cicer arietinum* (chickpea), *Lens culinaris* (lentil), *Pisum sativum* (pea) 'Koroza', 'Rondo', 'Onyx', 'Syrian Local' and 'IFPI 2903', *Vicia faba* (faba bean) 'Kompakta' and 'Syrian Local'.
   (b) Forage and wild legume species: *Lathyrus annuus, L. aphaca, L. cicera, L. gorgoni, L. ochrus, L. odoratus, L. sativus, Vicia ervilia, V. mollis, V. narbonensis, V. narbonensis* var. *aegyptiaca, V. narbonensis* var. *narbonensis, V. narbonensis* var. *salmonea, V. noeane, V. palaestina, V. sativa, V. sativa* ssp. *amphicarpa, V. sativa* ssp. *sativa, V. villosa, V. villosa* ssp. *eriocarpa, V. villosa* ssp. *villosa.*

(3) Symptomless infection: *Lathyrus hiersolymitanus, Medicago rigidula* var. *agrestis, M. scutellata, Vicia hybrida, V. pannonica, V. peregrina, Trifolium spumosum.*

(4) No infection:
   (a) Non-legume species: *Brassica juncea, B. napus, Carthamus* sp., *Crambe abyssinica, Cucumis sativus, Dolichus lablab, Lycopersicon esculentum* 'Marglobe S-16' and 'Syrian Local', *Nicotiana clevelandii, Spinacia oleracea* 'Bloom Long Standing' and 'Syrian local'.
   (b) Food-legume species: *Glycine max* 'Davis', *Phaseolus vulgaris* 'Amanda', 'Apolo', 'Bo 19', 'Canadian Wonder', 'Dubbele Witte', 'Jubila', 'Great Northern 31', 'Great Northern 123', 'Michelite', 'Monroe', 'Pinto 111', 'Pinto 114', 'Prelude', 'Redland Greenleaf B', 'Redland Greenleaf C', 'Red Mexican 34', 'Red Mexican 35', 'Sanilac', 'Sutter Pink', 'Top Crop', 'Widusa'.
   (c) Forage and wild-legume species: *Medicago arabica, M. rigidula* var. *cinerascens, M. rigidula* var. *submitus, M. sativa, M. turbinata* var. *spinulosa, Trifolium lappaceum, T. nigrescans, T. pauciflorum, T. resupinatum, T. scutatum, T. subterraneum.*

Aphid transmission. Three weeks after inoculation feeding, PSbMV symptoms were produced on faba bean plants. Rates of infection recorded were based on characteristic symptoms produced and confirmed by ELISA 4 weeks after inoculation. Results obtained indicated that 50, 47, 45, 44 and 24 plants became infected out of 50 plants inoculated with *Myzus persicae, Aphis fabae, Acyrthosiphon pisum, Aphis craccivora* and *Rhopalosiphum padi*, respectively. Accordingly, efficiency of transmission expressed in percentage of successful transmission was 100, 94, 90, 88 and 48% for the above-mentioned aphid species, respectively. *Diuraphis noxia* did not transmit the virus to any of the 50 inoculated plants.

Yield-loss evaluation. In experimental plots (3.5 × 1.2 m) seed yield losses of chickpea, faba bean, lentil and pea, inoculated with PSbMV at flowering stage, were 66.0, 40.5, 44.6 and 49.2%, respectively. In experimental plots (2 × 1.2 m) of *Vicia narbonensis*,

V. sativa, *Lathyrus ochrus* and *L. sativus*, yield losses were 31.7, 7.5, 35.7 and 12.0%, respectively (Table 1).

Seed transmission. Seeds were harvested from plants of the nine legume species inoculated with PSbMV SP9-88 at flowering stage. Seedling parts (cotyledon, root, seed coat and shoot) of 300, 300, 500, 500, 300, 400, 500 and 500 germinated seeds from plants of chickpea, faba bean, lentil, pea, *V. narbonensis*, *V. sativa*, *L. ochrus* and *L. sativus*, respectively, were tested in groups of ten by ELISA. PSbMV was isolated from the shoot of the above species except chickpea, and the virus was detected in the seed coat of all species. All parts of lentil and pea seedlings tested contained the virus. The rates of seed transmission were highest in pea (11%) and lentil (6%) (Table 2).

