Pea seed-borne mosaic virus : a review

Ravinder Kumar KHETARPAL & Yves MAURY

I.N.R.A., Pathologie végétale, Centre de Recherches de Versailles, 78000 Versailles

SUMMARY

Pea seed-borne mosaic virus (PSbMV), an economically significant seed-transmitted virus of pea has been commonly found in pea germplasm collections of many countries. The virus is suspected to have spread world-wide due to the exchange of infected germplasm material. Owing to the secondary spread of the virus in the field by aphid vectors, a low level of seed infection leads to a high proportion of the disease. The symptoms produced on sensitive pea cultivars are often not very characteristic.

Resistance to this virus, known to be governed by a recessive gene 'sbm', is in the process of being incorporated into improved pea varieties. Before accomplishing this genetic programme, testing of seed lots by Enzyme Linked Immunosorbent Assay can greatly contribute in limiting the spread of the virus.

In view of the importance of the disease and of the area of pea under cultivation, the present review has sought to deal at length with diverse aspects of the disease and the virus and also the sources and inheritance of resistance to PSbMV in pea germplasm collections.

Additional key words : *Economic significance, distribution, host range, strains, transmission, physico-chemical properties, detection technique, resistance.*

RÉSUMÉ

Pea seed-borne mosaic virus : revue bibliographique.

La récente augmentation des surfaces réservées à la production de Pois protéagineux et la fréquente contamination des collections de gènes par un virus transmissible par les graines, le Pea seed-borne mosaic virus ont conduit à compiler l'ensemble de l'information disponible à ce jour sur ce virus, d'autant que la difficulté à voir les symptômes et l'efficacité avec laquelle les pucerons transmettent le Pea seed-borne mosaic virus sont deux facteurs très favorables à sa dissémination.

L'influence de ce virus sur le rendement est notable. Un gène de résistance « sbm » est en cours d'incorporation dans des variétés améliorées. Avant l'aboutissement de ce programme génétique, un contrôle des lots de semences par le test ELISA peut limiter efficacement la dissémination du Pea seed-borne mosaic virus dans les cultures de Pois.

Mots clés additionnels : *Importance économique, distribution géographique, gamme d'hôtes, transmission, propriétés physicochimiques, techniques de détection, résistance.*

I. INTRODUCTION

Pea (*Pisum sativum* L.) is grown all over the world. As a source of food it ranks next to cereals in importance. In nature, the crop is vulnerable to a large number of economically important diseases, of which the diseases caused by viruses have gained importance in recent years owing to their systemic nature, their rapidity of dissemination via natural transmission mechanisms and their marked effect on yield both qualitatively and quantitatively.

Pea plants are susceptible to viruses described under more than 50 names among which 35 viruses are specifically defined (HAMPTON, 1984). Among the seed-transmitted viruses of pea (which include pea

seed-borne mosaic virus, pea early browning virus, pea enation mosaic virus and pea seed-borne symptomless virus), so far only two are known to be seed-transmissible at significant frequencies i.e. pea seed-borne mosaic virus (PSbMV, an aphid-transmitted virus) and pea early browning virus (transmissible by stubby root nematode). The transmission of a virus through seed assumes special importance because it is mostly in the form of seeds that the germplasm collections are conserved and exchanged internationally. The increasing area of pea under cultivation and the frequent occurrence of PSbMV in various germplasm collections has led to the compilation of this review which highlights the information available on various aspects of PSbMV in pea.

II. DISCOVERY OF PSbMV AND NOMENCLATURE

PSbMV was initially reported under different names by different workers based on their observations on symptomatology, mode of transmission and particle length. MUSIL (1966) first discovered this virus in Czechoslovakia and proposed the name "Virus des Blattrollens der Erbse" known as "pea leaf rolling virus" (KVICALA & MUSIL, 1967). INOUE (1967) in Japan called it "pea seed-borne mosaic virus". Soon after THOTTAPPILLY & SCHMUTTER (1968) in W. Germany found "Falsche Blattrollvirus der Erbse" (false pea leaf roll virus) and supposed it might be identical with pea seed-borne mosaic virus. In USA the virus was simultaneously detected during the same year by different workers from different places; HAMPTON (1969) from Oregon named it as "pea fizzle top virus", and MINK *et al.* (1969) from Washington called it "a seed-borne virus of pea" whereas STEVENSON & HAGEDORN (1969) from Wisconsin reported it as "a new seed-borne virus of pea". BOS (1970) in Netherlands called it "pea leaf roll mosaic virus" and justifiably suspected it to be same as those described by MUSIL (1966) and INOUE (1967). MUSIL (1970) renamed it to "pea leaf-rolling mosaic virus" to distinguish from the agents of "pea leaf roll" and "broadbean leaf roll". The seed-borne nature of the virus was, however, observed by all the workers.

It was MINK *et al.* (1974) who proposed that the name 'pea seed-borne mosaic virus' (as reported by INOUE in 1967) be adopted for all these viruses which are principally seed-transmitted in pea and have many characteristics in common. They also proved the serological relatedness of the virus in Japan (INOUE, 1967) and USA (HAMPTON, 1969). Since then the name 'pea seed-borne mosaic virus' (PSbMV) has been recognised internationally (HAMPTON & MINK, 1975).

