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SOME PROPERTIES OF BROME STREAK MOSAIC VIRUS

DAVOR MILIČIĆ, ĐORĐE MAMULA and MIŠKO PLAZIBAT

(Department of Botany, Faculty of Science, University of Zagreb)

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Brome streak mosaic virus (BSMV) has been found in nature on *Bromus mollis* L. and *Hordeum murinum* L. Mechanically it can be transmitted only to the members of *Poaceae* including the cereals wheat, barley and oat. BSMV has elongated and flexible particles c. 693 nm long. It builds characteristic cylindrical cytoplasmic inclusions which belong to the second subdivision according to Edwardson (1974). Two methods were found to be favourable for the purification of BSMV. By means of spectrophotometric analysis it was possible to establish the approximate virus concentration in partially purified preparations.

Introduction

Two years ago it was noticed by Miličić et al. (1980) that in the surroundings of Zagreb a virus was found on *Bromus mollis* (Fig. 1 A) and *Hordeum murinum* L. This virus attacks only members of *Gramineae*. It was mechanically transmitted to wheat, barley and oat, and therefore it could be important for agriculture.

During electron microscopic investigations it was established that this virus had an elongated particle. It provoked the formation of characteristic cylindrical cytoplasmic inclusions with laminated aggregates but without scrolls. On the basis of this property it could belong to the second group of viruses inducing pin-wheel structures (cf. Edwardson 1974). As this virus has not been given a scientific name till now, we propose to name it brome streak mosaic virus (BSMV).

Material and Methods

BSMV was cultivated on *Briza maxima* L. and *Hordeum vulgare* L. in an insect proof glasshouse. These two plants were used also for purification and other experiments.

The purification was performed first with *H. vulgare* and then with *B. maxima* by the following procedure. In each trial 50 g of leaves were homogenized in 60 ml of cold phosphate buffer 0,06 M (pH 7.8) which contained DIECA (0.2 %), Na_2SO_3 (0.03 %) and EDTA (0.01 M). After this treatment the volume of solution was 90 ml. Then 30 ml of chloroform was added to this solution. Afterwards the mixture was emusified in a magnetic stirrer for 20 min at 6° C and subsequently the low speed centrifugation was performed. Thereafter, the emulsion was centrifugated at 29,000 rev./min for 105 min (rotor 30) in a Spinco Beckman ultracentrifuge. The pellets were dissolved in 4 ml of 0.06 M phosphate buffer pH 7.8. This was followed by low speed centrifugation at 7,000 rev./min. for 20 min. Finally, the second cycle of differential centrifugation was performed and the pellets were dissolved in 0.3 ml of phosphate buffer 0.06 M (pH 7.8).

The second method used for purification of BSMW was that described by Slykhuis (1973) for purification of agropyron mosaic virus and was performed by us only with the material cultivated on *B. maxima*.

Spectrophotometric analysis of virus solutions was performed with a Beckman DB-GT spectrophotometer.

Electron microscopic investigations were made by means of a Siemens Elmiskop I and by using potassium phosphotungstate for negative staining.

Results

Investigation of host range

BSMV has been found till now only in the surroundings of Zagreb where it attacked two species, *B. mollis* L. and *H. murinum* L. Many hosts of BSMV were noted by Miličić et al. (1980) and were there listed on a table. In order to complete the host list and data, we present here the names and data of BSMW hosts known till now (Tab. 1).

Dicotyledon and monocotyledon plants which cannot be infected with BSMV have been noted by Miličić et al. (1980). The host plant *Briza maxima* which is very frequent in the Mediterranean region reacts with very strong symptoms after infection with BSMV (Fig. 1 B). On the leaves, very strong chlorosis appears with yellow or white streaks and spots. These alterations are sometimes followed by death of the plant. However, a large number of plants survive so that *B. maxima* can be used at least during autumn for purification and other purposes.

Length of virus particles

The length of 50 BSMV particles has been measured and it has been established that the length of particles is c. 693 nm.

Purification of virus

Purification performed by the first procedure gave a good result. The attempt to use borate buffer instead of phosphate one was not successful. The suspension with phosphate buffer was treated two times with chloroform. Final virus suspension contained quite a number of virus particles as well as some impurities (Fig. 2). Nevertheless, the virus suspension was suitable for spectrophotometric analysis and it was possible to establish roughly the concentration of virus in barley leaves.

Table 1. Host range of brome streak mosaic virus.

