Acta Bot. Croat. 37 (1978) 9—19

CONTRIBUTION TO THE KNOWLEDGE OF PEA SEED-BORNE MOSAIC VIRUS

DAVOR MILIČIĆ and BILJANA PLAVŠIĆ

(Institute of Botany, Faculty of Science, University of Zagreb, and Institute of Biology, University of Sarajevo)

Received February 6, 1978

Introduction

Pea seed-borne mosaic virus (PSMV) was first described by Musil (1966) in Slovakia under the name of pea leaf roll virus. Since the latter name was earlier used for another pea virus (syn. pea top yellow virus), Bos (1970) and Hagedorn (1974) suggested the name pea seed-borne mosaic virus to be employed for Musils virus. In 1967 PSbMV was investigated by Inouye in Japan who ascertained that the virus had the form of flexible filaments and was able to produce pin-wheel structures that are characteristic of the group of potyviruses. It has been also found that PSbMV is wide spread in various parts of the USA (Stevenson and Hagedorn 1969), in the Netherlands (Bos 1970) and some other countries.

The ability of PSbMV to be transmitted by seeds is its most important characteristic. Stevenson and Hagedorn (1973) established that the small seeds and seeds with growth cracks in their coats were infected more frequently with PSbMV than the seeds of normal size and with intact coat. In infected seeds PSbMV is found in the cotyledons and the embryo; in the coat the virus is present only in the immature seeds but is absent from the mature ones.

The main symptoms of the disease in pea and broad bean are downward rolling of leaflets and transient colour change on young leaflets. The second symptom mostly consists of vein clearing in pea and of mottle in broad bean. These symptoms have been described by Musil (1966, 1970), Hampton and Mink (1975) and others. The host range of PSbMV was studied especially by Aapola et al. (1974) who established the interesting fact that the virus could be transmitted in some species only mechanically but not by aphids and inversely. Like many other potyviruses, PSbMV causes characteristic cell inclusions in the form of pin-wheels, circles etc. In this paper we shall try to present some investigations of PSbMV carried out in Yugoslavia.

Material and Methods

The pea plants used in the investigations were cultivated in an insect proof greenhouse and on experimental fields in Zagreb and belonged to the cultivars Stern, Sprinter and Mingomark. Especially two isolates of the type PSbMV obtained from Sprinter (isolate LR) and from Stern seeds were investigated intensively. The seed-lots investigated were provided by the Faculty of Agriculture in Zagreb. Only the seeds of the cultivar Kleiner Rheinländer derived from the Institute of Agriculture at Butmir near Sarajevo.

In addition, a virus isolate from pea cv. Stern named pea latent strain (PLS) of PSbMV will be shortly described here.

The serum against PSbMV was kindly supplied by Professor R. O. Hampton (Corvallis, Oregon, USA).

For electron microscopic investigation leaves of pea and broad bean with obvious symptoms were taken. Small leaf parts were fixed in 1.5% glutaraldehyde with 0.1 M sodium cacodylate and then postfixed in 2% OsO_4. Fixed parts were dehydrated in an alcohol series and afterwards embedded in Epon 812. Ultrathin sections were cut with a diamond knife and stained with uranyl magnesium acetate (Kimura et al. 1975). The sections were examined in an electron microscope JEM 100 B.

Results

1. Investigation of the type strain of PSbMV

Occurrence of PSbMV in Yugoslavia

Pea plants growing in the field and belonging to the cultivar Sprinter were tested for the presence of viruses by means of *Ch. amaranticolor*. A very large number of the plants tested (cca 75%) showed a positive reaction in *Chenopodium* producing characteristic local lesions. Therefore, it was suspected that the seed-lot, with which this field was sown, was infected by PSbMV.

The virus isolate LR was investigated for identification purposes on several herbaceous plants. The result of this analysis is presented in Table 1.

Table 1. Reaction of some herbaceous plants after inoculation with the isolate LR of PSbMV

Plant	Reaction
Chenopodium album	L: chlorotic local lesions
Ch. amaranticolor Coste et Reyn.	L: small local lesions with brown centre well visible in the passing light
Ch. murale L.	L: chlorotic local lesions
Ch. quinoa Willd.	L: chlorotic local lesions
Lens culinaris Med.	S: slight stunting
Pisum sativum L.	S: leaf rolling, transient vein clearing, stunting
Tetragonia expansa Thunb.	symptomless
Vicia faba L.	S: slight leaf rolling, transient mottling

L = local infection, S = systemic infection

The LR isolate was not transmissible to *Nicotiana tabacum* White Burley and Samsun, *N. glutinosa, Datura stramonium, Lactuca sativa,* and *Petunia hybrida.*

Several hundreds of local lesions with a diameter of 0.8 mm apeared on the leaf of *Ch. amaranticolor.* These lesions were very distinct and could be used for detection purposes (Hampton 1976) and for quantitative investigation of PSbMV. However, the number of local lesions in *Ch. quinoa* was smaller.

The transient symptoms appearing after infection on the top leaves of pea and broad bean were very characteristic of the disease. They consisted of vein clearing and banding on the pea and mottling on the broad bean, but sometimes they were masked.