III. GEOGRAPHICAL DISTRIBUTION

HAMPTON & MINK (1975) reported that the virus is possibly distributed world-wide because seeds of many pea cultivars have been exchanged internationally. However, a perusal of the literature reveals that PSbMV is reported from Czechoslovakia (MUSIL, 1966), Japan (INOUE, 1967), West Germany (THOTTAPPILLY & SCHMUTTER, 1968), USA (HAMPTON, 1969; MINK *et al.*, 1969; STEVENSON & HAGEDORN, 1969); Netherlands (BOS, 1970), Canada (ZIMMER & ALI-KHAN, 1976), Yugoslavia (MILICIC & GRBELJA, 1977), GDR (KARL & SCHMIDT, 1978), Poland (KOWALSKA, 1979), Switzerland (PELET, 1980), New Zealand (FRY & YOUNG, 1980), UK (MATTHEWS *et al.*, 1981) and India (THAKUR *et al.*, 1984). LINDSTEN *et al.* (1976) suspected its possible occurrence in Sweden. Later MUNRO (1978) in Australia detected PSbMV in seeds imported from Sweden. HAMPTON & BRAVERMAN (1979) in USA detected PSbMV in two germplasm lines introduced from Peru and MATTHEWS *et al.* (1981) in UK detected it in a line from Sudan, although there is yet no record of occurrence of PSbMV from Peru and Sudan.

According to HAMPTON (1984) PSbMV has probably been disseminated all over the world through seeds, perhaps initially from India to Western Europe in breeding lines and subsequently from Europe to other parts of the world, including Japan and N. America.

IV. ECONOMIC SIGNIFICANCE

PSbMV is a pathogen of potential importance because of its high rate of transmission through seeds. It has posed a threat to the pea seed and processing industry in USA (MINK *et al.*, 1969; STEVENSON & HAGEDORN, 1971; HAMPTON *et al.*, 1976; KRAFT & HAMPTON, 1980) and has resulted in the loss of some breeding lines as well as delayed the advancement of new cultivars in Canada (HAMILTON, 1977).

Pea germplasm lines are particularly prone to PSbMV. The virus has been detected in pea germplasm collections of Canada (ALI-KHAN & ZIMMER, 1979), USA (HAMPTON & BRAVERMAN, 1979), New Zealand (FRY & YOUNG, 1980), UK (MATTHEWS *et al.*, 1981) and of France and India (KHETARPAL & MAURY, unpublished results).

In USA, after the discovery of PSbMV in commercial pea cultivars, the seed companies responded to this threatening disease outbreak by destroying virus-infected seed stocks, and PSbMV was not detected in pea fields or breeding lines between 1969 and 1974 (HAMPTON *et al.*, 1976). However, in 1974 several USDA-ARS breeding lines of canner, freezer and dry edible peas were found infected by PSbMV at Washington and this infection was traced to California where they were grown near imported pea breeding lines that were confirmed as the inoculum source. Seeds of all contaminated lines were destroyed and prompt eradication measures were taken.

Commercial seeds lots of pea containing as high as 90 % infected seed have been reported from USA (MINK *et al.*, 1969; KNESEK & MINK, 1970) and Switzerland (PELET, 1980) and 55 % infected seed in Czechoslovakia (MUSIL, 1970). CHIKO & ZIMMER (1978) found that average yield of pea plants mechanically inoculated with PSbMV were reduced by 11 to 36 % in commercial cultivars due to reduction in seed weight. Similarly MAURY & BOSSENNEC (unpublished) observed a yield reduction of 16 % per infected plant and 28 % reduction in 1 000 grain weight in the case of a commercial cultivar. The late maturing, more determinate cultivars (Mars, Cornway and Corfu) were more severely affected than early maturing indeterminate cultivars (Small Sieve Alaska and A-45) (KRAFT & HAMPTON, 1980).

MINK *et al.* (1974) reported the annual occurrence of PSbMV in epiphytotic proportions in a pea-growing region near Gobo, in Japan. The natural incidence of the disease has been also recently recorded in two commercial cultivars from northern India (THAKUR *et al.*, 1984). Above all PSbMV is one of the economically most important aphid-borne virus on broadbean in Japan (TACHIBANA, 1981) and in USA the lentil crop of the Pacific Northwest was found to be vulnerable to PSbMV (HAMPTON & MUEHLBAUER, 1977).

V. SYMPTOMATOLOGY

PSbMV is reported to produce rather mild, transitory symptoms in pea which are quite non-typical for virus symptoms and this is often a limiting factor for detecting the disease in the field. However, under ideal conditions characteristic symptoms of PSbMV are apparent both in field and glasshouse. The different symptoms on sensitive *Pisum* cultivars as reported by various workers (MUSIL, 1966 ; INOUE, 1967 ; HAMPTON, 1969 ; MINK *et al.*, 1969 ; STEVENSON & HAGEDORN, 1969 ; HAMPTON & BAGGET, 1970 ; BOS, 1970 ; HAGEDORN, 1974 ; HAMPTON & MINK, 1975 ; ZIMMER & ALI-KHAN, 1976 ; KRAFT & HAMPTON, 1980 ; PELET, 1980 ; HAMPTON, 1984) are as follows :

- slight chlorosis and various degree of stunting of the plants,
- shortening and downward rolling of the leaflets,
- transient clearing and swelling of the veins,
- tendril curling and rosetting (apical malformation),
- mosaic often not conspicuous,
- symptoms, as above, conspicuous within 5 to 10 days of seedling emergence,
- production of distorted flowers by infected plants that often give rise to small distorted pods having seeds with split seed coats (ovule development being uneven or incomplete),
- failure to set pods by some cultivars,
- masking of infected and malformed plants by taller, normal plants in the field,
- fading of leaf symptoms when plants approach bloom stage,
- slight stunting and leaf-shortening of field-grown plants by late bloom stage whereas greenhouse-grown plants usually reveal noticeable downward rolling of the lateral leaf margins, tendril curling and vein clearing.