Tribus	Species	Reaction
Agrostideae	*Lagurus ovatus* L.	++
Aveneae	*Avena barbata* Pott.	+
	Avena sativa L.	++
	Avena sterilis L.	++
Festuceae	*Briza maxima* L.	++
	Bromus molliformis Lloyd	++
	Bromus mollis L.	++
	Bromus sterilis L.	++
	Bromus tectorum L.	+
	Vulpia ligustica (All.) Lk.	++
	Vulpia membranacea (L.) Lk.	++
Hordeae	*Lolium multiflorum* Lam.	+
	Lolium perenne L.	+/−
	Lolium temulentum L.	+
	Triticum dicoccum Schrank	+
	Triticum durum Desf.	+
	Triticum vulgare Vill.	++
	Hordeum murinum L.	++
	Hordeum vulgare L.	++

Explanation of signs: Species which have been confirmed as BSMV hosts, by means of a back infection of barley, are marked ++. Species which reacted with obvious symptoms but the back infection was not accomplished, are marked +. Species with very weak symptoms and without back infection is marked +/−.

The second method used for purification of BSMV derives from Slykhuis (1973). Using this method we obtained a fairly pure virus suspension with a large number of virus particles. The particles were often arranged longitudinally end to end. However, it was not possible to investigate the virus solution spectrophotometrically because some unknown factors prevented clear results.

Spectrophotometric analysis

The virus suspension which was purified according to the first method using infected barley leaves was investigated by means of a spectrophotometer. For this purpose the absorption of UV light in the range of 220 to 300 nm was determined. The quotient A 260/A 280 = 1.28 was established, on the basis of absorption data. Since normal proteins were present in the virus suspension, this quotient served only for orientation purposes. The absorption quotient of anisometric viruses ranges from c. 1.2 to 1.3.

By means of these light absorption data it was possible to establish roughly the virus concentration in the suspension. For this purpose the formula $C = 1 \times \frac{A\ 260}{E}$ was used and it was found that from 1 kg of barley leaves by the used method of purification it could be obtained about 4 to 5 mg of virus. It was supposed that the extinction coefficient

(E) was 2.8, i. e. the average between 2.4 and 3.3 in which range lie the values of the extinction coefficient for elongated viruses.

Investigation with electron microscope

As *B. maxima* was very sensitive to infection with BSMV and reacted with strong symptoms and necrosis the electron microscopic examination of its tissues was performed. It was established that characteristic pin-wheel structures with relatively thick laminated aggregates were very frequent. However, pin-wheel structures with relatively thinner and longer laminated aggregates were also visible. The laminated aggregates of this type were often joined together in a complicated manner. The shape of these inculsions corresponds very well with inclusions found earlier in barley leaves (Miličić et al. 1980, Fig. 2—4).

Discussion

BSMV was considered to be a member of the potyvirus group in a previous paper of Miličić et al. (1980). However, some changes occurred with regard to the membership of some viruses to the poty-group in the near past.

Hollings and Brunt (1981) think that the transmission by aphids in a non-persistent manner is a very important property of potyviruses. This virus group is transmitted most efficiently after aphid feeding of only a few minutes. During feeding the stylet is inserted into the epidermis. There is no evidence for virus multiplication in the vectors of potyviruses. It has been established that for transmission all potyviruses require a helper factor which is a labile protein (Govier, Kassanis and Pirone 1977).

According to the data of Hollings and Brunt (1981) the potyviruses have also a characteristic length of 720 to 900 nm.

However, some viruses — which build well developed pin-wheel inclusions but which are not transmitted by aphids — have not been included in the list of potyviruses presented by Hollings and Brunt (1981). One of these not included groups attacks plants which are members of the family *Poaceae*, especially some cereals. The group includes some well known viruses, such as wheat streak mosaic (Brakke (1971), agropyron mosaic (Slykhuis 1973) and ryegrass mosaic (Slyk-

Fig. 1. Leaves of *Bromus mollis* (A) and *Briza maxima* (B). On the left the leaves with symptoms of brome streak mosaic disease, on the right a healthy leaf as control.

Sl. 1. Listovi vrsta *Bromus mollis* (A) i *Briza maxima* (B). Na lijevoj strani listovi sa simptomima crtičavog mozaika ovsika, a na desnoj strani radi usporedbe po jedan zdrav list.

Fig. 2. Elongated particles of partially purified brome streak mosaic virus. Bar marker represents 300 nm.

Sl. 2. Produžene čestice djelomično purificiranog virusa crtičavog mozaika ovsika. Skala predočuje 300 nm.

