COMPARISON OF THE CZECH *SCROPHULARIA* ISOLATE WITH THE ITALIAN *ANAGYRIS* STRAIN OF SCROPHULARIA MOTTLE VIRUS

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Summary. – Scrophularia mottle virus (ScMV) was newly found in the Czech Republic in *Scrophularia nodosa* L. plants. The Czech isolate (ScMV-C) was serologically identical and similar in symptoms and host range to the Italian *Anagyris* strain (ScMV-I). *Nicotiana tabacum* L. cv. Samsun, *Nicotiana glutinosa* L., *Nicotiana tabacum* L. cv. White Burley, *Physalis floridana* Rybd. and *Cucumis sativus* L. are described as new host plants of ScMV. Double-stranded RNA patterns and the isoelectric point of this virus are characterized.

Key words: tymovirus; *Scrophularia nodosa* L.; serology; purification; host range

Introduction

ScMV belongs to the tymovirus group according to its isometric particles of diameter about 27 nm, one genomic single-stranded RNA (ssRNA) of positive polarity and M_r about 2.1×10^6, and single coat protein of M_r 21.6 K (Bercks, 1973). The virus is readily transmissible by mechanical inoculation and by beetles *Cionus* sp. (Hein, 1959; Bercks, 1973). According to the phylogenetic analysis of the coat protein ScMV belongs to the Andean potato latent virus (AnPLV) subgroup of tymoviruses (Rybicki, 1991) and could provide a serological link between Turnip yellow mosaic virus (TYMV) and an PLV subgroups (Bercks *et al.*, 1971; Koenig, 1976; Koenig and Givord, 1974). Until now ScMV has been found in Germany (Hein, 1959) and Italy (Rana *et al.*, 1988).

The characterization of a Czech isolate from *Scrophularia nodosa* L. and its comparison with the *Anagyris* strain of the ScMV from Italy are reported in this paper.

Materials and Methods

Viruses. The Czech isolate of ScMV obtained from *S. nodosa* (ScMV-C) and the Italian isolate of the ScMV from *Anagyris foetida* L. (ScMV-I) (Rana *et al.*, 1988) were maintained on *Datura stramonium* L. plants.

Host plants. Fifteen plants of 19 different host plants (Table 1) were mechanically inoculated by the sap from ScMV-I and ScMV-C infected plants. Inoculum was obtained by homogenization of the leaves with disease symptoms in Sörensen phosphate buffer pH 7.0 (1:1 w/v). Reactions of host plants were evaluated 21 days after inoculation and the presence of the virus in symptomless plants was determined serologically. The experiments were repeated three times.

Virus purification. Both isolates were purified from *D. stramonium* leaves 21 days after inoculation. One hundred grams of leaves were homogenized in 0.5 mol/l phosphate buffer pH 7.2 (3:1 v/w) containing 1 % mercaptoethanol and stirred in ice bath for 1 hr. After centrifugation at 7000 rpm for 10 mins the supernatant was clarified by butanol/chloroform (10:6 v/v) extraction for 30 mins at room temperature. The virus was precipitated with 4 % PEG 6000 and 4 % NaCl for 1 hr, collected by centrifugation at 16 000 rpm for 15 mins and resuspended in 10 mmol/l phosphate buffer pH 7.2. Further purification was done by gel exclusion chromatography on Sephadex G-50 (Pharmacia) column and elution with 10 mmol/l phosphate buffer pH 7.0. The firstly eluted highly absorbing material was collected and used for preparation of antisera. Virus components were separated by a 10–30 % sucrose density gradient centrifugation. The sedimentation coefficient corrected for the standard conditions (S_{20}, w) was determined in a Spinco analytical ultracentrifuge at 25 000 rpm in 10 mmol/l phosphate buffer pH 7.0 at 15 °C from three experiments.