The symptoms of the disease vary among cultivars. MINK *et al.* (1969) and HAMPTON & BAGGET (1970) reported that symptoms on early cultivars are mild, as compared to those observed on midseason 'Perfection' types where rosette symptoms are more intense. STEVENSON & RAND (1970) reported that the progression of symptoms on susceptible pea cultivars was accelerated at higher temperature but the final severity of the symptoms was not significantly affected by temperature. They observed that vein clearing, the most striking early symptom, reached maximum severity 3 days after inoculation at 28 °C, 4 days at 24 °C and 5 days at 20 and 16 °C. The apical malformation was evident at all temperatures within 4 weeks after inoculation, but was apparent first at higher temperature.

Beside this, symptomless infections are also common. HAMPTON (1972) reported that the virus may be present without inducing symptoms in 5 to 10 % of the plants from infected seed lots. Symptomless infection has been observed in breeding lines in USA (HAMPTON & BRAVERMAN, 1979, HAMPTON *et al.*, 1981) and also in plants raised in quarantine from pea seeds imported from Sweden into Australia (MUNRO, 1978). Similarly among 208 ELISA-positive plants, 54 % escaped detection on the basis of symptomatology (MAURY *et al.*, 1987).

Pea seeds were found to have a necrotic line pattern

on the seed coat similar to that described for broadbean seed infected with broadbean stain virus (DEVERGNE & COUSIN, 1966). Testas of such seeds from most of the pea lines were found to be ELISA-positive for PSbMV indicating that such seeds had originated from infected plants (MAURY *et al.*, unpublished results).

Seeds also showing crack of the seed coat were found to transmit PSbMV more frequently (33 %) than normal-looking seeds (4 %) (STEVENSON & HAGEDORN, 1970). However, the symptoms on the seed cannot be considered as a reliable index for the seed transmission of the virus.

VI. HOST RANGE

The cultivars of *Pisum sativum* are the natural hosts of PSbMV. The virus has been also isolated from broad bean (*Vicia faba* L.) grown under natural conditions in Denmark (LUNDSGAARD, 1981) and Japan (TACHIBANA, 1981). Under experimental field conditions PSbMV was found to overwinter effectively in hairy vetch (*Vicia villosa* Roth.) (STEVENSON & HAGEDORN, 1973a).

AAPOLA *et al.* (1974) found that the virus can infect 47 species of plant belonging to 12 dicotyledonous families and most of the non-leguminous hosts are infected without producing symptoms. According to the available literature a total of 51 plant species (table 1) can be infected by PSbMV either by mechanical inoculation or by aphids or by both procedures. In table 1 the reason for certain plant species falling in more than one symptom type can be attributed to the difference in the mode of inoculation adopted by different workers (INOUE, 1967 ; MUSIL, 1970 ; AAPOLA *et al.*, 1974) and also to variation in strains of the virus.

Among the various plant species that can be infected by PSbMV, the "diagnostic species" widely utilized are Perfection type pea cultivars & *Vicia faba* var. *minor* (systemic hosts), *Chenopodium amaranticolor* Corte et Reyn. (necrotic local lesion host) and *C. quinoa* Wild. (chlorotic local lesion host) (HAMPTON & MINK, 1975).

VII. STRAINS

The differences in particle length measurement, reported to be 700 nm in Czechoslovakia (MUSIL, 1970), frequently shorter than 700 nm in USA (HAMPTON, 1969 ; STEVENSON & HAGEDORN, 1969 ; HAMPTON *et al.*, 1974) and 750 nm in Japan (INOUE, 1967) and so also the differences in ultrastructural cytology (HAMPTON *et al.*, 1973), initially suggested the existence of strains in PSbMV. However, discrepancies in reported particle lengths for PSbMV were partially resolved by HAMPTON *et al.* (1974) who found that particle lengths of PSbMV were significantly shorter in leaf-dip and in partially purified purifications when fixed with formalin than those derived from either preparation when fixed with glutaraldehyde for electron microscopy. MILIČIĆ & PLAŠIĆ (1978) in Yugoslavia found a PSbMV isolate named as "pea latent strain"

TABLE 1

Plant species found to be susceptible to pea seed-borne mosaic virus.*
Espèces sensibles au pea seed-borne mosaic virus.

