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A POTYVIRUS ISOLATED FROM *BROMUS MOLLIS*

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Introduction

Although very large areas are covered with cereals and grasses in Yugoslavia, a relatively small number of viruses of *Gramineae* has been identified so far (Šutić 1974). The identified viruses are: European corn mosaic virus (Panjan 1960, Štefanac 1967), wheat streak mosaic virus (Tošić 1971, Juretić 1979, barley stripe mosaic virus (Šutić and Tošić 1964), brome mosaic virus (Miličić et al. 1966), and cucumber mosaic virus (Panjan 1966). Therefore, we decided to investigate these plants and we found a virus apparently new for this region.

In spring 1977 we succeeded in isolating one and the same virus first from *Bromus mollis* and later from *Hordeum murinum*. The isolate from *Bromus mollis* was found in the agricultural region south of Zagreb and the isolate from *Hordeum murinum* alongside a road in the surroundings of Zagreb. The isolates were maintained on barley and wheat. The isolate from *Bromus mollis* was used for further investigations.

Material and Methods

The isolate from *Bromus mollis* was cultivated in an insect proof glasshouse on barley. As barley was a very favourable plant for the cultivation of virus, we used it for investigations of the virus stability in sap and of the morphology of virus inclusions.

For electron microscopy leaves of barley with intensive symptoms were taken. Small pieces of leaves were fixed in 1% (v/v) glutaraldehyde in cacodylate buffer for 30 min and then postfixed in 1% (w/v) osmium tetroxide for 2 h. Fixed pieces were dehydrated in an alcohol series and afterwards embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate. Finally, the sections were examined in a Siemens Elmiskop I and in an electron microscope JEM 100 B.

Results

Investigation of host range

The *Bromus mollis* isolate was first transmitted to wheat and barley. During the warm season the first symptoms appeared on young leaves 10 days p. i. Approximately 60 per cent of inoculated barley plants showed clear symptoms of infection in the form of chlorotic lines. The symptoms were distinct and appeared first on young leaves but persisted also in old leaves (Fig. 1A).

During 1979 we established that many gramineous species were hosts to *Bromus* isolate (Tab. 1). The virus was successfully transmitted to some species which appertained to the genera *Avena, Bromus, Hordeum, Vulpia and Triticum.* That some of infected species were really

Table 1. Host range investigation of the *Bromus mollis* isolate

Tribus	Species	Reaction
Maydeae	*Zea mays* L.	—
Phalarideae	*Phalaris canariensis* L.	—
Aveneae	*Avena barbata* Pott.	+
"	*Avena fatua* L.	—
"	*Avena sativa* L.	—
"	*Avena sterilis* L.	++
Festuceae	*Melica nutans* L.	—
"	*Melica transsilvanica* Schur	—
"	*Briza maxima* L.	++
"	*Bromus erectus* Huds.	—
"	*Bromus molliformis* Lloyd	++
"	*Bromus mollis* L.	++
"	*Bromus secalinus* 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.	+
"	*Agropyron caninum* (L.) Pal.	—
"	*Secale cereale* L.	— —
"	*Triticum dicoccum* Schrank	+
"	*Triticum durum* Desf.	+
"	*Triticum monococcum* L.	—
"	*Triticum vulgare* Vill.	+ +
"	*Hordeum bulbosum* L.	—
"	*Hordeum jubatum* L.	—
"	*Hordeum murinum* L.	+
"	*Hordeum vulgare* L.	++

infected with *Bromus* isolate was confirmed by means of back infection of barley. These plants are marked in Table 1 with the sign ++. However, some *Gramineae* showed virus symptoms but the test of back infection was not accomplished. This group of plants is marked in Table 1 with the sign +. The third group remained without symptoms and was marked with —. Alltogether 30 gramineous species were tested from which 15 species reacted positively. Two species, *Lolium multiflorum* and *L. perenne* showed very weak symptoms and therefore were signed with +/—. In this case, the back inoculation tests were not carried out and thus it remains unknown whether these plants are virus hosts or not.

