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CHARACTERIZATION, PURIFICATION AND SEROLOGY OF THE CZECHOSLOVAK ISOLATE OF RADISH MOSAIC VIRUS

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Summary. - The radish mosaic virus 1 (RMV 1) isolate was characterized according to the reaction of differential test plants and then purified by the chloroform-butanol method. UV-analysis of sucrose density gradients at 254 nm revealed three components. The values of $A_{260/280}$ ratio were 1.42 for the top, 1.49 for the middle and 1.67 for the bottom component, respectively. Electron microscopy of the purified virus revealed isometric particles about 30 nm in diameter. Two antisera were prepared with the titre 1:1024 and 1:2048, respectively. The RMV 1 antigen formed a distinct precipitation line with the antiserum against the California neo-type strain of RMV.

Key words: radish mosaic virus; purification; serology; Brassicaceae

Introduction

The occurrence of radish mosaic virus (RMV) - a member of the comovirus group - on the territory of the CSFR was found for the first time in 1984. The isolate of the virus designated as RMV 1 was obtained from winter turnip rape (*Brassica napus* L. var. silvestris (Lam.) Briggs) cv. Perko PVH near Český Krumlov in South Bohemia (Fig. 1). The isolate was identified on the basis of the host plant's reaction, electron microscopy and transmission by vectors (Špak, 1986).

The preparation of an antiserum was a prerequisite for further studies on the occurrence and distribution of RMV on Brassica crops and the characterization of serological properties and virulence of Czech and Slovak isolates. The results obtained during characterization of the RMV 1 isolate, purification and preparation of antisera are presented in this paper.

Materials and Methods

Inoculation of test plants. Test plants (Table 1) were grown throughout the experiments in an insect-free glasshouse. In the phase of four true leaves the plants were dusted with carborundum

600 mesh and inoculated with the virus isolate using a glass rod. Inoculum was prepared by grinding in a mortar 1 g of the leaves of white mustard *Sinapis alba* L. cv. Přerovská, 14 days after inoculation, with 2 ml of 0.1 mol/l phosphate buffer pH 7.0. The symptoms on inoculated leaves and of systemic reaction were evaluated 10 and 30 days after inoculation, respectively. Five plants from each species were used in each experiment which was repeated twice. The sap from symptomless plants was inoculated back on *S. alba* plants in order to reveal latent infection of plants.

Purification. S. alba L. cv. Přerovská leaves with symptoms of systemic infection 14 to 16 days after mechanical inoculation with the RMV 1 isolate were collected and stored at -20 °C. The material was blended at 4 °C in 0.066 mol/l potassium phosphate buffer pH 7.6 (1.5 ml/g of leaf tissue). The same buffer was applied for all steps of preparation. After filtration through a cheese cloth, a chloroform - n-butanol mixture 1:1 was added to make a final concentration of 20 %. This liquid was stirred for 2 hrs at 4 °C and the resulting emulsion was separated by low speed centrifugation at 8500 rev/min for 10 min in a Janetzki K 24 centrifuge. The clear yellow supernatant aqueous phase was subjected to high-speed centrifugation (35 000 rev/min for 100 min in a Beckman Ti 50.2 rotor). The pellets were resuspended in the buffer and stirred at 4 °C overnight. The virus was separated from the host components by a 10-40 % sucrose density gradient centrifugation at 24 000 rev/min for 195 min in a Beckman SW 28 rotor. Gradients were analysed on a UV-detector where UV absorption at 254 nm was measured and recorded. Virus containing fractions were collected, diluted 4 times and concentrated by centrifugation at 50 000 rev/min for 100 min in a Beckman Ti 50.2 rotor. The final pellets were resuspended and characterized spectropho-