Symptoms produced on the host by PSbMV	Names of the host species
1. <i>Systemic</i> (leaf roll, chlorotic mottle, stunting, vein clearing and vein chlorosis)	<i>Chenopodium botrys</i> , <i>C. foetidum</i> , <i>C. urbicum</i> , <i>Cicer arietinum</i> , <i>Lathyrus odoratus</i> , <i>L. ciceria</i> , <i>Lens culinaris</i> , <i>Vicia angustifolia</i> , <i>V. articulata</i> , <i>V. benghalensis</i> , <i>V. cornigea</i> , <i>V. dasycarpa</i> , <i>V. ervilia</i> , <i>V. faba</i> , <i>V. globosa</i> , <i>V. hirsuta</i> , <i>V. hybrida</i> , <i>V. lutea</i> , <i>V. macro-</i> <i>carpa</i> , <i>V. monantha</i> , <i>V. narbonensis</i> , <i>V. onobrychoides</i> , <i>V. pannonica</i> , <i>V. persica</i> , <i>V. peregrina</i> , <i>V. sativa</i> , <i>V. villosa</i> , <i>Portulaca grandiflora</i> , <i>Astragalus</i> sp., <i>Proboscidea</i> <i>jussieui</i> .
2. <i>Local lesion</i> (chlorotic or necrotic)	<i>Tetragonia expansa</i> , <i>Chenopodium album</i> , <i>C. quinoa</i> , <i>C. capitatum</i> , <i>C. murale</i> , <i>C. amaran-</i> <i>ticolor</i> , <i>C. urbicum</i> .
3. <i>Symptomless</i> (plants infected but without apparent symptoms)	<i>Tetragonia expansa</i> , <i>Vinca rosea</i> , <i>Gomphrena globosa</i> , <i>Chenopodium capitatum</i> , <i>Senecio</i> <i>vulgaris</i> , <i>Zinnia elegans</i> , <i>Brassica pekinensis</i> , <i>Capsella bursa-pastoris</i> , <i>Cucurbita maxima</i> , <i>Medicago lupulina</i> , <i>M. sativa</i> , <i>Aquilegia</i> sp., <i>Humulus lupulus</i> .

* After INOUE (1967) ; BOS (1970) ; HAMPTON & BAGGET (1970) ; MUSIL (1970) ; PROVVIDENTI & SCHROEDER (1972) ; AAPOLA *et al.* (1974) ; ZIMMER & ALI-KHAN (1976) ; ALCONERO *et al.* (1986).

which often caused transient vein clearing but no leaf rolling or stunting, and had shorter stability *in vitro*.

The first detailed evidence of existence of strains in PSbMV was demonstrated by HAMPTON *et al.* (1981). They compared seven virus isolates presumed to be PSbMV, namely, virus isolate PsS₂ from Czechoslovakia (MUSIL, 1970), isolate WA-1 from Washington (KNESEK *et al.*, 1974), isolate WI-1 from Washington (STEVENSON & HAGEDORN, 1969), isolate P.202 from Japan (INOUE, 1967), a deviant type E.224 of isolate E.210 from Netherlands (BOS, 1970) and isolate C-4-24 from Oregon (HAMPTON *et al.*, 1974). They found that all 7 isolates were characterized by 750-780 nm particle length and were closely related serologically. They evaluated the suitability of 19 *Pisum sativum* lines as PSbMV "strain differentials" and simultaneously examined the magnitude of variation among several isolates. They observed that the responses in all the 19 *P. sativum* differentials inoculated with the 7 isolates ranged from rapid development of whole-plant necrosis to immunity. They concluded that :

- the USA isolates varied about as much as those from Japan, Czechoslovakia and Netherlands ;
- isolate E.210 and E.224 were the least typical by the criterion of symptoms induced in plant hosts but were serologically indistinguishable from other isolates ;
- isolate E.210 failed to infect pea cultivar 447, which was found to be susceptible to all other isolates. This isolate also induced systemic infection in *Chenopodium quinoa* (BOS, 1970), a characteristic not found in other PSbMV isolates ;
- isolate E.224 induced unusually mild symptoms in all susceptible *P. sativum* differentials, including those that had died rapidly when inoculated with all other isolates ;
- lines P.I. 140297 and P.I. 263033 might indeed be useful in distinguishing PSbMV isolates or strains as these lines exhibited remarkable difference in type of symptoms expressed by different isolates ;

They also inferred that the 'pea latent strain' of PSbMV reported in Yugoslavia by MILIČIĆ & PLAVSIC (1978), presumably represents a significant departure from characteristics previously described for other PSbMV isolates.

Besides, HAMPTON (1982) detected a lentil strain of PSbMV (designated as PSbMV-L) in 38 of the 580 accessions of lentil tested. He observed that PSbMV-L was distinguishable from the standard USA strain of PSbMV by non-pathogenicity to most pea cultivars ; also in lentil collections, the sources of immunity to PSbMV (pea) were found to be independent from that of PSbMV-L. The inoculum reservoir for the latter was apparently restricted to lentil seed.

Recently three more pathotypes of PSbMV (namely P-1 and P-4 from pea, and L-1 from lentil) have been characterized on the basis of their infection of pea genotypes (ALCONERO *et al.*, 1986). Interestingly the pathotype L-1 isolated from lentil was much more severe on pea cultivars.

VIII. TRANSMISSION AND SPREAD

Seeds of pea infected by PSbMV are considered to be the initial source of inoculum reservoir. Once the virus is transmitted through the seed to the germinating seedling it is spread to nearby healthy peas in the field by aphid vectors.

A. Transmission through seeds

1. Rate of seed transmission

The transmission of PSbMV through seeds was first demonstrated by MUSIL (1966) in Czechoslovakia. INOUE (1967) reported that in Japan the rate of seed transmission was about 30 % in cv. Oranda, 10 % in Sanjunichi-Kunusaya and Futsukoku-Osaya, and over 20 % and 10 % in New Season and Perfected Wales,

respectively. In Wisconsin, the virus was found to be transmitted through seeds harvested from infected pea plants to at least 10 % of the developing seedlings (STEVENSON & HAGEDORN, 1969). Seed transmission rate of as high as 100 % have been found in some pea introductions to USA (HAMPTON, personal communication).

PSbMV was also found to be seed-transmitted in lentils at frequencies of 32-44 % (HAMPTON & MUEHLBAUER, 1977) and through a low percentage of seeds of *Vicia articulata*, *V. narbonensis* and *V. pannonica* (HAMPTON & MINK, 1975).