In addition to that, 14 dicotyledons were tested but none of them reacted positively after inoculation. These dicotyledons were: *Brassica rapa* L., *Chenopodium amaranticolor* Coste et Reyn., *Ch. quinoa* Willd., *N. glutinosa* L., *N. megalosiphon* Heurck et Muell. Arg., *N. tabacum* L. Hicks, *N. tabacum* L. Samsun, *N. tabacum* L. White Burley, *Petunia hybrida* hort. ex Vilm., *Pisum sativum* L., *Vicia faba* L., *Vigna cylindrica* (L.) Skeels, *V. sinensis* Savi ex Hassk. var. *variegata*. Thus, it seems that the *Bromus mollis* isolate has not an affinity for the dicotyledon plants.

Investigation of virus stability in sap

For this investigation the leaves of barley with obvious symptoms were homogenized in a mortar, and the mash was squeezed through a cheese-cloth to obtain the infective sap. Afterwards, the sap was centrifugated for 15 min at 3000 rpm and the supernatant was used. As test plants young barley plants were inoculated.

During these experiments the thermal inactivation point, the longevity in vitro and the dilution end-point of infective sap were investigated. The results are presented in Table 2. The inoculation of barley was performed by rubbing the leaves with carborundum as abrasive.

Table 2. Investigation of the stability of *Bromus mollis* virus in sap

Thermal inactivation point		Longevity in vitro at 23° C	
Control	13/20	Control	16/20
40° C	12/20	2 h	17/20
45° C	9/20	1 day	6/20
50° C	12/20	2 days	4/20
55° C	4/20	3 days	2/20
60° C	0/20	4 days	2/20
		5 days	2/20

Dilution end-point	
Control	12/20
10^{-1}	19/20
10^{-2}	2/20
10^{-3}	2/20
10^{-4}	0/20

The numerator denotes the number of infected plants, and the denominator the number of inoculated plants.

As it is visible from the data presented in Table 2, the isolate from *Bromus mollis* is a rather stable virus.

Investigation of transmission possibility of the Bromus isolate by aphids

An attempt was made to transmit the *B. mollis* isolate with *Myzus persicae* as vector. The experiments were first performed in a not persistent manner using a short feeding time which lasted 3 min. In other experiments we tried to transmit the virus after a long feeding time which lasted three days. These two experiments did not give positive result with *Myzus persicae* as a vector.

Electron microscopic investigations

The infected barley leaves with obvious symptoms were used for investigations of the morphology of virus particles. The leaves were cut and dipping preparations were made by using potassium phosphotungstate. In preparations filamentous and flexible virus particles were present (Fig. 1 B). The average length of 20 particles measured was 670 nm.

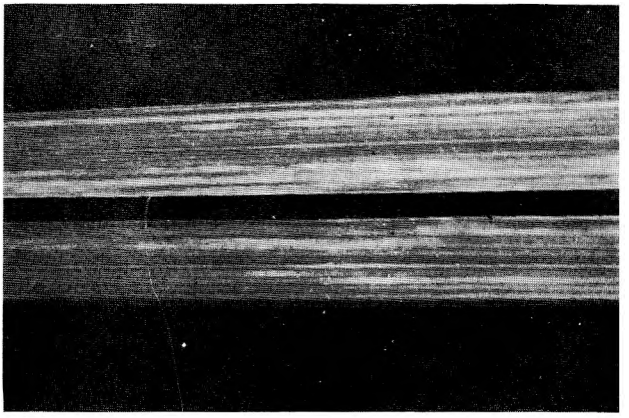
Barley leaves were also used for a study of alterations in infected cells. A large number of pin-wheel structures were found in the cells. The pin-wheels consisted of a large number of laminated aggregates which were ordinarily not curved but had very often the form of plane and fairly thick sheets connected round a centre (Figs. 2 and 4 i). The laminated aggregates were relatively long and sometimes ended on the surface of small vacuoles (Fig. 2 v). However, relatively thin laminated aggregates were sometimes curved (Fig. 2 and 3 c). Complicated formations would sometimes appear because of the dense position of pin-wheel structures and a large number of connections among their laminated aggregates (Fig. 3 f).