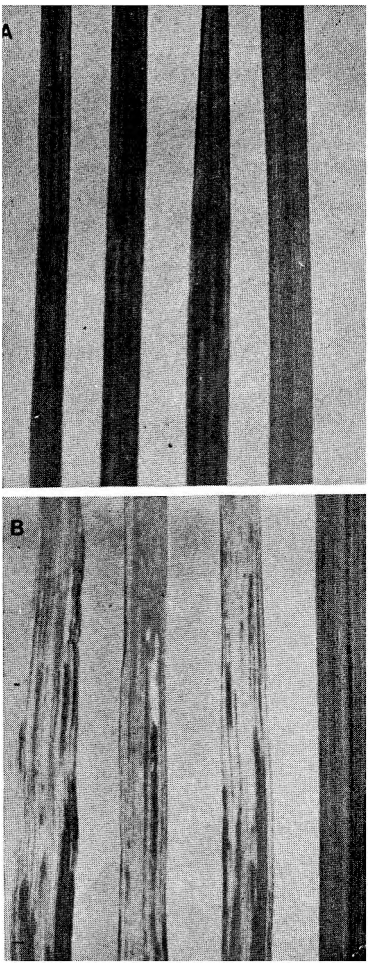

Fig. 1 — Sl. 1.

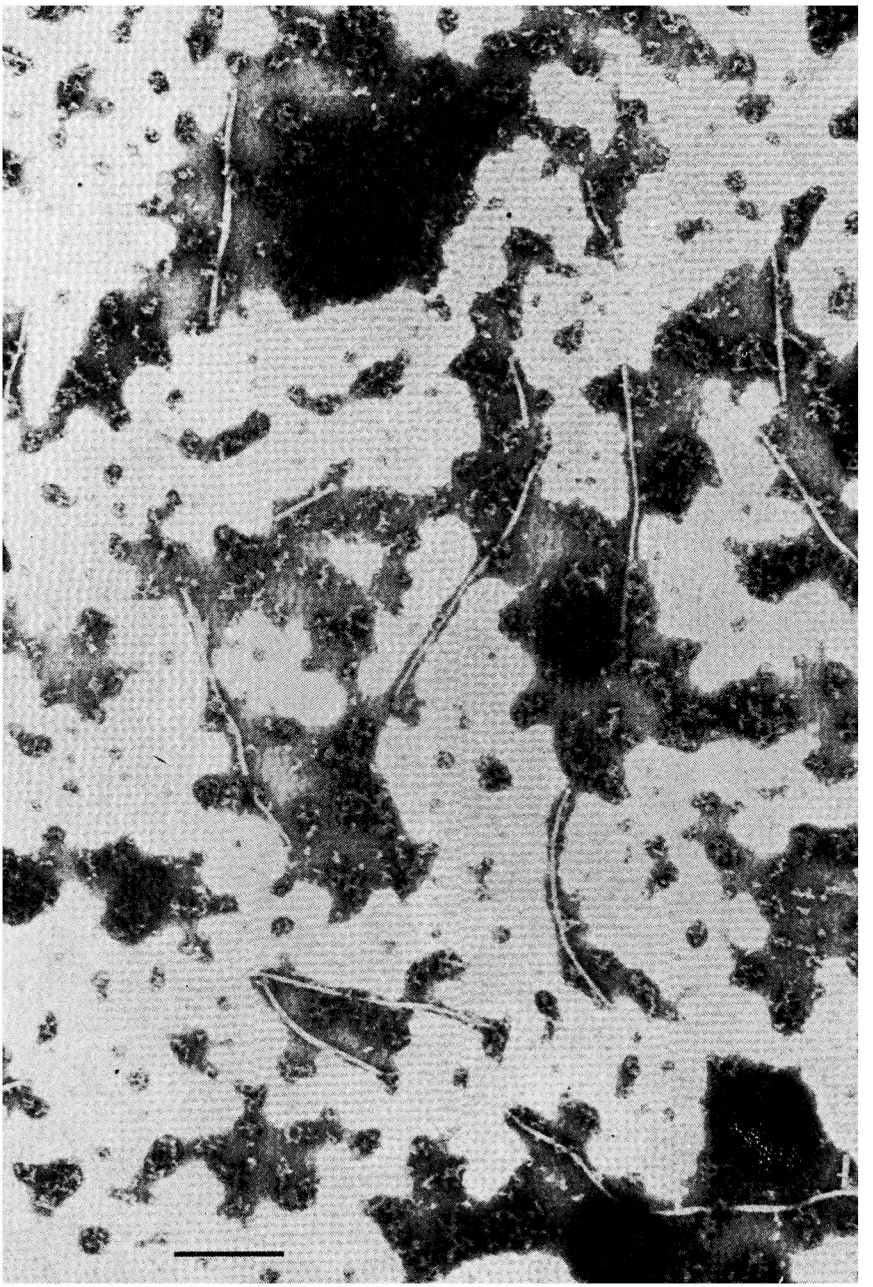

Fig. 2. — Sl. 2.

huis and Paliwal 1972). All these viruses have some mite species as their vectors. Wheat streak mosaic virus is transmitted by *Aceria tulipae* and *A. tossichella*. However, agropyron mosaic and ryegrass mosaic viruses are transmitted by means of *Abacarus hystrix*.