2. Possible mode of seed transmission of PSbMV

STEVENSON & HAGEDORN (1973b) found that all parts of the inflorescence from infected plants, including carpels, filaments, petals, pollen and sepals contained virus. It was transmitted to seed both by the female parent and by pollen. However, for a seed transmission rate of 6 %, pollen was found to contribute less than 1 %.

3. Factors affecting seed transmission

With respect to growth conditions, MINK *et al.* (1969) who observed seed transmission of 16-25 % in certain seed lots, found that when certain of these lots were grown at 29 °C and 14 000 lux approx., the seed transmission rate increased to 65-90 %.

The studies of STEVENSON & HAGEDORN (1973b) revealed a difference in rate of seed transmission of PSbMV among cultivars. High rates of seed transmission were found to be generally associated with early maturing cultivars (Alaska and Alsweet types). Besides, the time of inoculation had no significant effect on rate of transmission in the late maturing cultivar Dark Skin Perfection.

With respect to seed characteristics, STEVENSON & HAGEDORN (1973b) observed that seed transmission of PSbMV was confined to small-sized seed (100 %) in pea cv. Star and to growth-cracked seed coats (31 %) in cv. Cascade. However, MAURY & BOSSENEC (unpublished results) while analysing the seeds from infected plants of cv. Amino found that seeds of all sizes could transmit PSbMV, though the rate of transmission was significantly higher for smaller seeds.

B. Transmission by Aphid vectors

1. Aphid species that transmit PSbMV

Locally, PSbMV is principally spread by aphids which transmit the virus from seed-infected plants to adjoining healthy peas and other hosts such as *Vicia* spp. So far 21 aphid species have been reported to be capable of transmitting the virus in different countries as given in table 2.

2. Mode of Transmission by Aphids

The aphid vectors of PSbMV were first reported to transmit the virus in a typical non-persistent manner.

In USA colonies of *Macrosiphum euphorbiae*, the potato aphid, tended to be more efficient vectors than *Myzus persicae*, the green peach aphid, which in turn was more efficient than those of *Acyrtosiphon pisum*, the pea aphid, but there were some variation between colonies of each species. Alatae were generally more efficient vectors than Apterae (GONZALEZ & HAGEDORN, 1971). It was reported by GONZALEZ & HAGEDORN (1970) that aphids acquired and inoculated PSbMV in 10-90 s feeding periods and no latent period was required. MINK *et al.* (1969) observed 1-3 h of acquisition period by the green peach aphid, *M. persicae*. CHIKO & ZIMMER (1978) reported that *M. persicae* transmitted the virus from healthy to infected plants following acquisition feeds of 1-2 mn, 10 mn, 3 h or 2 days in Manitoba, transmission being most efficient following acquisition feeding periods of 10 mn and 3 h. The wide differences in mode of aphid transmission in PSbMV as observed by different workers indicated that perhaps there was no single mode of transmission involved.

LIM & HAGEDORN (1974) first indicated that PSbMV is transmitted by the New London biotype of potato aphid, *Macrosiphum euphorbiae* in a bimodal manner. [The term 'bimodal transmission' as coined by CHALFANT and CHAPMAN (1962) refers to aphid transmission of viruses after both short and long acquisition feeds.] However, similar studies carried on by GONZALEZ & HAGEDORN (1971) with the Madison biotype of the same aphid species had earlier not given any indication of bimodal transmission. This might imply that the manner of transmission does not depend upon the properties of the virus or the vector alone, but is a function of the virus-vector interaction.

LIM *et al.* (1977) showed the evidence of attachment of PSbMV on the stylet of the aphid *Macrosiphum euphorbiae* by scanning electron microscopy. They found that PSbMV was localized by an indirect immunospecific latex label on stylet of its vectors, the New London and Madison biotypes of *M. euphorbiae*. On the efficient New London biotype, large quantities of label were observed mostly on the inner surfaces of the mandibles at mandibular ridges, maxillary projections, food duct, common food chamber or extraneous materials carried externally on the stylets. The transmissible nature of the virus labelled at 5 mn acquisition was supported by the decreased labelling after 2 different inoculation feedings of 5 mn and 1 h each. The sparse label observed on the Madison biotype aphids indicated that this vector was inefficient because insufficient virus was acquired for effective transmission. LIM & HAGEDORN (1977) concluded that the reason why the Madison biotype of potato aphid could not transmit bimodally like the New London biotype may be due to the qualitative difference in stylet surface.

IX. PURIFICATION

Attempts to purify PSbMV were first made by INOUE (1967) who tested some organic solvents for the clarification of extract (carbon tetrachloride-ether combination, butanol and chloroform) and observed that all these solvents except chloroform destroyed the infectivity of PSbMV-infected extracts. STEVENSON &

TABLE 2

Aphid vectors known to transmit PSbMV on pea.
Pucerons vecteurs capables de transmettre le PSbMV au Pois.