All these symptoms indicated that the field peas mentioned above were infected with PSbMV. Therefore, we acquired the seed-lot of cv. Sprinter with which the field was sown. It is interesting to note that a large number of these seeds had cracked testa. About 200 seeds were sown in the sterilized soil and kept in the greenhouse. After three weeks ten plants remained stunted with leaves rolled downwards and showed other symptoms of PSbMV infection. *Ch. amaranticolor* leaves were inoculated with the sap of these plants and after one week the characteristic lesions described appeared.

On the basis of these experiments it was likely that the field plants and seed lot were infected with PSbMV. To provide further evidence for this opinion we examined the field plants and young greenhouse plants grown from infected seeds with the serum against PSbMV. The serological reactions were performed in microprecipitin tests. The infective plant sap was previously centrifugated for 15 min at 5,000 rpm. The reaction was made in various dilution stages of serum and virus and it was constantly positive. Chloroplast agglutination reactions were also always positive. The sap of healthy pea plants, however, never reacted with the serum against PSbMV.

Transmission of PSbMV by seeds

It has already been mentioned that the seed lot of cv. Sprinter was infected with PSbMV and that 5% of the seedlings had obvious symptoms.

An analysis of the seed-lot of the cultivar Stern showed that a large number of seeds had cracked testa. We separated the seeds with cracked testa from the seeds with intact ones and these two groups of seeds were sown apart from one another. After a time it was found on the basis of characteristic symptoms that in both groups of seedlings there were specimens infected with PSbMV. However, the number of diseased plants was greater in the group deriving from cracked seeds (cf. Stevenson and Hagedorn 1970).

A seed-lot of cv. Mingomark was also investigated for the presence of PSbMV. On the basis of characteristic symptoms it was found that about 5% of seeds contained this virus.

In order to investigate the PSbMV in a pea seed-lot it is not necessary to use young seedlings because it is possible to identify the virus directly in the seeds. According to Knesek and Mink (1970) in a cluster of 10 pea seeds it is possible to prove the presence of only one infected seed. To attain this, it is necessary to moisten the 10 seeds dur-

ing the night, then to homogenize the swollen seeds in a mortar and at last to inoculate the homogenized material into young pea plants or *Ch. amaranticolor*.

As mentioned, PSbMV is not only present in the embryo but also in the cotyledons (Knesek and Mink 1970). Sometimes it is important to know which pea seed in a seed-lot is infected with PSbMV in order to use the infected seed for studying the virus location, the development of symptoms and other processes in young plant grown from infected seed. We have found that it is possible to divide the pea seed in two parts so that in one part the embryo remains intact, i. e. capable of germination and experiments; the other seed part without the embryo containing mainly the cotyledons can serve to prove the presence of the virus.

During the experiments with whole and divided seeds it was established that the quantity and virulence of PSbMV in seeds was very different. For the valuation of the virus in seeds, local lesions of *Ch. amaranticolor* were used. Different homogenized seeds produced a rather variable number of local lesions in this plant. So e. g. one infected seed produced less than 10 local lesions per leaf and the other more than 100 local lesions. On the contrary, the two parts of the same seed, i. e. the corresponding "embryo" and "cotyledon" parts, provoked a similar number of local lesions.

Fig. 1. *A* and *B*. Photomicrographs of epidermal broad bean cells showing X-bodies (i) in contact with the nuclei (n). Bar represents 20 μm. *C*. Ultrathin section of a cell of broad bean. Scrolls (s) and pin-wheels (w) are often connected together. Bar represents 200 nm.

Sl. 1. *A* i *B*. Svjetlosno-mikroskopska slika epidermskih stanica boba. Vide se X-tijela (i) u dodiru s jezgrom (n). Skala je duga 20 μm. *C*. Ultratanki presjek kroz stanicu lista boba. Vide se smoci (s) i vrtuljci (w) koji su često povezani. Skala iznosi 200 nm.

Fig. 2. Longituninal section of a very long scroll (s). Next to the scroll a longitudinally sectioned pin-wheel (w) is shown. Bar represents 100 nm.

Sl. 2. Uzdužni presjek kroz vrlo dugi smotak (s). Pokraj smotka vidi se uzdužno presječeni vrtuljak (w). Skala iznosi 100 nm.

Fig. 3. Regular hexagons (h) representing deposits of crystalline protein in pea leaves. Bar represents 300 nm.

Sl. 3. Pravilni heksagoni (h) koji predstavljaju depozite kristaličnog proteina u listovima graška. Skala iznosi 300 nm.

Fig. 4. *A* and *B*. Submicroscopic inclusion bodies of pea latent strain of PSbMV. *A*. Regularly formed pin-wheel structures in transversal and longitudinal sections. *B*. Many longitudinally sectioned pin-wheel structures in contact (arrows) with the rough endoplasmic reticulum. Bars represent 400 nm.

Sl. 4. *A* i *B*. Submikroskopske inkluzije latentnog soja graška koji pripada virusu mozaika graška koji se prenosi sjemenom. *A*. Pravilno građeni vrtuljci prerezani poprečno i uzdužno. *B*. Strelice pokazuju mjesta gdje je veći broj uzdužno presječenih vrtuljaka prislonjen na hrapavu endoplazmatsku mrežicu. Skale iznose 400 nm.

