Acta Bot. Croat. 45, 7—19, 1986.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 576.858.8:582.662(497.1) = 20

CHARACTERISTICS OF THE TOMBUSVIRUS FROM SPINACH (*SPINACIA OLERACEA*)

*ŽIVOJIN ERIĆ, ZLATA ŠTEFANAC** and *BILJANA PLAVŠIĆ*

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

Received February 24, 1986

The biological, serological and some biophysical properties of the tombusvirus causing severe disease of spinach (*S. oleracea* L.) and here designated TBSV-S were studied. The virus was compared with 11 other isolates belonging to six serologically interrelated tombusviruses.

In double diffusion serological tests, TBSV-S was shown to be identical to PAMV-Type, TBSV-Grape vine and TBSV-Cherry from PAMV sub-group of tombusviruses, and formed a spur with AMCV of the same sub-group and other reference tombusvirus isolates. It differed, however, from the members of the PAMV sub-group in the reaction of test-plants and electrophoretic mobility. Electron microscopy of purified preparations and density gradient centrifugation showed that TBSV-S was considerably stable and in this respect similar to AMCV. Its DEP was between 10^{-5} and 10^{-8}, TIP between 84°C and 88°C and stability in sap at 20°C was 46 and 58 days, depending on the host-plant source. In addition to the ultrastructural modifications observed earlier with infections by other tombusviruses, TBSV-S provoked in the cytoplasm the appearance of distinct concentrically arrayed membranes deriving from the proliferation of endoplasmic reticulum. In some places virus particles were found in monolayers sandwiched between pairs of these membranes. Such structures could not be found in tissues infected with the reference isolates. On the basis of the properties investigated and current knowledge of tombusviruses, we consider the tombusvirus from spinach in a broader sense as a special spinich strain of TBSV. This paper also shows the remarkable variability of tombusviruses, which is expressed in the overlapping of their numerous properties, and which makes their classification difficult.

Introduction

In a previous paper (Štefanac 1978b; cf. Martelli 1981) the occurrence of a tombusvirus denoted as tomato bushy stunt (TBSV) from spinach (*Spinacia oleracea* L.) with severe virus symptoms was reported. Here we describe comparative reactions of test-plants, serological experiments, ultrastructural modifications in infected cells and some data which show that following the classification of tombusviruses presented by Matthews (1982) and with the current knowledge of tombusviruses (Gallitelli et al. 1985, Koenig and Gibbs 1986) this virus can be considered a distinct strain of TBSV.

Materials and Methods

Tombusvirus isolates and culture. The spinach isolate (TBSV-S, in the previous paper TBSV) studied in this work was collected with spinach plants near Zagreb in March 1977, and some of its biological and serological properties have already been briefly reported (Štefanac 1978a,b, Erić et al. 1985). Before these studies it was passed through three single lesions of *Nicotiana glutinosa*. For its accurate identification and comparative purposes the following isolates belonging to six serologically interrelated tombusviruses were used: the type strain of TBSV (TBSV-Type, Smith 1935), the type isolate of PAMV (PAMV-Type, Lovisolo 1957), carnation Italian ringspot virus (CIRV, Hollings and Stone 1965), artichoke mottled crinkle virus (AMCV, Martelli 1965), isolate of pelargonium leaf curl virus (PLCV-456, Hollings and Stone 1975), isolates described as TBSV from grape vine (Bercks 1967), cherry (small lesion strain, Allen and Davidson 1967), tulip (Mowat 1972), pepper (Fischer and Lockhart 1977), TBSV-Pb from piggyback (Henriques and Schlegel 1978), and eggplant mottled crinkle virus (EMCV, Makkouk et al. 1981). All isolates were kept in dry leaves under the silica gel and from this source the cultures were occasionally renewed.

Inoculation tests. For transmissions, infected leaves were ground in a mortar by adding 0.06 M phosphate buffer, pH 7.6 with 0.1% TGA and slurry rubbed on carborundum dusted leaves. At least five plants of each test species were inoculated with a particular virus isolate. When symptoms had not developed, return inoculations were made to *Chenopodium amaranticolor*.