Fig. 1. **A.** Barley leaf parts with disease symptoms in the form of chlorotic streaks and stripes. **B.** Filamentous virus particles in crude sap treated with potassium phosphotungstate. Bar marker represents 300 nm.

Fig. 2. Electron micrograph of barley leaf cell showing mostly cross sectioned pin-wheel structures. Laminated aggregates are often thick and flat (i). Frequently two pin-wheels are connected with each other (t). The centre where laminated aggregates of one pin-wheel join together is sometimes fairly large (j). Some laminated aggregates end on the surface of vacuole (v). Bar marker represents 300 nm.

Fig. 3. **A.** Pin-wheel structure in longitudinal section forming a bundle (b). The peripheral parts of adjacent cells are altered. Specific from of a plasmodesma (p) in the altered region. **B.** Several cross sectioned pin-wheels are placed near each other and therefore show a very complex structure (f). Some laminated aggregates are very long (l) and curved (c). Bar marker in figure (A) also relates to figure B and represents 300 nm.

Fig. 4. Different forms of pin-wheels. Many laminated aggregates are flat and thick (i). Bar markers represent 200 nm.



A

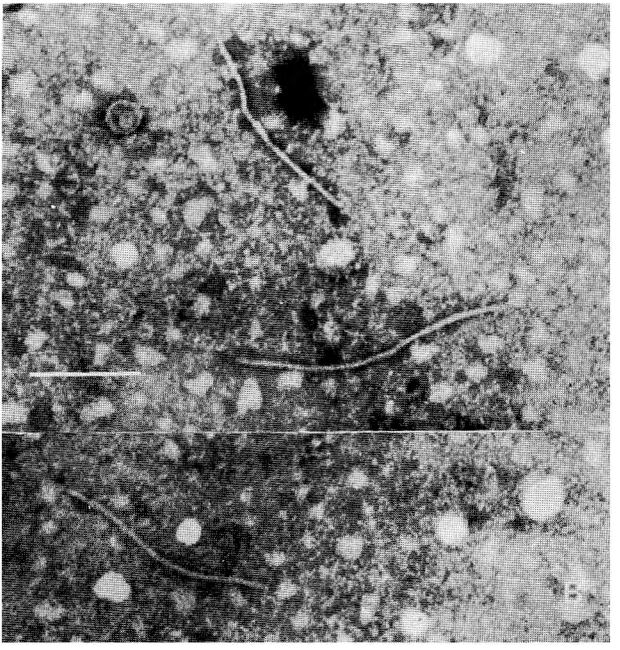


Fig. 1.