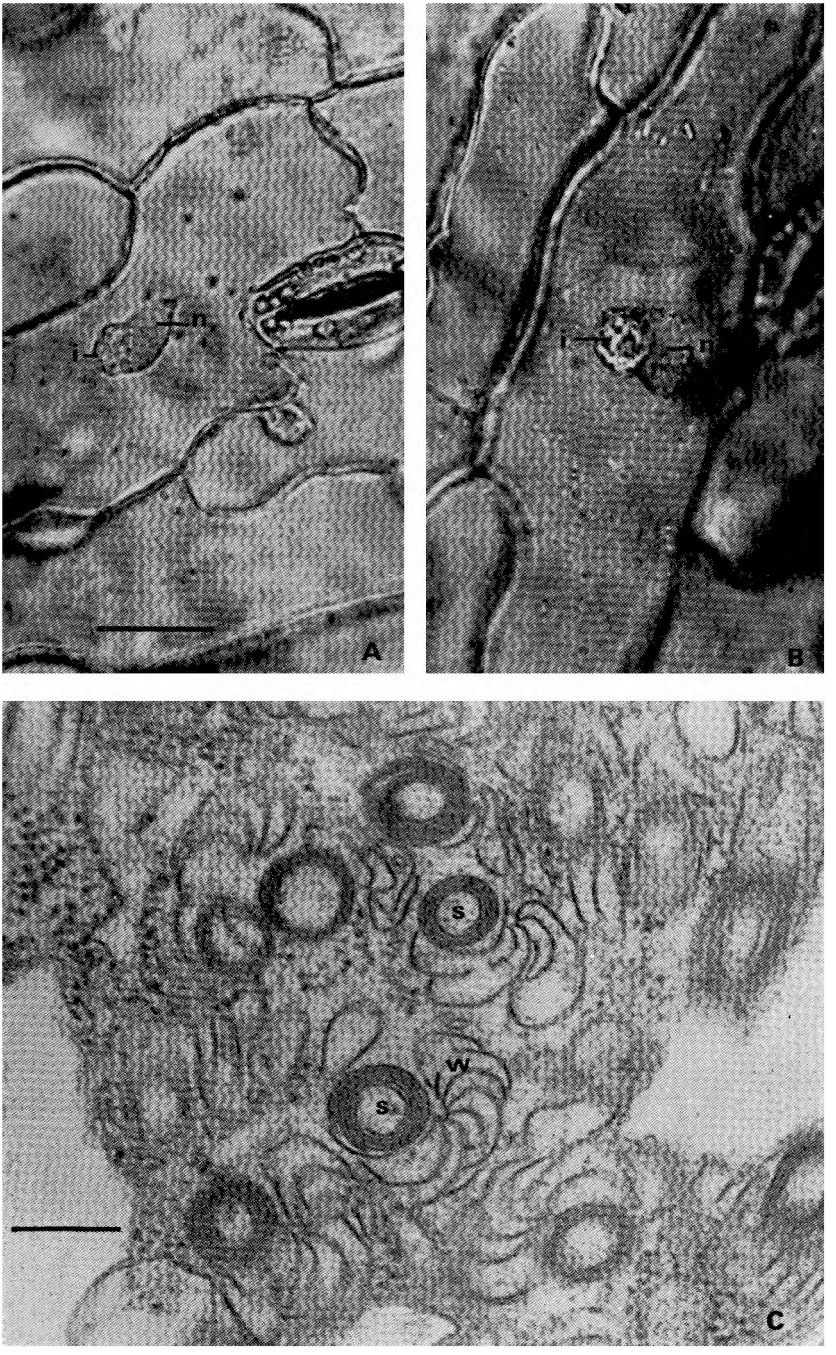

Fig. 1. — Sl. 1.