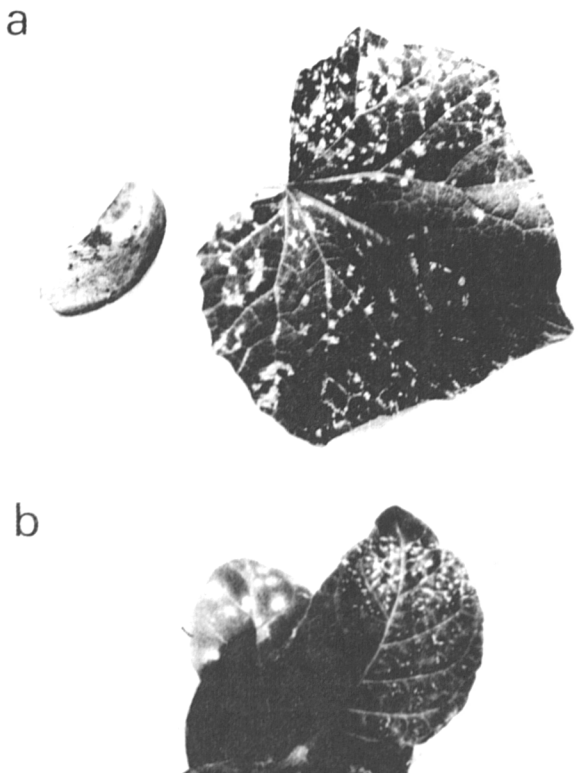

Fig. 1
Reactions of host plants after inoculation with ScMV-C
(a) *Cucumis sativus*, necrotic lesions on inoculated leaves (left) and vein clearing and mosaic (right). (b) *Nicotiana tabacum* L. cv. Samsun, necrotic rings on inoculated leaves and mosaic.

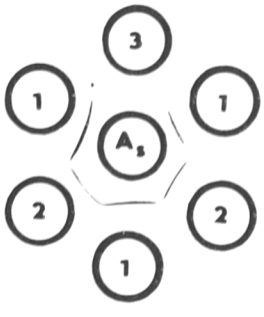

Fig. 2
Double diffusion test
Sap from ScMV-C infected *D. stramonium* (1), sap from ScMV-I infected *D. stramonium* (2), sap of healthy *D. stramonium* (3), antiserum to ScMV-C (middle well).

Antisera against ScMV-C and ScMV-I isolates were prepared each in two rabbits immunized by three intramuscular injections of the virus emulsified in Al-Span Oil incomplete adjuvant, followed by two intravenous injections given in 14 days intervals without adjuvant. For each injection 0.4 mg (ScMV-C) and 1.3 mg (ScMV-I) of purified virus were used.

Double diffusion tests in agar were conducted as described by Rana *et al.* (1988). Both the purified virus and sap from *D. stramonium* leaves infected with the two isolates of ScMV served as antigens.

Isoelectric point of the isolates was determined by nonequilibrium pH gradient electrophoresis in 1 % agarose gel with 12 % (w/v) sorbitol and 3.0 % (v/v) of the 4–9 Ampholyte (Serva), and with 0.5 N NaOH and 0.02 N H_2SO_4 as electrolytes.

Nucleic acids were isolated from purified viruses by phenol-chloroform extraction and ethanol precipitation (Maniatis, 1982) and separated in 1 % agarose gel. Double-stranded RNAs (dsRNAs) were isolated from 1 g of infected *D. stramonium* leaves and separated by 5 % polyacrylamide gel electrophoresis (PAGE) according to Valverde *et al.* (1990). M_r of the coat protein was estimated by 7.5–15 % gradient PAGE (Laemmli, 1970).

Electron microscopy. Precipitations for transmission electron microscopy were stained with 1 % uranyl acetate. Thin sections were prepared from small pieces of infected *D. stramonium* leaves fixed in 0.1 mol/l phosphate buffer pH 7.3 with 5 % glutaraldehyde and 4 % sucrose for 20 mins at room temperature under vacuum. The samples were postinfixed in 1 % osmium tetraoxide, dehydrated by washing in solutions with increasing ethanol content and embedded in Durcupan (Fluka). Thin sections were double-stained with uranyl acetate in 70 % ethanol and lead citrate and examined in Philips 420 electron microscope.

Results and Discussion

Host symptoms

Naturally infected plants of *S. nodosa* with dark green mottling mosaic on leaves were found in a plant community Quercus-Betula in South Bohemia. Only isometric particles of diameter about 27 nm were observed in electron microscope in sap from these plants. These particles did not react with antisera against TYMV, Erysimum latent virus, Arabis mosaic, Broad bean wilt, Cucumber mosaic, Radish mosaic and Turnip mosaic viruses.