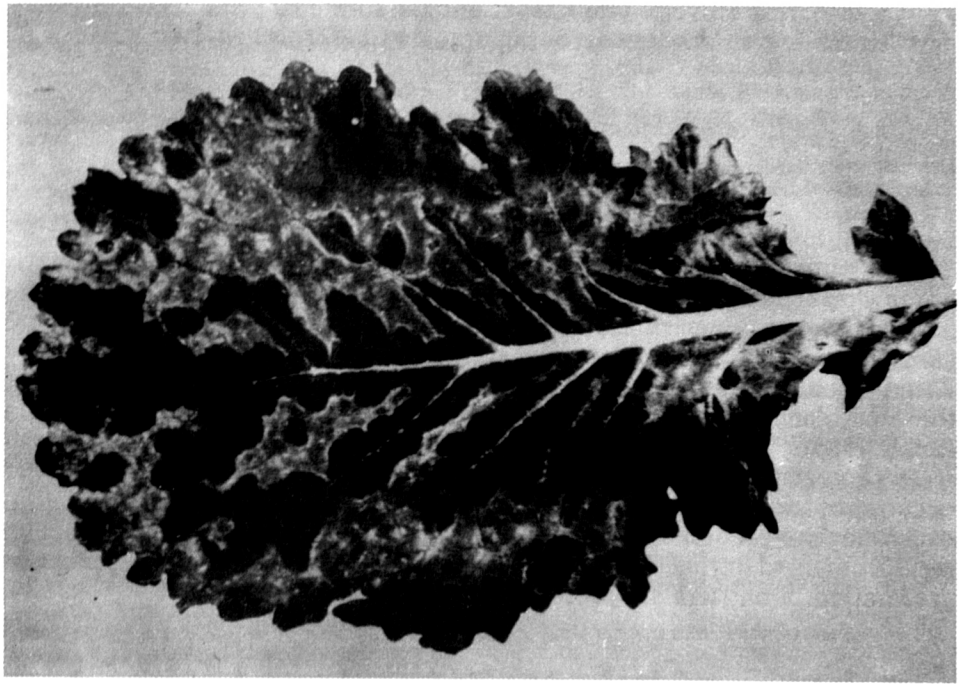

Fig. 1
Symptoms of radish mosaic virus infection on the leaf of winter turnip rape cv. Perko PVH

tometrically from 230 to 330 nm on a Varian UV-spectrophotometer. The virus concentration was determined using the E_{260} coefficient (1 mg/ml, 1 cm light path) 9 for the middle and 11 for the bottom component, respectively (Campbell, 1973).

Electron microscopy. The virus particles were visualized using the Philips electron microscope. Preparations were stained with 2 % uranyl acetate.

Preparation of antisera. Antisera were obtained by immunizing two New Zealand white rabbits with two intramuscular injections in a two-week interval. Each injection contained 1 mg/ml of the virus emulsified with Freund's incomplete adjuvant. A third, intravenous injection containing 5 mg of the virus (top, middle and bottom component) was applied a month later. The titre of antisera was determined by double diffusion test against sap from mustard leaves 14 days after inoculation with RMV 1 isolate. Infectious leaves were ground and 0.9 % Difco Noble agar was prepared in 0.01 mol/l Mc Ilvaine buffer pH 7.0 containing 0.02 % sodium azide.

Results

Reactions of differential host plants on the mechanical inoculation with the RMV 1 isolate are summarized in Table 1. The isolate was of relatively low virulence to cultivated brassicas.

The partially purified virus was found to consist of several components when subjected to density gradient centrifugation. Two clearly defined opalescent bands were visible 23 and 28 mm below the meniscus. Fractionation of the

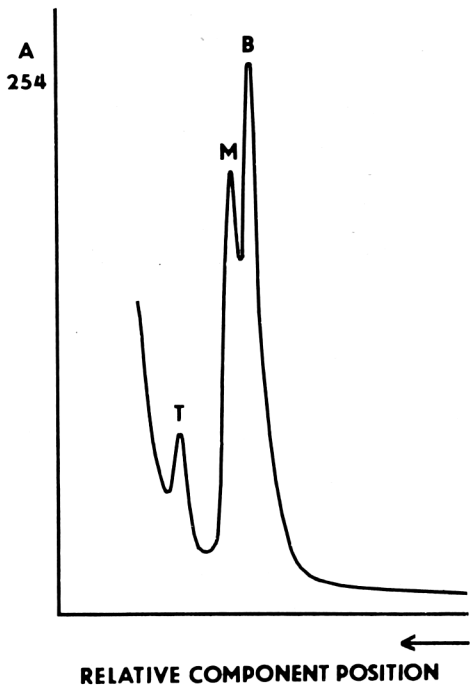

Fig. 2
Positions of the isolate RMV 1 components after centrifugation in 10-40 % sucrose density gradient

gradients at 254 nm revealed three components, which are hereafter referred to as the top (T), middle (M) and bottom (B) components, respectively (Fig. 2). The T component was found just below the meniscus.