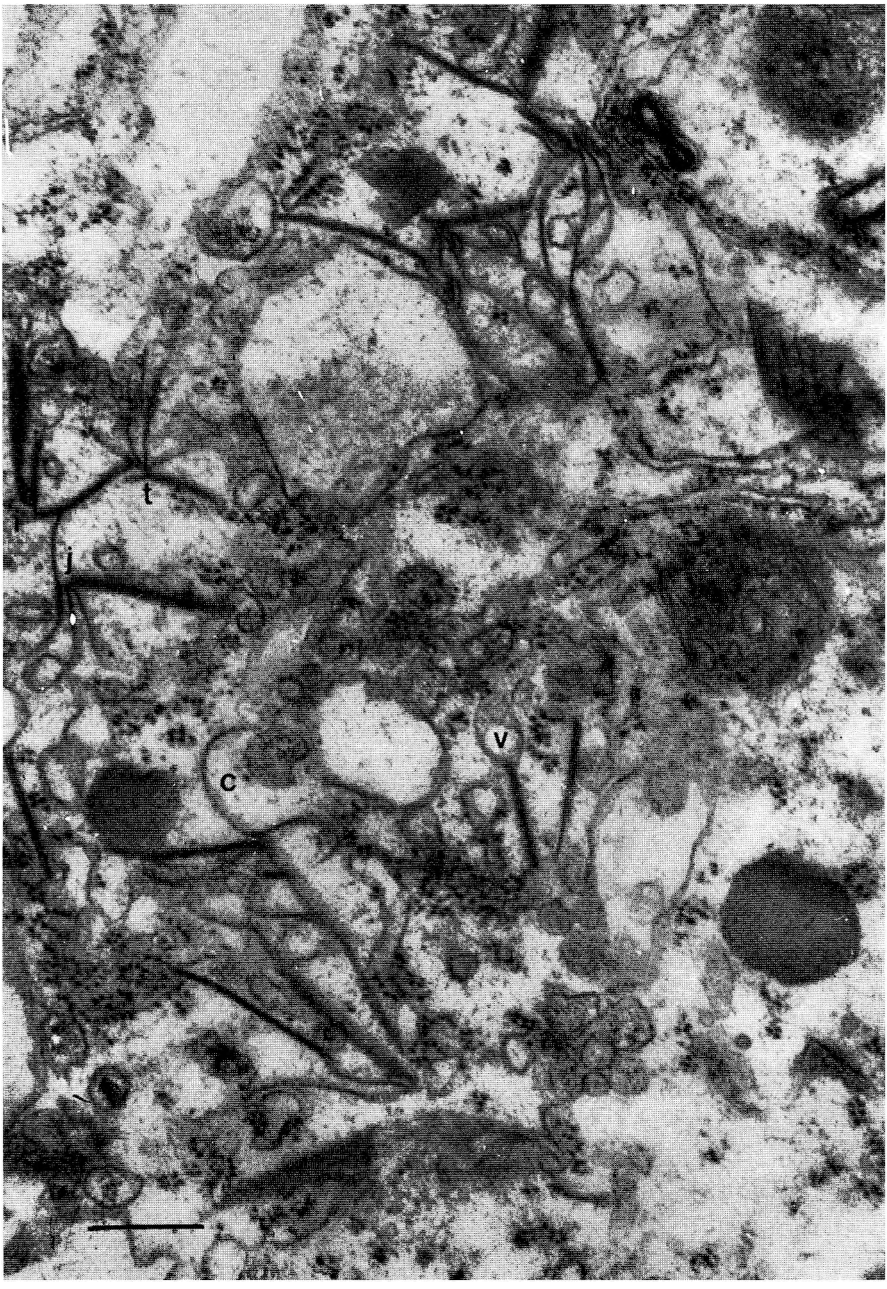


Fig. 2.