The Czech and Italian isolates successfully infected 10 from 19 different host plant species tested (Table 1). *Chenopodium amaranticolor* Coste et Reyn., *C. quinoa* Willd., *Sinapis alba* L., *Lactuca sativa* L. and *Spinacia oleracea* L. were not susceptible to infection. Unfortunately, we only had the possibility to compare host reactions of ScMV-I and ScMV-C isolates as our effort to obtain the third isolate from Germany (ScMV-G) described by Hein (1959) has been unsuccessful.

Table 1. Reactions of host plants to the infection with ScMV-C and ScMV-I

Host plant	Symptoms			
	ScMV-C		ScMV-I	
	local	systemic	local	systemic
Nicotiana glutinosa L.	NL	Mb,St	NL	Mb,St
N. clevelandii Gray	NR	NV	NL	Mb
N. tabacum L. cv. Samsun	NR	Mb	NR	Mb
N. tabacum L. cv. White Burley	CR,NR	Mb	CR,NR	Mb
N. benthamiana Domin.	CL	Mb,LC	CL	Mb,LC
Datura stramonium L.	NL	Mb	NL	Mb
Physalis floridana Rybd.	CL	Mb	CL	Mb
Petunia hybrida Hort et Vilm.	LI	M, LC	LI	M, LC
Cucumis sativus L.	NL	VC, M	n.i.	n.i.
Antirrhinum majus L.	LI	LI	LI	VC, M
Phaseolus vulgaris L.	LI	St	LI	St

CL – chlorotic lesion; LI – latent infection; NL – necrotic lesion; St – stunting
CR – chlorotic rings; M – mosaic; NR – necrotic rings; VC – vein clearing
LC – leaf curl; Mb – blister mosaic; NV – necrosis along veins; n.i. – no infection

ScMV-C infected *C. sativus* plants displayed necrotic lessions on cotyledons and vein clearing and mosaic on leaves (Fig. 1a), whereas ScMV-I infected plants remained symptomless similarly as with ScMV-G (Hein, 1959).

ScMV-C produced necrotic rings and systemic vein necrosis on *N. clevelandii*. This plant species infected with ScMV-I in our experiments developed necrotic lesions and leaf blister mosaic in contrast to symptomless infection in inoculated leaves found by Rana *et al.* (1988).

We found *Antirrhinum majus* L. to be a latent host of ScMV-C and a systemic host of ScMV-I. *Nicotiana glutinosa* L. and *Physalic floridana* Rybd. were systemic hosts of ScMV-C and ScMV-I, whereas Rana *et al.* (1988) found these three species not to be susceptible to ScMV-I. ScMV-G pronounced necrotic and chlorotic lessions on *A. majus* and brown local lessions on *N. glutinosa* (Hein, 1959).

Petunia hybrida Hort et Vilm. was not infected systemically with ScMV-G (Hein, 1959), whereas ScMV-C and ScMV-I developed on *P. hybrida* mosaic, vein clearing and leaf curl in our experiments. Rana *et al.* (1988) described symptomless systemic infection on *P. hybrida*. Our results did not confirm the susceptibility of *Pisum sativum* L. and *Vicia faba* L. var. *equina* Pers. to ScMV-C and ScMV-I as found by Rana *et al.* (1988) and Hein (1959).

N. tabacum L. cv. Samsun (Fig. 1b), *N. glutinosa* L., *N. benthamiana* Domin, *N. tabacum* L. cv. White Burley, *Datura stramonium* L. and *Physalis floridana* Rybd. produced a similar systemic green blister mosaic when infected with ScMV-C and ScMV-I.

N. tabacum L. cv. Samsun, *N. tabacum* L. cv. White Burley, *N. glutinosa* L., *Physalis floridana* Rybd. and *Cucumis sativus* L. were previously not described as host plants of ScMV.

Serology

The prepared antisera had titer 1:256 and did not react with the sap form healthy plants of *D. stramonium*. Both the isolates reacted with these antisera, did not form spur lines and were serologically indistinguishable (Fig. 2).

Purification, electron microscopy and isoelectric point

The $A_{260/280}$ ratio of purified ScMV-I and ScMV-C was 1.65 and 1.63, respectively. The virus yield was 22.4 mg from 100 g leaves with ScMV-C and 41.1 mg with ScMV-I.