Some faba bean and pea seeds obtained from PSbMV-infected plants had a necrotic line pattern on the seed coat (Fig.1A and B). The seed-coats of such seeds were found to be ELISA-positive for PSbMV. However, the virus was not detected in the shoot of faba bean seedlings, but was detected in the shoots of pea seedlings which emerged from such seeds.

Rates of PSbMV seed transmission in pea and *V. narbonensis* seeds with split testas (cracked seed coats) (Fig.1C and D) were similar to those of normal seeds. When embryo axes from 42 and 48 germinated pea seeds with and without split testas, respectively, were tested in groups of two by ELISA, virus was detected in 5 of 21 groups of the seeds with cracked seed coats, and in 5 of 24 groups of normal seeds. Seed-transmission rates of PSbMV were estimated to be 12.7% for seeds with cracked seed coat and 11.0% for seeds with normal seed coat. Necrotic line pattern also was not correlated with PSbMV seed-transmission rates.

Table 1. Seed yields[a] of food and forage legume species in experimental plots inoculated with pea seed-borne mosaic virus (PSbMV) during the growing season 1990–1991. Values in brackets are yields in percents of healthy control.

Host	Genotype	Seed yield (g)	
		Healthy	PSbMV-infected
Food legume			
Cicer arietinum	Syrian Local	516.3	175.8** (34.0)
Lens culinaris	Syrian Local	535.3	296.3* (55.4)
Pisum sativum	Syrian Local	505.8	256.8** (50.8)
Vicia faba	Syrian Local	1047.0	622.5* (59.5)
Forage legume			
Lathyrus ochrus	IFLA 585	354.5	228.0** (64.3)
L. sativus	IFLA 513	501.5	441.5** (88.0)
Vicia narbonensis	IFVI 2933	495.5	338.3* (68.3)
V. sativa	IFVI 594	151.3	140.0 (92.5)

[a] Yield in grams per plot of 3.5 × 1.2 m for food legumes, and 2 × 1.2 m for forage legumes, replicated four times.

* Significant at $P = 0.05$ when compared with the healthy control.

** Significant at $P = 0.01$ when compared with the healthy control.

Table 2. Detection of pea seed-borne mosaic virus (PSbMV) by ELISA in parts of germinated seeds collected from PSbMV-infected food and forage legume species.

Host	Rates of infection in percent			
	Cotyledon	Root	Seed coat	Shoot
Food legumes				
Cicer arietinum	0.0[a]	0.0	1.8	0.0
Syrian Local	(0/30)[b]	(0/30)	(5/30)	(0/30)
Lens culinaris	3.0	2.7	5.0	6.0
Syrian Local	(13/50)	(12/50)	(20/50)	(23/50)
Pisum sativum	4.4	8.8	7.9	10.8
Syrian Local	(18/50)	(30/50)	(28/50)	(34/50)
Vicia faba	0.0	0.0	1.1	0.7
Syrian Local	(0/30)	(0/30)	(3/30)	(2/30)
Forage legumes				
Lathyrus ochrus	0.0	0.0	0.4	0.2
IFLA 585	(0/50)	(0/50)	(2/50)	(1/50)
L. sativus	0.0	0.0	0.6	0.4
IFLA 513	(0/50)	(0/50)	(3/50)	(2/50)
Vicia narbonensis	0.0	0.4	0.7	1.1
IFVI 2933	(0/30)	(1/30)	(2/30)	(3/30)
V. sativa	0.0	0.0	0.5	0.3
IFVI 594	(0/40)	(0/40)	(2/40)	(1/40)

[a] Rates of infection were estimated by the following formula (Maury et al., 1985):
$P = [1 - (H/N)^{1/n}] \times 100$ (see Materials and methods).
[b] Values between brackets are number of groups where PSbMV was detected over number of groups tested. Each group consisted of ten seedlings.