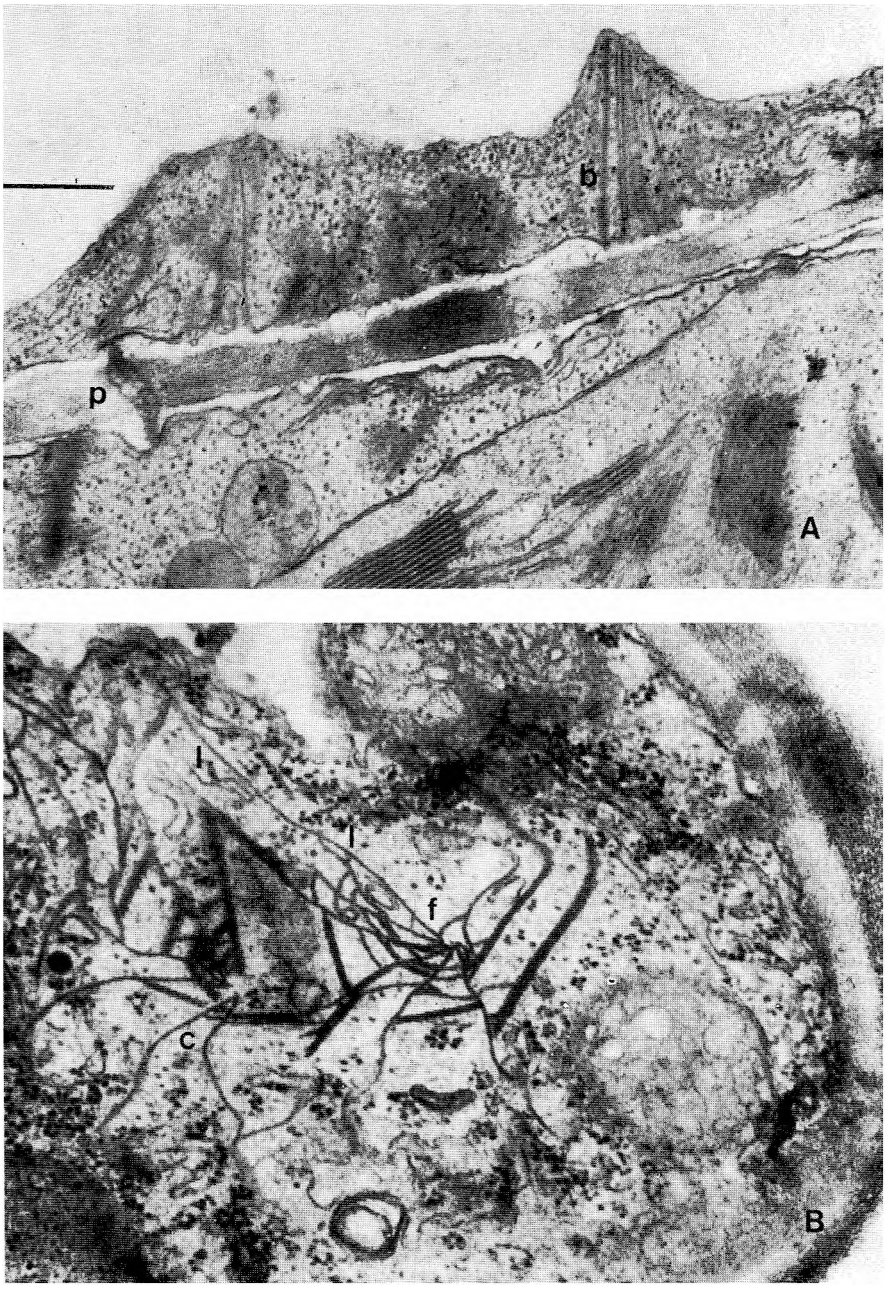


Fig. 3.