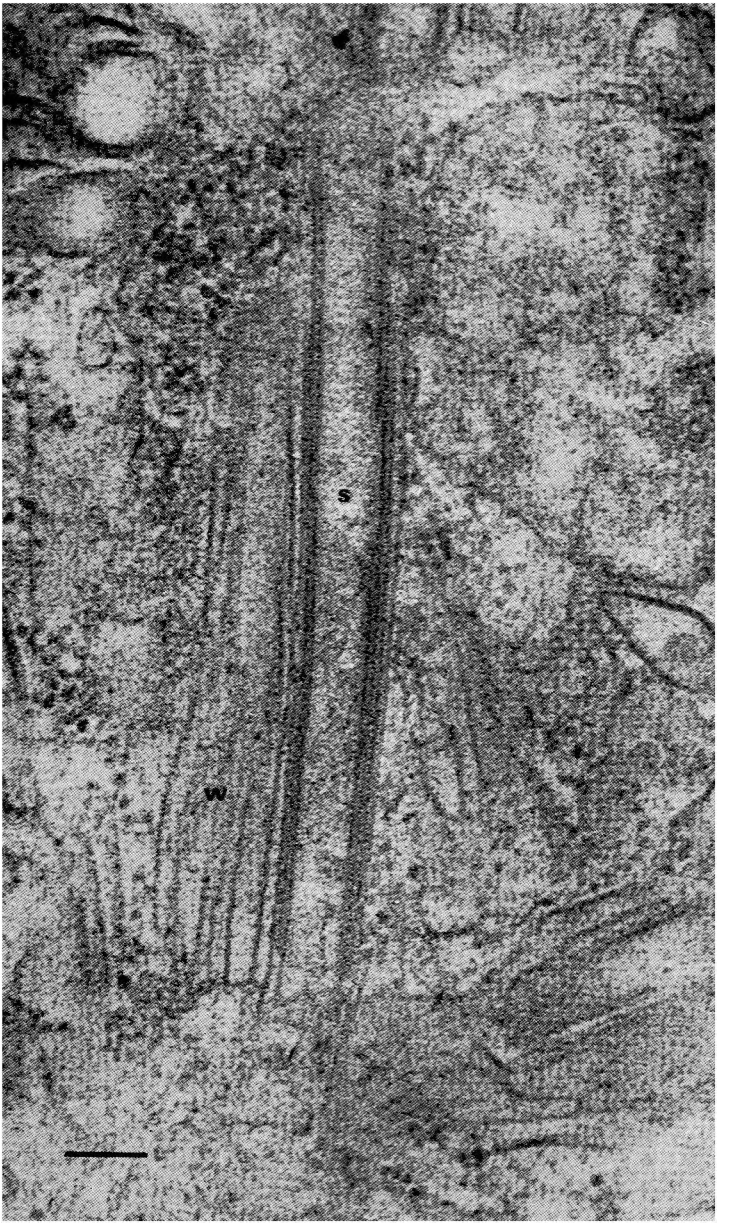

Fig. 2. — Sl. 2.

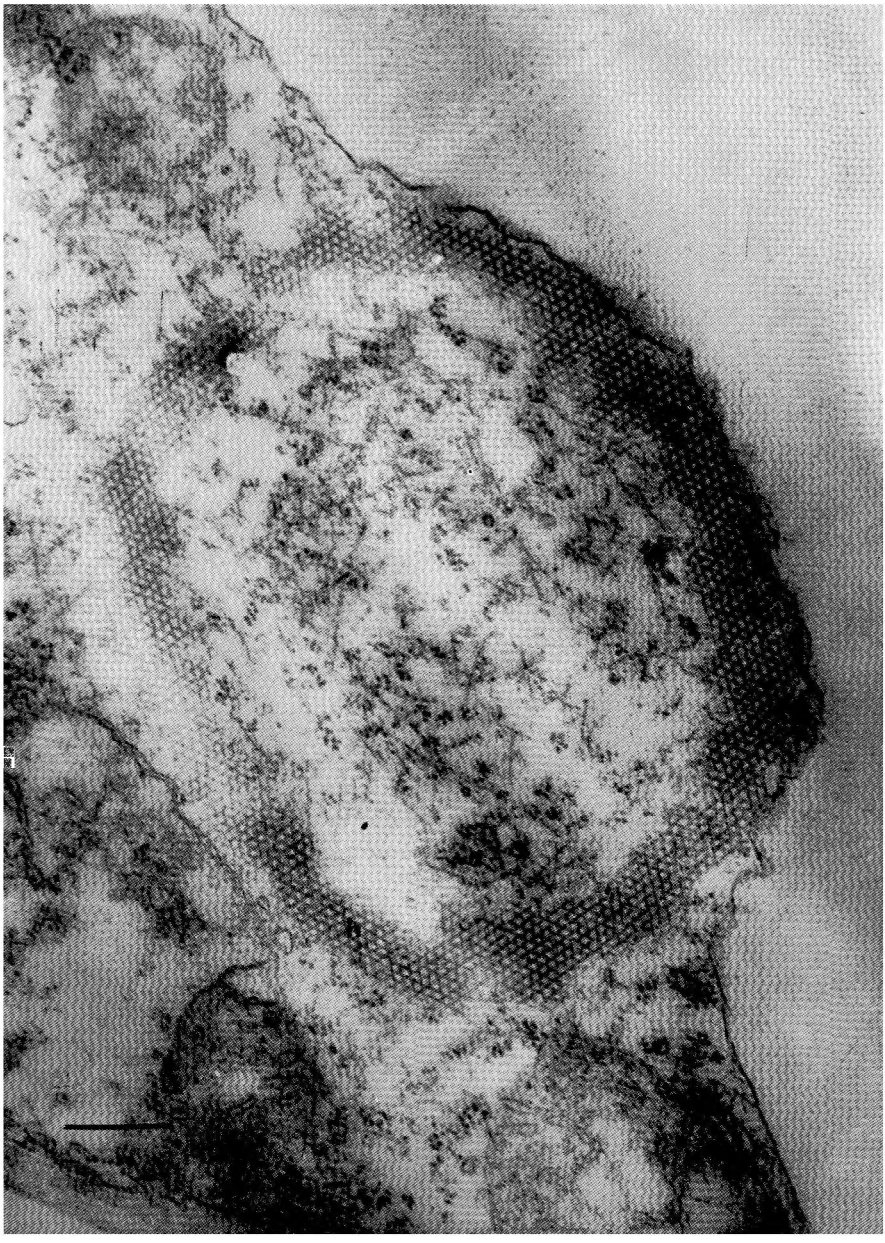

Fig. 3. — Sl. 3.