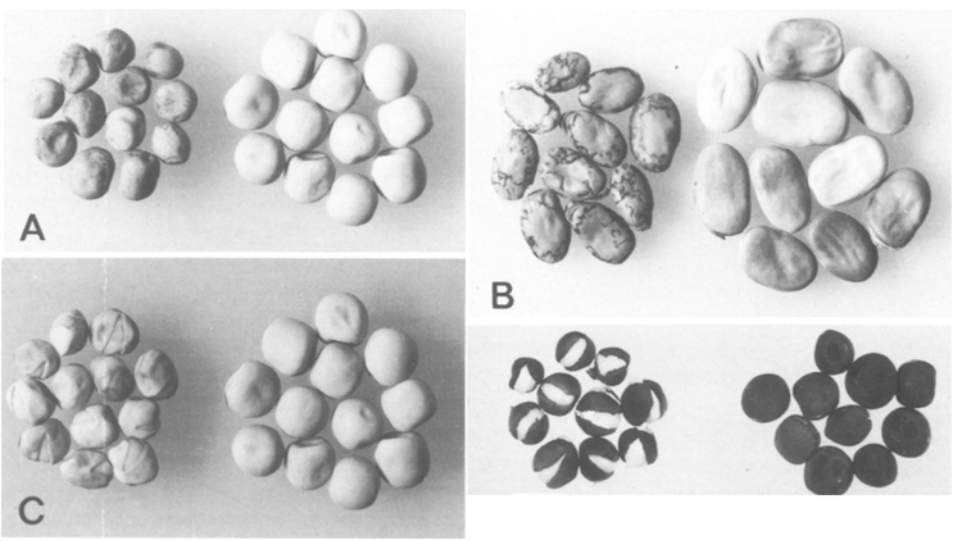

Fig. 1. Seed-coat symptoms in seeds of *Pisum sativum* (A and C), *Vicia faba* (B) and *Vicia narbonensis* (D) from plants infected with pea seed-borne mosaic virus; at right, healthy controls.

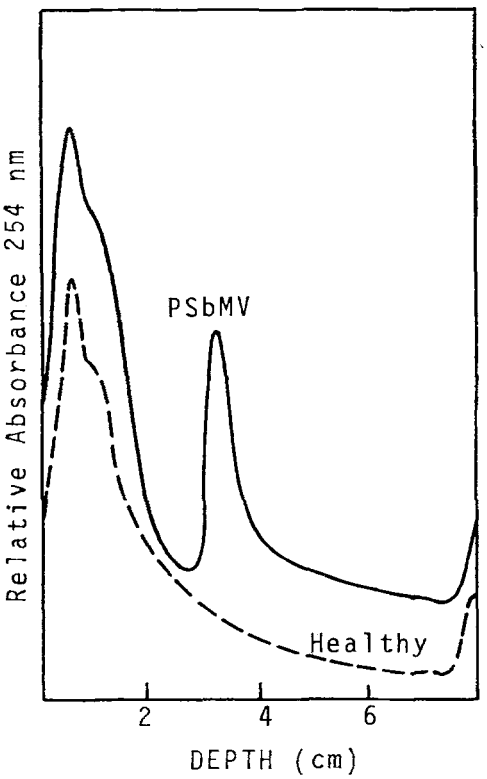

Fig. 2. Rate-zonal sucrose density-gradient centrifugation analysis of purified pea seed-borne mosaic virus. Preparations of infected and healthy faba bean tissue were centrifuged for 2 h at 23 000 rpm in a 10–70% sucrose gradient in a Beckman SW28 rotor. Tubes were scanned through an ISCO density-gradient fractionator with UV monitor.

Virus purification. Faba bean leaves were a good source for PSbMV purification. Inoculated plants of faba bean were tested by ELISA 8, 10, 12, 14, 16, 18, 20 and 22 days after inoculation. The highest virus concentration was detected 18 days after inoculation as measured by ELISA. Thereafter, virus concentration declined. Accordingly, infected tissue for virus purification was harvested 15–20 days after inoculation.

The purification procedure produced one distinct virus band during sucrose density-gradient centrifugation (Fig.2). Presence of PSbMV in the band was confirmed by ELISA, and virus from the band was further purified by high-speed centrifugation and resuspension of virus pellets in 0.01 M borate buffer, pH 8.2. The UV absorbance ratio A260/A280 of purified PSbMV was 1.18. Assuming an extinction coefficient for PSbMV of 2.5 (Hampton and Mink, 1975), the yield of purified virus was calculated to be 10–15 mg/kg of leaves.

Electron microscopy. Faba bean infected with SP9-88 contained filamentous virus particles c. 750 × 11 nm, typical of a potyvirus. The particles were strongly decorated with the PSbMV antiserum (E210).

Serology. Gammaglobulin from the antiserum to SP9-88 produced an ELISA reading of A405 = 0.86 with SP9-88-infected tissue and 0.27 with healthy tissue, whereas gammaglobulin from the Dutch E210 antiserum gave A405 = 0.73 with infected faba bean and 0.15 with healthy tissue.