UV-absorption spectra of single components are demonstrated on Fig. 3. The values of $A_{260/280}$ ratio were 1.42 for the T, 1.49 for the M and 1.67 for the B components, respectively. The yield of purification of the M and B component was 7.48 mg for the former and 3.61 mg for the latter from 100 g of fresh material. The proportion of M:B components was 1:2. The yield of the virus was about 10 mg per 100 g of fresh leaf material.

Electron microscopy of a suspension of the purified virus revealed isometric particles about 30 nm in diameter. Some of them appeared to be hollow and partially destroyed. Unfortunately electron microscopy of single components

Table 1. Reactions of the test plants on the inoculation with the RMV 1 isolate

Plant species	I	II
Brassicaceae		
B. napus L. cv. Tandem	nL	N
B. napus L. var silvestris (Lam.) Briggs cv. Perko PVH	no	M,N
B. nigra (L.) Koch	cL	M,Ch
B. pekinensis (Lour.) Rupr.	no	M
B. rapa L. var. rapa	nL	M,N
B. oleracea L. var. gongylodes	no	+
B. oleracea L. var. sabauda	no	no
B. oleracea L. var. capitata	no	no
B. oleracea L. var. botrytis	no	no
Sinapis alba L.	cL	cR,M,C,N,P
Raphanus sativus L.	no	no
Non-Brassicaceae		
Nicotiana megalosiphon Heurck et Muell.	nR	M,nR
N. tabacum L. cv. Samsun	nL	no
N. glutinosa L.	nL	no
Gomphrena globosa L.	no	no
Chenopodium quinoa Willd.	nL	no
Chenopodium murale L.	nL	no
Reseda odorata L.	no	no

no = symptoms not observed
nL = local necrotic lesion
cl = local chlorotic lesion
cR = chlorotic rings
nR = necrotic rings
I = symptoms on inoculated leaves
+ = latent infection
N = necrosis
P = pattern
M = mottling
Ch = chlorosis
C = crinkling
II = systemic symptoms

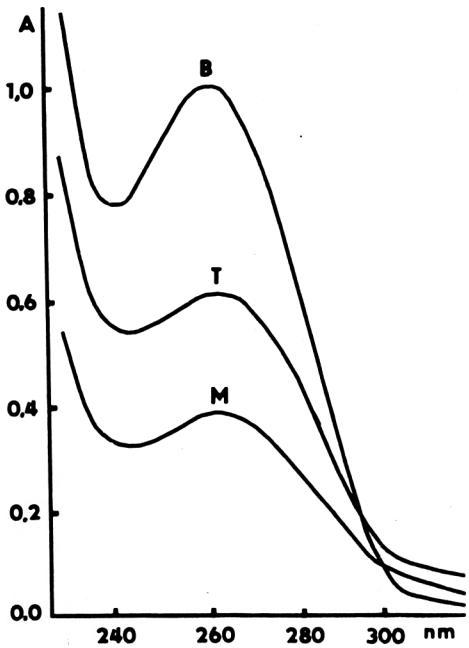

Fig. 3
UV-absorption spectra of the RMV 1 isolate top (T), middle (M) and bottom (B) components

which could confirm the possibility of larger damage of the M component during the purification was not made.

The virus was a good immunogen. After three injections the titre of antisera reached 1:1024 and 1:2048, respectively. Antisera reacted neither with healthy plant sap from *S. alba*, nor with the turnip yellow mosaic, cauliflower mosaic, turnip mosaic and Erysimum latent viruses. The virus antigen formed a distinct precipitation line in a double-diffusion test with the antiserum against the California neo-type strain of RMV (provided by Prof. R. N. Campbell).

Discussion

The symptoms of infection on the differential host plants were very similar to those obtained by Shukla and Schmelzer (1974) who compared the Yugoslavian (HZ) and German (DT 4) isolates of RMV. In particular „maple leaf" like patterns on *S. alba* leaves and necrotic rings on *N. megalosiphon* leaves were identical with those of the DT 4 isolate on these plants on the photographs published by Shukla and Schmelzer (1974).