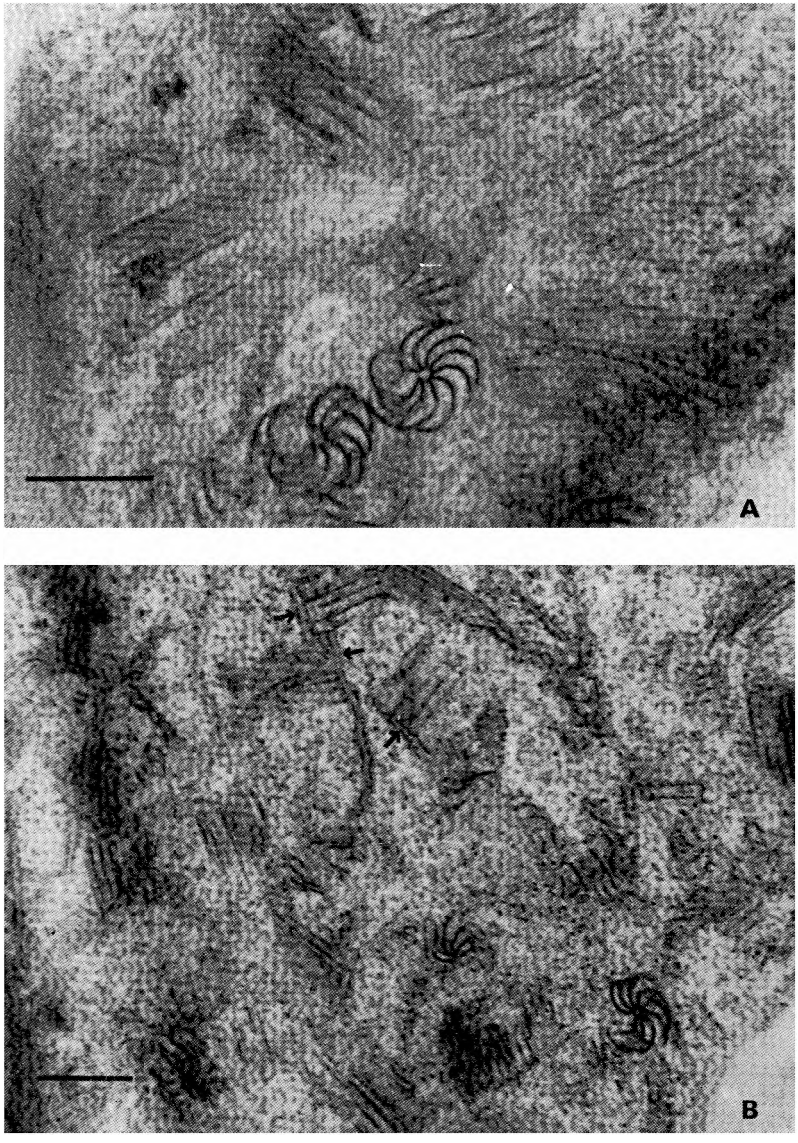

Fig. 4. — Sl. 4.

Intracellular inclusion bodies and data on virus particles

Light microscopic cell inclusions of PSbMB were first observed by Bos (1970). Intracellular inclusions were regularly found also by us in the leaf and stem epidermis of pea and broad bean infected with the LR and Stern isolates. They appeared together with the first symptoms, and were mostly round, amorphous and slightly smaller than the nucleus (Fig. 1, A, B). It was often possible to see little lipid drops in the X--bodies. In older infections the substance of X-bodies became denser and small irregular vacuoles originated inside them. In these X-bodies a large number of crystalline needles was found, and they were sometimes parallelly arranged. In the end, the bodies assumed the form of needle aggregates. The last inclusions were very similar to the light microscopic cell inclusions formed in crucifer plants infected with turnip mosaic virus (Štefanac and Miličić 1965). The crystalline needles represented very probably the pin-wheels and long scrolls which appear abundantly in the leaf tissues infected with PSbMV.

The presence of interesting inclusions visible in the light microscope stimulated us to undertake electron microscopic investigations. By means of the dip method and treatment with phosphotungstic acid it was established that in the infected sap there was a moderate number of filamentous virus particles. The virions were about 750 nm long and 13 nm wide.

In the parenchyma cells of broad bean leaves pin-pheel structures were very frequent and regularly had curved laminae (Fig. 1C). Pin--wheels were very often sectioned longitudinally and then they assumed the form of bundles (Fig. 2 w).

The second form of submicroscopis inclusions appeared sometimes as very long scrolls (Fig. 2). Every scroll apparently consisted only of one thin lamina which was many times bent round the axis of the scroll. The peripheral margin of the scroll lamina was sometimes connected with a lamina of pin-wheels (Fig. 1 C). The scrolls certainly are structures closely related to the circles which have been described by Hampton et al. (1973). Some "circles" of these authors (Hampton et al. 1973, fig. 6) correspond very well to our scrolls. Therefore, it seems that the scrolls are also characteristic inclusions of PSbMV. On the other hand it was not possible to find in our material the 'convoluted and densely stained structures' resembling the convoluted endoplasmic reticulum.

However, we sometimes observed the crystalline inclusions first found by Hampton et al. (1973) in pea cells infected by the isolate S 1. In our material they consisted of regular hexagons in some sectional planes (Fig. 3). According to the opinion of Hampton et al. (1973) the hexagons represent deposits of crystalline protein and appear in the late phases of PSbMV infection. Our deposits had sometimes a circular form and inside the circle many virus-like particles were present (Fig. 3).