Discussion

PSbMV was detected in leaf samples of faba bean from eight out of ten countries and in leaf samples of lentil from six out of eight countries surveyed. The number of faba bean samples tested from Algeria, Jordan and Morocco was small, and no lentil samples were tested from Ethiopia, Jordan and Sudan. Further sampling and testing is required for more reliable reporting of the relative occurrence of PSbMV in those countries. The present results show that PSbMV naturally infects faba bean in Egypt, Ethiopia, Lebanon, Libya, Morocco, Sudan, Syria and Tunisia; and lentil in Algeria, Egypt, Morocco, Syria, Tunisia and Turkey. This is the first report of PSbMV infection of faba bean in Ethiopia and Libya, and of lentil in Algeria and Turkey. Within the region the virus had been previously reported on faba bean in Egypt, Lebanon, Sudan, Syria, Tunisia (Makkouk et al., 1988) and Morocco (Fortass and Bos, 1991), and reported earlier on lentil in Syria (Makkouk et al., 1992a).

The host range, transmission characteristics, induced symptoms, particle size, morphology, physical properties, and ELISA serology of the selected PSbMV isolate SP9-88 indicated a close resemblance to the PSbMV described by Musil (1966), Inouye (1967) and Hampton and Mink (1975). Isolate SP9-88 was strongly cross-reactive with the Dutch E210 strain of the virus (Bos, 1970).

In the experimental host-range studies, reactions obtained in many species were similar to those reported earlier (Aapola et al., 1974; Musil and Lešková, 1969; Kvičala, 1969; Inouye, 1967; Musil, 1966; Stevenson and Hagedorn, 1973). An additional 19 leguminous species are now reported as susceptible, and 10 legume and 5 non-legume species as non-susceptible to PSbMV.

Some of our host reactions differed from those reported earlier. *S. oleracea* has been reported to be susceptible to PSbMV infection (Bos, 1970), whereas in this study the cultivars Bloom Long Standing and Syrian Local were found to be immune. PSbMV has been reported to infect *N. clevelandii* (Bos, 1970), *P. vulgaris* (Musil, 1966), *M. sativa* (Musil and Lešková, 1969; Aapola et al., 1974), whereas the cultivars of these species tested in this study were not systemically susceptible to infection. However, the virus was reported by other workers not to infect *N. clevelandii, S. oleracea, P. vulgaris* (Kvičala et al., 1973), and *M. sativa* (Stevenson and Hagedorn, 1973), which is in agreement with the results now obtained with the Syrian isolate (SP9-88).

In this study, 29 wild leguminous species appeared to be susceptible to PSbMV infection. Such wild species may therefore be important natural PSbMV reservoirs especially when the virus is seed-borne, as already found for some species of *Vicia* and *Lathyrus* (Makkouk et al., 1992b).

The five aphid species reported here as vectors of PSbMV had been reported earlier (Musil, 1966; Inouye, 1967; González and Hagedorn, 1971; Aapola and Mink, 1973; Karl and Schmidt, 1976; Thakur et al., 1976). *M. persicae* has been found to be the most efficient vector of PSbMV (González and Hagedorn, 1970), which is in agreement with our results.

The purification procedure yielded fairly pure virus preparations as indicated by a well-separated virus band in sucrose gradients. The A260/A280 ratio of the band was 1.18 which is very close to the value reported for PSbMV (Hampton and Mink, 1975).

Our results show that PSbMV is seed-transmissible in faba bean, lentil, pea, *V. narbonensis, V. sativa, L. ochrus* and *L. sativus*, which is in agreement with earlier reports (Mink et al., 1969; Hampton and Mink, 1975; Hampton and Muehlbauer, 1977; Musil, 1980; Cockbain, 1988; Alconero and Hoch, 1989; Makkouk et al., 1992b). However, PSbMV seed-transmission rates of 0.7, 6.0 and 10.8% reported here for faba bean, lentil

and pea, respectively, were lower than those reported earlier for these crops (Hampton and Muehlbauer, 1977; Musil, 1980; Cockbain, 1988), but this might have been due to the advanced stage of development of our plants at the time of inoculation. In addition, it has been reported earlier that seed transmission of PSbMV in pea was eight times more frequent in seeds showing cracked seed coats (33%) than through normal-looking seeds (4%) (Stevenson and Hagedorn, 1970), whereas we found no appreciable difference in seed-transmission rates between the two seed categories. Accordingly, symptoms on the seed cannot be considered as a reliable parameter of the seed transmission of the virus.

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