Country	Aphids transmitting PSbMV	Reference
Japan	<i>Aphis craccivora</i> Koch * <i>Myzus persicae</i> Sulz. <i>Rhopalosiphum padi</i> L. <i>Macrosiphum</i> spp.	INOUE (1967)
Czechoslovakia	<i>Myzus persicae</i> Sulz. * <i>Acyrtosiphon pisum</i> Harr. <i>Aphis fabae</i> Scop. <i>A. craccivora</i> Koch <i>Cryptomyzus ribis</i> L. <i>Macrosiphum rosae</i> L.	KVIČALA & MUSIL (1967) ; KVIČALA (1969) ; MUSIL (1970)
United States		
Wisconsin	<i>Acyrtosiphon pisum</i> Harr. <i>Myzus persicae</i> Sulz <i>Macrosiphum euphorbiae</i> Thom.	GONZALEZ & HAGEDORN (1971)
Washington	<i>Acyrtosiphon pisum</i> Harr. <i>Dactynotus escalantii</i> <i>Macrosiphum euphorbiae</i> Thom. <i>M. rosae</i> L. <i>Myzus persicae</i> Sulz. <i>Ovatus crataegarius</i> <i>Rhopalosiphum padi</i> L.	AAPOLA & MINK (1973)
Canada	<i>Myzus persicae</i> Sulz.	CHIKO & ZIMMER (1978)
GDR	<i>Acyrtosiphon pelargonii rogersii</i> Theob. <i>A. pisum</i> Harr. <i>Aphis craccivora</i> Koch <i>A. fabae</i> Scop. <i>A. frangulae beccabungae</i> Koch <i>A. frangulae gossypii</i> Glov. <i>A. nasturtii</i> Kalt. <i>Aulacorthum circumflexum</i> Bekt. <i>Brevicoryne brassicae</i> L. <i>Macrosiphum avenae</i> F. <i>M. euphorbiae</i> Thom. <i>Metopolophium dirhodum</i> Walk <i>Myzus persicae</i> Sulz. <i>Phorodon cannabidis</i> Pass. <i>Rhopalosiphum padi</i> L. <i>Semiaphis dauci</i> F.	KARL & SCHMIDT (1978)
Switzerland	<i>Acyrtosiphon pisum</i> Harr.	PELET (1980)
India	<i>Acyrtosiphon pisum</i> Harr. <i>Myzus persicae</i> Sulz. <i>Aphis craccivora</i> Koch	THAKUR <i>et al.</i> (1984)

* Known to transmit PSbMV on lentil too (HAMPTON, 1982 ; HAMPTON & MUEHLBAEUR, 1974).
 (Connu pour la transmission du PSbMV à la lentille.)

HAGEDORN (1973c) used a high molarity buffer, chloroform and low speed centrifugation for initial clarification of pea extracts prior to Polyethylene Glycol (M.W. 6 000) precipitation followed by two cycles of differential ultracentrifugation for the concentration of particles. This procedure yielded a partially purified preparation.

KNESEK *et al.* (1974) investigated several procedures for purification and found that best results were obtained when infected tissues were ground (root tissue in 0.01 M sodium diethyldithiocarbamate (NaDIECA) + 0.01 M cysteine or leaf tissue in NaDIECA + cysteine containing 0.01 M ethylenediamine tetraacetic acid (EDTA)) and clarified with one-half volume of chloroform. The clarified suspensions were concentrated by one cycle of differential ultracentrifugation, and further purified by centrifugation on columns containing 30 % sucrose, 4 % PEG (M.W. 6 000) and 0.12 M

NaCl. The virus thus precipitated was suspended in 2 % sucrose containing 0.1 % Igepon T-73 (sodium N-methyl-N-oleoyl taurate) at pH 7. This procedure yielded 1 to 1.5 mg virus from 18 g fresh tissue.

HAMILTON & NICHOLS (1978) also obtained a substantial yield of virus by a modification of the method of HUTTINGA (1973). They homogenized the infected leaves in an ice-cold mixture of 0.1 M tris-HCl pH9 containing 0.2 % 2-mercaptoethanol (150 ml), carbon tetrachloride (40 ml) and chloroform (40 ml) in a Waring blender and centrifuged the resulting slurry at 10 000 g for 20 mn. The supernatant thus obtained was centrifuged at 26 500 g for 1.5 h and the resulting pellet containing virus was resuspended in 0.1 M tris-HCl pH 8 to make a 10-fold concentration. After clarification by centrifugation at 10 000 g, the supernatant was made 0.1 % with Igepon T-73 and centrifuged for 5 h at 25 000 rpm through a sucrose cushion (5 ml virus

suspension floated on 6.0 ml of 0.1 M tris-HCl + 0.1 % Igepon T-73, pH 8, containing 45 mg sucrose/ml). The pellet was resuspended in 0.1 M tris HCl, pH 8 to which was added a few crystals of chlorobutanol to prevent microbial growth. Virus yields were approximately 5 mg/100 g tissue.

Recently the cytoplasmic inclusion protein (CIP) induced by two isolates of PSbMV has also been purified (ALCONERO *et al.*, 1986).

X. PHYSICO-CHEMICAL PROPERTIES

On the basis of the properties outlined below, PSbMV belongs to the potyvirus group.

A. Particle shape and size

The average size of the particle is 770×12 nm (HAMPTON & MINK, 1975). Discrepancies in particle length observed by different workers (as discussed earlier under 'strains') have been attributed to the difference in methods adopted for electron microscopy (HAMPTON *et al.*, 1974 ; MINK *et al.*, 1974).