During our investigation laminated aggregates were never noticed in the cells. This type of inclusions was very rarely observed also by Hampton et al. (1973) so that these authors considered it as abnormal and not characteristic of PSbMV infection. The laminated aggregates were first found by Inouye (1967) in PSbMV infected plants from Japan. On the basis of this finding Edwardson (1974) included the submicroscopic inclusions of PSbMV according to their morphology in the third subdivision of potyvirus inclusions. The presence of pin-wheels, bundles, scrolls and laminated aggregates are characteristic of this sub-

division. However, since it is known today that the laminated aggregates are rarely present in plants infected with PSbMV, it would be well worth revising their position in this system.

Stability in vitro

In the autumn of 1976 the stability in vitro of the Sprinter isolate of PSbMV was determined. In this experiment the infective sap of broad bean plants was used and its infectivity was investigated by means of *Ch. amaranticolor.* The result is presented in Table 2.

Table 2. Stability in vitro of the Sprinter isolate of PSbMV by 22 — 25 °C

Time in hours	Average number of local lesions on one leaf of *Chenopodium amaranticolor*	
	1 trial	2 trial
Control	201	233
1	118	—
2	71	—
3	39	—
24	27	51
48	1	34
72	0	2
96	0	0

Consequently, the sap of broad bean remained infective for more that 72 hours. This result is similar to the one of Knesek et al. (1974) who found that the sap did not lose the infectivity for more than 96 hours. It must be mentioned that the experiments of Knesek et al. were performed by means of the sap of pea plants.

2. *Pea latent strain of PSbMV*

Two years ago a strain of PSbMV named pea latent virus was isolated from pea cv. Stern (Miličić et al. 1976). A special characteristic of this virus was very weak symptoms on pea so that it was difficult to distinguish the diseased plants from healthy ones by means of this plant. However, the freshly infected pea plants often displayed a vein clearing 6 — 8 days after inoculation. The vein clearing gradually extended toward the intercostal leaf regions so that a fairly thick light green net appeared on the upper leaves. Those symptoms soon disappeared so that after one to two days the plants regained their normal appearance.

In order to investigate the properties of this virus thoroughly we transmitted it to some herbaceous plants (tab. 3).

As shown in Tables 1 and 3, the pea latent strain differs from the type strain especially in the symptoms appearing on the pea plants. While the type strain caused leaf rolling and stunting of plants, the pea latent strain (PLS) caused only the common transient vein symptoms. Besides, these strains differed in regard to symptoms on broad bean where the transient symptoms on young leaves differed more and were stronger under the influence of PLS.

Table 3. Symptoms of pea latent strain of PSbMV in some plants

Plant	Reaction
Chenopodium amaranticolor Coste et Reyn.	L: moderate number of local lesions with brown centre. Lesions well visible in the passing light
Ch. murale L.	L: chlorotic lesions with necrotic centre
Ch. quinoa Willd.	L: chlorotic local lesions
Lens culinaris Med.	S: slight stunting of plants
Pisum sativum L.	S: transient vein clearing
Tetragonia expansa Thunb.	symptomless
Vicia faba L.	S: slight leaf rolling; transient mosaic, spotting or yellow net on upper leaves

On the inoculated leaves of *Ch. amaranticolor* the PLS provoked local lesions which were very similar to the lesions of the type strain, but only larger; they had a diameter of 1.2 mm and those of the type strain only 0.8 mm. Moreover, the number of local lesions was smaller with PLS as follows from a comparison of Tables 2 and 4.

Serological relationship between the type PSbMV and the PLS

Since the PLS showed many common characteristics with PSbM, we tried to compare them in serological test. For this purpose broad baen saps containing PSbMV and PLS were examined with the serum against PSbMV in microprecipitin tests. The trials were repeated many times and were performed in the manner as described in this paper earlier. In most trials a positive reaction appeared between the PSbMV antiserum and PLS which showed that these viruses were serological related.

Submicroscopic inclusion and data on virus particles

Since the PSbMV causes the formation of light microscopic cell inclusions, we tried to find them in pea and broad bean cells infected with PLS. In most attempts we did not find amorphous cell inclusions in the epidermis of broad bean and pea, only in one case we found a small amount of them.

However, an electron microscopic examination of pea and broad bean leaf parenchyma tissue revealed a large number of pin-wheels in the infected cytoplasm (Fig. 4). The pin-wheels were regularly built of many simple laminae which were all bent towards the same side. Sometimes, when the pin-wheels were visible in the side view, it was obvious that they stood in contact with the endoplasmic reticulum (Fig. 4 B). Moreover, circles and scrolls were sometimes found in the infected tissue but laminated aggregates were never observed.

By means of the dip method and treatment with phosphotungstic acid a moderate number of virus particles was found. The particles of PLS were filamentous and about 750 nm long.

Stability in vitro

This property of PLS was examined on the isolate B1. In this experiment the infective sap of broad bean plants was employed. The sap infectivity was again investigated in *Ch. amaranticolor*. The result of this experiment is shown in Table 4.

Table 4. Stability in vitro of the B1 isolate of pea latent strain by 22 — 25°C

Time in hours	Average number of local lesions on one leaf of *Chenopodium amaranticolor*
Controls	60
1	62
2	45
3	21
24	2
48	0

The virus infectivity in vitro disappears in one to two days. Thus, the stability is lower than the stability of LR isolate of PSbMV.