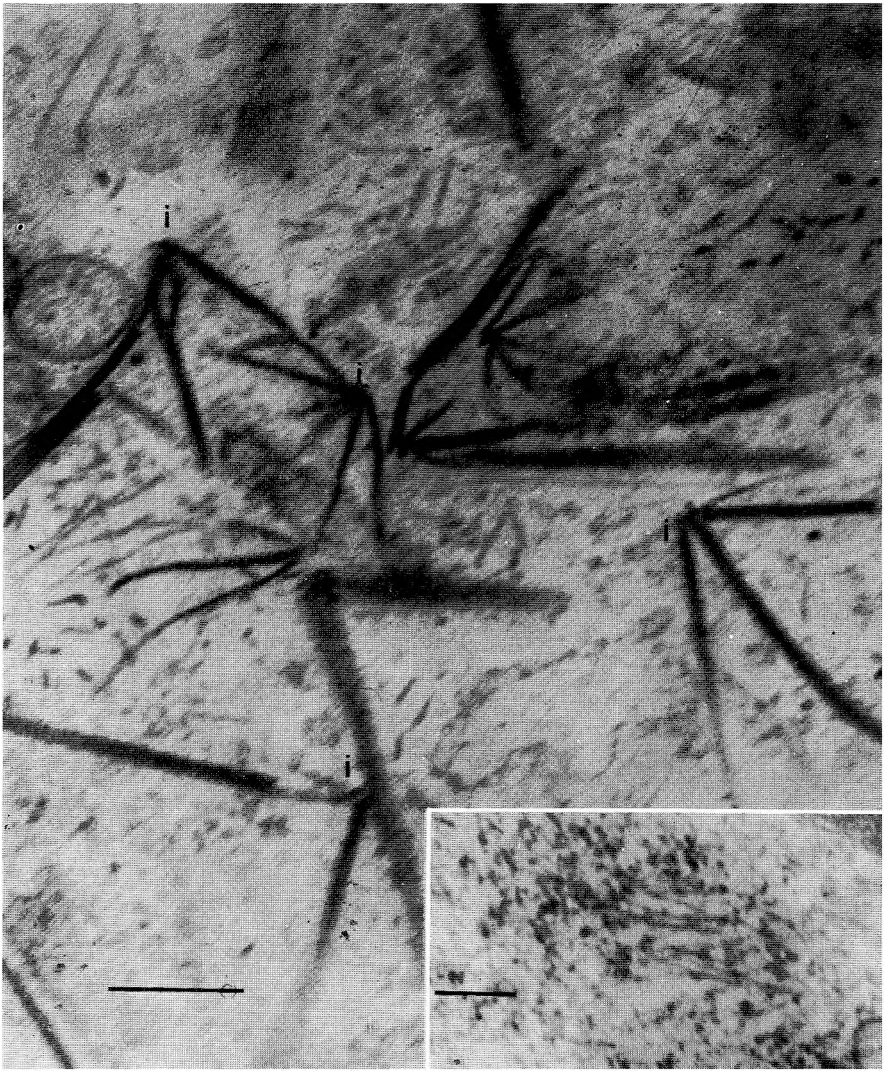


Fig. 4.

Edwardson (1974) devide the potyviruses into three subdivisions with regard to the morphology of their pin-wheel structures. The isolate of *B. mollis* surely belongs to the second subdivision. The characteristic of this subdivision is the presence of laminated aggregates and bundles and the absence of scrolls and tubes. In the work of Edwardson (1974) various types of inclusions belonging to the second subdivision are illustrated.

Discussion

Although the *B. mollis* isolate has not been identified till now, we want to point out the direction in which the next investigations should be carried out. We have most intensively studied the inclusions, and we have established that these bodies belong to the second subdivision of Edwardson (1974). According to Chamberlain (1977) the form of inclusions is diagnostic for dividing elongated viruses in subdivisions. Therefore, it is necessary to mention that Edwardson quotes three viruses of *Gramineae* which appertain to the second subdivision: anthoxanthum mosaic virus, oat mosaic virus and ryegrass mosaic virus. The *B. mollis* isolate differs from oat mosaic virus in the property that it attacks various genera of *Gramineae* while oat mosaic virus infects only the genus *Avena* (Hebert and Panizo 1975). In addition, ryegrass mosaic virus does not attack the wheat (Mulligan 1960, Slykhuis and Paliwal 1972), while the *B.* isolate is easily transmissible to wheat. Thus, it seems that the *B.* isolate resembles more anthoxanthum mosaic virus described by Catherall (1970).

Summary

Three years ago a virus was isolated from *Bromus mollis* and *Hordeum murinum* in the surroundings of Zagreb in Yugoslavia. This *B. mollis* isolate is fairly stable and is easily mechanically transmissible to various species of *Hordeum, Bromus, Triticum, Avena,* and *Vulpia.* Its particles are filamentous and flexible with a length of 670 nm. In these experiments the virus was not transmissible by *Myzus persicae.* In the infected plants it causes characteristic pin-wheel structures with laminated aggregates but without scrolls. The virus can survive in the squeezed sap more than 5 days at room temperature and remain infective at a dilution 1:1000.

*

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SAŽETAK

POTYVIRUS IZOLIRAN IZ VRSTE *BROMUS MOLLIS*

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Prije tri godine izoliran je virus prvo iz vrste *Bromus mollis*, a zatim iz vrste *Hordeum murinum* u okolici Zagreba. Taj virusni izolat prilično je stabilan i lako se prenosi mehanički na različite vrste rodova *Hordeum, Bromus, Triticum, Avena* i *Lolium*. Čestice virusa su nitaste a njihova dužina iznosi oko 670 nm. Lisna uš *Myzus persicae* ne može prenositi izolirani virus.

U stanicama inficiranih biljaka virus stvara karakteristične vrtuljke (pin-wheel) s laminatnim agregatima ali bez smotaka (scrolls). Virus može zadržati infektivnost u sirovu soku više od pet dana pri sobnoj temperaturi a njegova krajnja točka razrjeđenja iznosi 1:1000.

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