B. Particle composition

The RNA is 5.3 ± 1 % of the particle weight with a base ratio of adenine 44.0 %, guanine 22.8 %, cytosine 17.6 %, and uracil 15.6 % (KNESEK *et al.*, 1974). The protein coat subunit has a molecular weight of 34 000 (HUTTINGA, 1975). The relative amino acid molar ratio as worked out by KNESEK *et al.* (1974) is alanine (3.3), arginine (2.5), aspartic acid (4.7), glutamic acid (4.9), glycine (2.7), histidine (1.0), isoleucine (1.8), leucine (2.2), lysine (1.5), methionine (1.9), phenylalanine (1.1), proline (1.4), serine (1.2), threonine (2.0), tyrosine (1.2) and valine (2.4). The method used for amino acid analysis resulted in the destruction of cysteine and tryptophan. The concentration of acidic amino acids (aspartic and glutamic acid) was found to be twice that of basic amino acids.

1. Sedimentation co-efficient

The virus preparations usually contain one sedimenting component with a sedimentation coefficient of 145 ± 1 S (KNESEK *et al.*, 1974) or 154 S (HUTTINGA, 1975).

2. Buoyant density

Buoyant density as determined in caesium chloride at 20 °C was found to be 1.329 g/cm^3 (HUTTINGA, 1975). It was also observed that there is no correlation between buoyant density and S value on one hand pathogenicity on the other hand.

3. Stability in sap

The dilution end point was observed to be 10^{-3} to 10^{-5} and the thermal inactivation point at 10 mn exposure was 55 to 60 °C (INOUE, 1967 ; MUSIL, 1970). THAKUR *et al.* (1984) found that virus could tolerate a

dilution between a narrow range of 10^{-2} to 10^{-3} and infectivity of sap is also abolished when maintained at 60 °C for 10 mn. The longevity *in vitro* in pea sap was observed to be 4-8 days at 20 °C by INOUE (1967), 2 days at 20-22 °C by MUSIL (1970) and 3 days at 12-18 °C by THAKUR *et al.* (1984). KNESEK *et al.* (1974) observed that freshly prepared juice from leaf or root was highly infectious and extracts of roots were found to be infective for more than 96 h, whereas undiluted leaf extracts became non-infective within 24 h at room temperature and also the infectivity was lost on freezing.

4. Production of inclusion bodies in cells and tissues

INOUE (1971) observed that PSbMV-infected pea leaves reveal inclusions of the shape of pinwheels, rings, circles, tubes or bundles in the cytoplasm, especially in the swollen parts. HAMPTON *et al.* (1973) reported that less common isolates induce tonoplast aggregates or dense bodies and laminated aggregates and one isolate characterized by induction of tonoplast aggregates also induced formation of convoluted endoplasmic reticulum. Also they observed that root parenchyma cells contained few inclusions, but extensive masses of virus-like particles. MILIČIĆ & PLAVSIC (1978) observed deposits of crystalline protein built from relatively large particles in the cytoplasm of diseased cells.

XI. DEVELOPMENT OF METHODS FOR DETECTING PSbMV

A. Assay on indicator hosts

The plant species which have been routinely employed as 'indicator hosts' of PSbMV are : Perfection type peas and *Vicia faba* var. *minor* (on which diagnostic systemic symptoms of PSbMV are produced on inoculation) and *Chenopodium amaranticolor* (necrotic local lesion host) and *C. quinoa* (chlorotic local lesion host) (INOUE, 1967 ; MUSIL, 1970 ; STEVENSON & HAGEDORN, 1970 ; HAMPTON & BAGGET, 1970 ; STEVENSON & HAGEDORN, 1971 ; BAGGET & HAMPTON, 1972 ; STEVENSON & HAGEDORN, 1973b ; AAPOLA *et al.*, 1974 ; KNESEK *et al.*, 1974 ; ZIMMER & ALI-KHAN, 1976 ; HAMPTON *et al.*, 1976 ; MINK & PARSONS, 1978 ; MUNRO, 1978 ; HAMILTON & NICHOLS, 1978 ; ALI-KHAN & ZIMMER, 1979 ; HAMPTON & BRAVERMAN, 1979 ; FRY & YOUNG, 1980 ; KRAFT & HAMPTON, 1980 ; MATTHEWS *et al.*, 1981 ; PELET, 1980 ; HAMPTON *et al.*, 1981).

MINK & PARSONS (1978) developed a procedure for detecting PSbMV in pea seeds by direct-seed assay, i.e. by indexing pea seed on *C. amaranticolor* and pea cultivar 447. They demonstrated that when the incidence of infected seed is consistently 0.3 % or greater, the direct indexing of 300 seeds is adequate to detect most infected seed lots.

B. Detection by serological techniques

Antisera against PSbMV were first obtained by MUSIL (1970) and by STEVENSON & HAGEDORN

(1973*d*). The latter group also obtained an antiserum to the capsid subunit and developed a fast procedure for the absorption of antibodies to host contaminants. Owing to the inability of the elongated particles of PSbMV to move in agar gels, even after several modifications of the procedure, the microprecipitation test was then used for large-scale screening of infected plants. It was also utilized to reveal the serological relatedness between isolates from USA and from Japan (MINK *et al.*, 1974) and 7 isolates from different countries (HAMPTON *et al.*, 1981).