It can be mentioned that PLS can maintain the infectivity for more than one year when the infective leaves are dried after the method of Mc Kinney (1947).

Discussion

In this paper the properties of two European strains of PSbMV are described, especially their submicroscopic cell inclusions. That strains of PSbMV could exist, Hampton and Mink (1975) supposed on the basis of differences among the intracellular inclusions of various isolates. In connection with these inclusions it can be mentioned that we have not found laminated aggregates in our material. Therefore, our results support the opinion of Hampton et al. (1973) that this inclusion type is very rare and abnormal for PSbMV.

The PL strain of PSbMV described here differs from the type strain in many properties. First, the PLS does not cause the rolling of pea leaves, or this symptom is very rare and weak. On the contrary, the leaf rolling is a very pronounced symptom of the type strain of PSbMV.

The PLS builds larger lesions of about 1,2 mm in diameter on *Ch. amaranticolor* while the lesions of the type strain are smaller with the diameter about 0.8 mm. The lesion number per leaf is also different; the type strain produces some hundreds of lesions per leaf, the PLS only some tens.

A further difference between the two strains is the presence of light microscopic inclusions in pea and broad bean cells infected with the type PSbMV, and the very rare finding of these inclusions in the cells infected with PLS.

The two strains differ from one another also with regard to their stability in vitro. The infectivity of the type strain disappears after 3 to 4 days and the infectivity of PLS after one day.

The strain PL can be dangerous because it can be easily overlooked owing to its latency in peas. It is interesting to note that Hampton (1972) established that 10% of pea plants infected with PSbMV were without any symptom. The question is whether a strain similar to PLS was present among the infected and symptomless pea plants in Hampton's investigation.

Summary

Pea seed-borne mosaic virus (PSbMV) was for the first time found in Yugoslavia last year. It was observed in the field and was also established in seed-lots of cultivars Sprinter, Stern, and Mingomark. The identification of the virus was ascertained by an analysis on herbaceous plants, its stability in vitro, serological properties, and transmissibility by seeds. It was also confirmed that PSbMV was able to produce amorphous X-bodies well visible in light microscope. By means of an electron microscope pin-wheel structures, bundles, circles, and scrolls were seen in the infected cells but never laminated aggregates. In addition to that, hexagonal deposits of crystalline protein built from relatively large particles were found in the cytoplasm of the diseased cells; these deposits were similar to those described by Hampton et al. (1973).

Another pea virus isolate which probably represents a strain of PSbMV is described in this paper. This virus is named pea latent strain (PLS) of PSbMV because it is mostly latent in pea. It does not cause the symptom of leaf rolling and stunting in infected peas but it often provokes the transient symptom of vein clearing. It builds rarely X-bodies visible in the light microscope but forms submicroscopic inclusion bodies in the form of typical pin-wheels. PLS differs from the type virus also by shorter stability in vitro and some other characteristics.

Literature

Aapola, A. A., J. E. Knesek, and *G. I. Mink,* 1974: The influence of inoculation procedure on the host range of pea seed-borne mosaic virus. Phytopathology *64,* 1003—1006.

Bos, L., 1970: The identification of three new viruses isolated from *Wistaria* and *Pisum* in the Netherlands, and the problem of variation within the potato Y group. Neth. J. Plant Pathol. *78,* 8—46.

Edwardson, J. R., 1974: Some properties of the potato virus Y-group. Florida Agric. Exp. Sta. Monograph Series, No. 4. University of Florida, Gainesville.

Hagedorn, D. J., 1974: Virus diseases of pea, *Pisum sativum.* Monograph *9.* Amer. Phytopath. Soc.

Hampton, R. O., 1972: Dynamics of symptom development of the seed-borne pea fizzletop virus. Phytopathology *62,* 268—272.

Hampton, R. O., 1976: Factors affecting detection of pea seedborne mosaic virus in *Pisum* seedlots. Proceed. Amer. Phytopath. Soc. *3,* 207—208.

Hampton, R. O., J. E. Knesek and *G. I. Mink,* 1974: Particle-length variability of the pea seedborne mosaic virus. Phytopathology *64,* 1358—1363.

Hampton, R. O. and *G. I. Mink,* 1975: Pea seed-borne mosaic virus. C. M. I./A. A. B. Description of Plant Viruses. No. *146.*

Hampton, R. O., Sandra Phillips, J. E. Knesek and *G. I. Mink,* 1973: Ultrastructural cytology of pea leaves and roots infected by pea seedborne mosaic virus. Archiv ges. Virusforsch. *42,* 242—253.

Inouye, T., 1967: A seed-borne mosaic virus of pea. Ann. Phytopath. Soc. Japan *33*, 38—42.

Kimura, M. L., L. Seveus and *K. Maramorosch*, 1975: Ferritin in insect vectors of the maize streak disease agent: electron microscopy and electron microprobe analysis. J. Ultrastruct. Res. *53*, 366—373.

Knesek, J. E. and *G. I. Mink*, 1970: Incidence of a seedborne virus in peas grown in Washington and Idaho. Plant Dis. Reptr. *54*, 497—498.