The BSMV described here is also a virus attacking plants which belong to the family *Poaceae*. It is also about 700 nm long and therefore shorter than the other potyviruses. BSMV also builds typical pin-wheel structures which belong to the second subdivision according to Edwardson (1974). With regard to these properties BSMV is similar to the cereal viruses cited. Therefore, BSMV cannot be considered a potyvirus before it is known by which vector BSMV is transmitted.

Accordingly, BSMV attacks only members of *Gramineae* and forms inclusions belonging to the second subdivision. Edwardson (1974) quotes three viruses which attack only *Poaceae* and form inclusions belonging to this subdivision. These viruses are: anthoxanthum mosaic virus, oat mosaic virus and ryegrass mosaic virus.

BSMV differs from oat mosaic virus in that it attacks various genera of *Gramineae* while oat mosaic virus infects only the genus *Avena* (Hebert and Panizo 1975). Ryegrass mosaic virus does not attack the wheat (Mulligan 1960), while BSMV is easily transmissible to wheat.

Anthoxanthum mosaic virus described by Catherall (1970) is the most similar to BSMV in the shape of inclusions. However, we have not succeeded in transmitting BSMV to *Anthoxanthum odoratum*. Above that these two viruses differ with regard to the particle length because anthoxanthum mosaic virus is longer and measures 740 to 760 nm.

The further investigation of BSMV will be carried in order to establish better the differences between BSMV and related viruses.

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SAŽETAK

NEKA SVOJSTVA VIRUSA CRTIČAVOG MOZAIKA OVSIKA

Davor Miličić, Đorđe Mamula i Miško Plazibat

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Virus crtičavog mozaika ovsika (VCMO = brome streak mosaic virus) nađen je prije više godina na vrsti *Bromus mollis* L. u okolici Zagreba. To je virus koji ima produženu i savitljivu česticu, dugu oko 693 nm. Virus je na mehanički način prenesen na otprilike 20 vrsta iz porodice *Poaceae*, među njima i na žitarice *Avena sativa* L., *Triticum vulgare* Vill. i *Hordeum vulgare* L. Karakteristično je da taj virus stvara cilindrične citoplazmatske inkluzije (pin-wheels) koje pripadaju drugoj podskupini prema razdiobi Edwardsona (1974).

Vrlo je pogodna pokusna biljka za VCMO *Briza maxima* L. koja reagira na infekciju vrlo jakim simptomima, a ponekad i nekrozama. U listovima te vrste stvaraju se također karakteristične cilindrične citoplazmatske inkluzije s laminatnim agregatima ali bez smotaka.

Da bismo bolje karakterizirali virus, purificirali smo ga na dva različita načina. Prvi način sastojao se najprije od homogeniziranja listova pomoću fosfatnog pufera uz dodatak potrebnih sastojina. Poslije toga je suspenzija bila dvaput obrađena kloroformom i izložena dvokratnom diferencijalnom centrifugiranju.

Drugu upotrebljenu metodu opisao je Slykhuis (1973), a korištena je samo za purifikaciju inficiranih listova biljke *B. maxima* L. Dosadašnja iskustva pokazuju da će i ta metoda biti upotrebljiva.

Spektrofotometrijska istraživanja obavljena su pomoću UV svjetla u području od 220 do 300 nm i pri tom je utvrđen apsorpcijski kvocijent A 260/A 280 = 1,28. Na osnovi tih istraživanja ustanovljeno je da se pomoću upotrebljene metode iz 1 kg listova inficiranog ječma dobiva oko 5 mg virusa.

Prof. dr. Davor Miličić
Đorđe Mamula, mr biol.
Miško Plazibat, inž. biol.
Botanički zavod PMF
Marulićev trg 20/II
YU-41000 Zagreb (Jugoslavija)

Note: During the print we received the paper »F. Rabenstein and A. Stanarius, 1981: Ein neuer Stamm des Weizenstrichelmosaik-Virus (wheat streak mosaic virus) von *Hordeum murinum* L. und *Bromus sterilis* L. Nachrichtenblatt f. Pflanzenschutz in DDR 35: 190«, according to which our brome streak mosaic virus is related to wheat streak mosaic virus.