The gel diffusion technique, however, was shown to be effective when a detergent (sodium dodecyl sulphate) was added to the agar gel (HAMILTON, 1977). This procedure could be optimized by selecting the source of agar and changing the concentration of sodium dodecyl sulphate and of sodium azide (ZIMMER, 1979). In UK, MATTHEWS *et al.* (1981) exploited this test to screen and eradicate PSbMV from germplasm collections. However, HAMILTON & NICHOLS (1978) did not find this method sensitive enough to screen reliably large population of plants by group testing. Using the enzyme-linked immunosorbent assay (ELISA) and immunosorbent electron microscopy (ISEM) techniques, they found that by group testing, plant populations containing 5 % infected plants could be detected readily by both methods. In case of latent infections of PSbMV it was reported that detection of the virus in leaves by ELISA was more effective on single-plant samples than on bulked samples of four to five plants, and from older plants rather than young seedlings (ALCONERO *et al.*, 1985). It has been also reported that antisera to the cytoplasmic inclusion proteins induced by the isolates of PSbMV reacted more strongly to the antigens in sap from infected plants than antisera prepared from the purified virus in indirect ELISA (ALCONERO *et al.*, 1986).

With homogenates of seeds, HAMILTON & NICHOLS (1978) could detect PSbMV in seed lots containing 1 to 5 % infected seed using ISEM whereas negative results were obtained with ELISA at this level. Adopting ELISA, MAURY *et al.* (1987) observed (1) a good correlation between seed transmission and ELISA-positive embryos; (2) different levels of embryo infection, the least infected embryo being detected even when mixed with 60 or 100 healthy embryos depending on the pea cultivar; (3) a large overestimation of the percentage of transmission of seed lots when groups of whole seeds were tested, due to the extraction of non-seed-transmissible virus from the testas. VAN BALEN & FRANKEN (1985) suggested supplementing ELISA by ISEM when a definitive proof of the presence of PSbMV in seed extracts is necessary.

XII. RESISTANCE TO PSbMV

A. Sources of resistance

Soon after the discovery of PSbMV and the confirmation of its easy transmission through seeds, a number of germplasm lines/cultivars were tested. The screening for resistance/immunity was made by considering both percentage of plants diseased and severity of symptoms after mechanical inoculation or by testing

on indicator hosts. Two lines out of 671 (STEVENSON & HAGEDORN, 1971); 4 out of 1 326 (BAGGET & HAMPTON, 1972); 3 out of 510 (MUSIL *et al.*, 1981) and 16 out of 1 835 (HAMPTON & BRAVERMAN, 1979) were found to be resistant. Moreover, HAMPTON (1980*a*) found, in 160 lines, heterogeneity in resistance to PSbMV that extended the possibility of choosing also the sources of resistance in a range of lines showing different horticultural characteristics.

In USA two PSbMV-resistant breeding lines, OSU B442-15 and B445-66 (BAGGET & HAMPTON, 1977), and two PSbMV-immune canner breeding lines, VR74-410-2 and VR74-1492-1 (KRAFT & GILES, 1978), all of which were unique in also having resistance to other important pea diseases and possessing desirable horticultural characteristics, were released to the breeders.

Recently, ALCONERO *et al.* (1986) found that a few of the plant introductions of pea that were earlier reported to be immune to PSbMV were infected by one or all three of their isolates. Also they reported seven lines of pea resistant to all their 3 isolates. They observed that resistance in peas was isolate-specific. Resistance to their PSbMV isolate from lentil (L-1) was found to be associated with bean yellow mosaic virus resistance and also with a delayed reaction to one of the PSbMV isolates (P-4) from pea.

B. Inheritance of resistance

STEVENSON & HAGEDORN (1971) reported that resistance to PSbMV was recessive as they found that F_1 progenies of reciprocal crosses between resistant P.I. accessions (P.I. 193 586 & 193 835) and susceptible pea cultivars were all susceptible. They also reported that the same gene for resistance was carried by both the accessions as the F_1 hybrids between the two P.I.s were resistant.

HAGEDORN & GRITTON (1973) reported that resistance to PSbMV is conditioned by a single recessive gene and is dependent upon the homozygous condition of that single recessive gene pair. They proposed that the recessive factor for resistance to PSbMV be designated *sbm*.

The gene *sbm* was later found to be linked with gene *wlo* on chromosome 6 (GRITTON & HAGEDORN, 1975; HAMPTON & MARX, 1981); *wlo*, in the homozygous state causes the upper surfaces of the leaflets to lack wax, while other plant parts are waxy. Also *sbm* was found to be located on the *p* side of *wlo* in the *Pisum* linkage group VI (gene *p* affects the type and amount of pod membrane).

HAMPTON & MARX (1981) emphasized that the breeders who take advantage of the linkage *wlo-p-sbm* for developing resistant cultivars should take the necessary precaution of testing their material for immunity at crucial steps. Infact, HAMPTON *et al.* (1981) observed a wide range of symptoms (ranging from whole-plant necrosis on one hand to very slight leaf rolling/or vein clearing or latent infection on the other) on pea cultivars acting as 'strain differentials'. Since resistance to PSbMV is conferred by a single recessive gene pair, they presumed that whole plant necrosis might be caused by modifier-genes of a unique germplasm that enhances host sensitivity to PSbMV, whereas tendencies towards latent infection or infection with mild symptoms might be caused by modifier-genes

that reduced host sensitivity. HAMPTON (1980b) pointed out that due to such a modifier-gene system in peas, it is likely that breeders in their efforts to develop PSbMV-immune cultivars, might mistake tolerance or resistance to PSbMV for immunity. This would be due to underestimation of the extent to which gene *sbm* can be modified. He also emphasized that immunity would be the only acceptable breeding objective against

PSbMV and this would warrant precise virus-detection methodology.

In case of lentils also, HADDAD *et al.* (1978) found four P.I. lines immune to PSbMV and showed that resistance in that case too was conditioned by a single recessive gene designated as 'sbv'.

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