McKinney, H. H., 1947: Stability of labile viruses in desiccated tissue. Phytopathology *37*, 139—142.

Miličić, D. i J. Grbelja, 1976: Virus mozaika graška koji se prenosi sjemenkama (pea seed-borne mosaic virus) u nekim sortama graška u Jugoslaviji (Pea seed-borne mosaic virus in some pea cultivars in Yugoslavia). Zaštita bilja (Beograd) *28*, 147—154.

Miličić, D., R. Lešić, Đ. Mamula, N. Pleše and *V. Hršak*, 1976: Occurrence of pea enation mosaic virus in Yugoslavia. Acta Bot. Croat. *35*, 25—31.

Musil, M., 1966: Über das Vorkommen des Virus des Blattrollens der Erbse in der Slowakei. Biológia (Bratislava) *21*, 133—138.

Musil, M., 1970: Pea leaf rolling mosaic virus and its properties. Biológia (Bratislava) *25*, 379—392.

Stevenson, W. R. and *D. J. Hagedorn*, 1969: A new seed-borne virus of peas. Phytopathology *59*, 1051—1052 (Abstr.).

Stevenson, W. R. and *D. J. Hagedorn*, 1970: Effect of seed size and condition on transmission of pea seed-borne mosaic virus. Phytopathology *60*, 1148—1149.

Stevenson, W. R. and *D. J. Hagedorn*, 1973: Further studies on seed transmission of pea seed-borne mosaic virus in *Pisum sativum*. Plant Dis. Reptr. *57*, 248—252.

Štefanac, Z. und *D. Miličić*, 1965: Zelleinschlüsse des Kohlrübenmosaikvirus. Phytopath. Z. *52*, 349—362. 1965.

SADRŽAJ

PRILOG POZNAVANJU VIRUSA MOZAIKA GRAŠKA KOJI SE PRENOSI SJEMENOM

Davor Miličić i Biljana Plavšić

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu i Biološki institut Univerziteta u Sarajevu)

Prošle je godine prvi put u Jugoslaviji pronađen virus mozaika graška koji se prenosi sjemenom (VMGPS). Virus je zapažen na polju, a nađen je i u komercijalnim uzorcima sjemena koji su pripadali sortama stern, sprinter i mingomark. VMGPS je najprije detaljno analiziran na zeljastim pokusnim biljkama. Pri tom je karakteristično reagirao grašak koji je pod utjecajem virusa zaostajao u rastu i savijao svoje liske prema dolje. Osim toga su se često prosvjetljavale žile lisaka graška nekoliko dana poslije infekcije, ali je taj simptom poslije kratkog vremena iščezavao. Na vrsti *Chenopodium amaranticolor* nastajale su karakteristične lokalne lezije koje su bile vrlo prozirne u prolaznom svjetlu. One su se sastojale od smeđeg nekrotičnog središta koji je bio okružen svijetlozelenim klorotičnim prstenom (usp. Miličić i Grbelja 1978).

Nazočnost VMGPS-a može se utvrditi i izravno u uzorcima sjemena graška. U tu se svrhu uzme po 10 sjemenaka, namoči ih se preko noći u vodi da nabubre, a zatim se homogeniziraju u tarioniku. Dobivenom kašom uz pomoć karborunda natrljaju se listovi graška ili vrste *Ch.*

amaranticolor. Ako je u sjemenkama bilo VMGPS-a, pojavit će se opisani simptomi bolesti na tim pokusnim biljkama.

Osim toga VMGPS smo identificirali i s pomoću određivanja njegove postojanosti in vitro, istraživanja njegovih seroloških svojstava i prenošljivosti sjemenom.

Prilikom istraživanja promjena u zaraženim stanicama ustanovili smo da VMGPS stvara amorfna X-tijela koja se dobro vide svjetlosnim mikroskopom (sl. 1 A i B). Elektronskim mikroskopom zapazili smo velik broj vrtuljaka, krugova i smotaka, ali u stanicama nikad nije bilo laminatnih agregata (sl. 1 C, 2). Osim toga zapazili smo u citoplazmi heksagonalne nakupine kristaličnog proteina koje su se sastojale od razmjerno velikih čestica (sl. 3). Te su nakupine bile slične onima koje su opisali H a m p t o n et al. (1973).

U ovom radu opisali smo i virusni izolat iz graška koji je sličan tipičnom VMGPS-u, tako da ga smatramo za novi soj VMGPS-a. Taj smo izolat nazvali latentnim graškovim sojem VMGPS-a, jer je većinom latentan u grašku. Latentni graškov soj ne izaziva na grašku simptom savijanja lisaka i kržljavljenja, ali često prouzrokuje prolazni simptom prosvjetljavanja lisnih žila. Običnim mikroskopom rijetko smo mogli pronaći X-tijela, ali smo elektronskim mikroskopom utvrdili da stvara inkluzije u obliku tipičnih vrtuljaka. Od tipičnog VMGPS razlikuje se latentni graškov soj još i kraćom postojanošću in vitro i nekim drugim svojstvima.

Prof. dr Davor Miličić
Botanički zavod PMF
Marulićev trg 20/II
YU-4100 Zagreb (Jugoslavija)

Prof. dr Biljana Plavšić
Biološki institut
Univerziteta u Sarajevu
YU-71000 Sarajevo (Jugoslavija)