The presence of two visible and three UV-detected components of purified RMV 1 corresponds with the results obtained by Hollings and Stone (1969), Campbell (1973), Krylov *et al.* (1981) and Mamula and Juretič (1985). Only

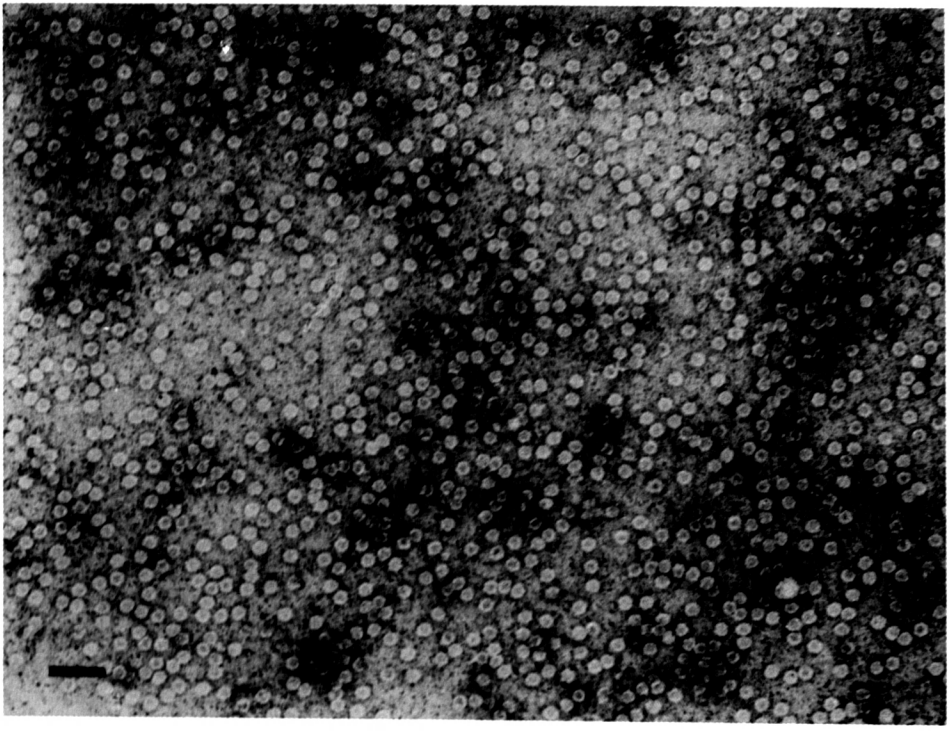

Fig. 4
Purified particles of the RMV 1 isolate. Bar represents 100 nm

some of the authors who have purified the RMV mentioned a ratio of single components of the virus and $A_{260/280}$ values.

The ratio of M:B components was quite different from the ratio 2:1 or 4:1 published by Campbell (1973), 1:1 found by Hollings and Stone (1969) and 1:37:1 or 1:16:1 by Juretič and Fulton (1974). Juretič and Fulton (1974) obtained four components with $A_{260/280}$ values of Ta 1.00; of T 1.25; of M 1.50; and of B 1.49, similarly as Krylov *et al.* (1981) who found values of Ta 1.00; of T 1.59; of M 1.7; and of B 1.77. Hollings and Stone (1969) published values of M 1.61 and of B 1.68, Campbell (1973) of M and B 1.65 and 1.78, respectively. This could be the result of a different purification procedure, virus isolate or length of virus reproduction in plants used in the experiments. Juretič and Fulton (1974) found that the relative component position and yield of single components are markedly influenced by purification buffer, time length of centrifugation and moreover by the season of virus production.

The yield of the purified virus is in good correlation with the results published by Campbell (1973). The chloroform-butanol purification method yielded an appropriate amount of the virus of sufficient purity similarly as in experiments described by Mamula and Juretič (1985) and Shukla *et al.* (1973). Further studies aimed on the occurrence of RMV on different host plants in the CSFR and characterization of their virulence and serological properties are in progress.

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