Electron micrographs of both isolates showed numerous "full particles" (nucleoprotein particles) and "empty shells" (capsids only) of diameter 25–27 nm (Fig. 3) in accord with data of Rana *et al.* (1988), Bercks *et al.* (1971) and Bercks (1973). In ultrathin sections alterations typically associated with tymovirus infections were seen. Chloroplasts developed small peripheral vesicles bounded by double membranes and later they rounded and clumped similarly as described by Koenig and Lesemann (1979) and Lesemann (1977). Numerous virus particles were observed in vacuoles and in the cytoplasm.

The isoelectric points of intact particles of ScMV-C and ScMV-I were 3.9 ± 0.1 and 4.4 ± 0.3, respectively, for both bottom (B) and top (T) components.

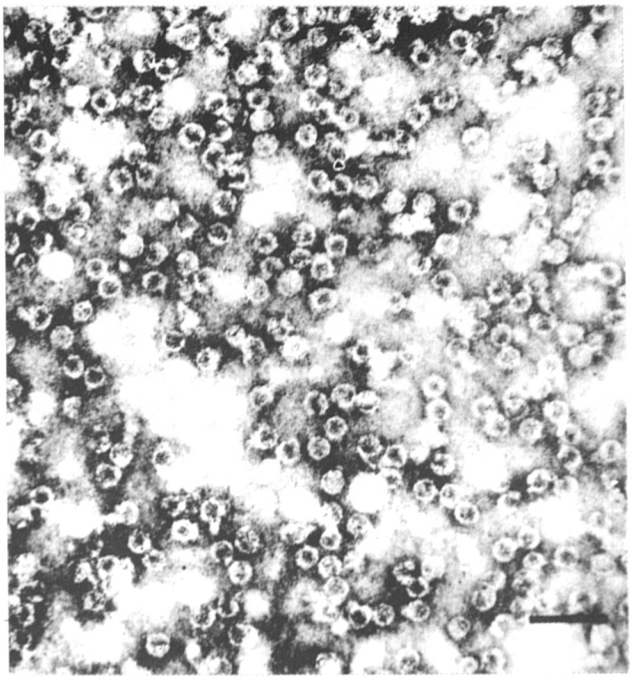

Fig. 3
Electron micrographs of purified ScMV-C
Bar = 100 nm.

Sedimentation coefficient of ScMV-C

The sedimentation coefficients ($S_{20,w}$) of B and T components of ScMV-C were 112 ± 3 and 57 ± 3 S, respectively. These results slightly differ from those published by Rana *et al.* (1988), who found 103 S and 49 S, respectively, for ScMV-I, and from those of Bercks *et al.* (1971), who determined 116 S and 54 S, respectively, for ScMV-G.

Viral coat protein and nucleic acids

The coat proteins of ScMV-C and ScMV-I formed in PAGE single band of M_r 21.3 K and 20.3 K, respectively in accord with M_r 20.0 K reported for ScMV-I by Rana *et al.* (1988). Virions contained ssRNA of just one size (M_r 2.0×10^6) and no subgenomic RNAs. On the other hand, several dsRNAs of various length were isolated from *D. stramonium* plants infected with ScMV-C. These dsRNAs had M_r 4×10^6, 3.5×10^6, 0.5×10^6. The dsRNAs isolated from plants inoculated with ScMV-I were identical.

There is only a limited information about the spectrum of dsRNAs in plants infected with tymoviruses. TYMV produces during its life cycle small amount of subgenomic ssRNAs, and several subgenomic dsRNAs were isolated *in vitro* (Gargouri *et al.*, 1989). Also dsRNA of double genomic lenght (M_r 4×10^6) was reported (Garnier *et al.*, 1980).

The comparison of physical characteristics showed differences in the sedimentation coefficient and isoelectric point of ScMV-C components and in M_r of ScMV-C coat protein as compared to other ScMV isolates. According to these results ScMV-C seems to be closely related to the German isolate, ScMV-G (Bercks *et al.*, 1971; Bercks, 1973).

On the other hand, ScMV-I and ScMV-C isolates were serologically indistinguishable, whereas ScMV-G and ScMV-I were serologically different in experiments conducted by Rana *et al.* (1988).

With respect to similarities in host plant reactions of ScMV-C and ScMV-I and their serological relatedness it seems that ScMV-C is more related to ScMV-I than to ScMV-G.

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