Purification. For most of the studies, TBSV-S and other tombusvirus isolates were purified from infected leaves of *N. megalosiphon* and some from *C. quinoa* by the n-butanol method of Hollings et al. (1970) used for purification of CIRV. Virus preparations were stored at +2 °C by adding 20% glycerol.

Spectrophotometry. The spectrophotometry was done with a DU-2 Beckman spectrophotometer using $E_{260}^{0.1\%} = 4.5$ and $A_{260}/A_{280} = 1.62 - 1.66$ from earlier literature (Martelli et al. 1971).

Density gradient centrifugation. It was done in a Beckman SW 25.1 rotor at 25 000 rpm for 150 min in 10—40% sucrose density gradient columns. The virus containing fraction was collected by puncturing the bottom of the tube, diluted in 0.03 M phosphate buffer, pH 7.6 and concentrated by centrifuging (120 min at 78 500 g).

Antisera and gel-diffusion tests. A broad spectrum antiserum to TBSV-S was prepared by giving a rabbit five intravenous injections at three day intervals with a total of about 50 mg purified virus. Antisera to PAMV-Type, CIRV and TBSV-Tulip were kindly provided by Drs. O. Lovisolo, A. Quacquarelli and W. P. Mowat, respectively.

Double diffusion tests were made in 0.9% (w/v) agar gels in saline with 0.02% (w/v) sodium azide. When estimating the titres of antisera, crude sap extracts were used as undiluted and diluted 1/2, 1/4 and 1/8. In spur tests antisera were employed as undiluted and antigens as raw sap or purified preparations.

Immunoelectrophoresis. Immunoelectrophoresis was done in an LKB--Gelman 6800A-1 microelectrophoresis apparatus in 0.9% Ionagar no. 1 (Oxoid) in 0.03 M phosphate buffer, pH 7.6 with purified virus preparations or crude virus sap and using antiserum to TBSV-S. Electrophoresis was performed at +2 °C for periods of 340 min and at c. 2.5 V/cm.

Electron microscopy. All preparations were examined in a JAM 100B electron microscope. For detecting virus in extracts of infected plants, they were expressed by squeezing leaves in the presence of 2% (w/v) potassium phosphotungstate (1 ml/1 g).

Purified virus preparations were mixed with either 2% (w/v) phosphotungstic acid, pH 6.9 or with 1% (w/v) magnesium uranyl acetate. Sometimes they were previously fixed with 10% formaldehyde for 20 min.

Ultrastructural observations of TBSV-S infected tissue were made from leaves of *C. quinoa, N. glutinosa* and *N. megalosiphon.* Tissue samples were fixed in 3% glutaraldehyde prepared in cacodylate buffer, postfixed in 2% osmium tetroxide, dehydrated in a grade series of ethanol and propylene oxide, and embedded in Epon 812. Ultrathin sections were cut by a LKB ultramicrotome with diamond knife and double stained with 10% magnesium uranyl acetate and 0.1% lead citrate.

Results

Reactions of test plants

The reactions of 17 species to mechanical inoculation with TBSV-S were as follows:

**Capsicum annuum.* Necrotic local lesions in 5—6 days. Systemic symptoms involved appearance of chlorotic spots rimmed with brown rings, mottling, leaf deformations (Fig. 1B) and inhibition of growth.

Chenopodium amaranticolor. Numerous small chlorotic local lesions in 2—3 days, soon turning necrotic; systemic infection with similar lesions and apical necrosis.

C. murale. Few translucent local lesions; systemic latent infection.

C. quinoa. Numerous chlorotic local lesions later turning necrotic; systemic infection similar to that in inoculated leaves with leaf distortion and apical necrosis.

* Symptoms described previously (Štefanac 1978b)

Datura stramonium. Diffuse chlorotic local lesions in 4—6 days; systemic yellow green mottle and inhibition of growth.

Gomphrena globosa. Necrotic local lesions later rimmed with red ring; not systemic.

Lycopersicum esculentum. Mechanically inoculated leaves showed larger diffuse chlorotic lesions. Systemic symptoms developed in about 40% of the plants and consisted of light chlorotic mottle, veinal necrosis and deformation of some leaflets. The main axis of the steam was zig-zag shaped. Inhibition of the growth was noticeable.

Nicotiana clevelandii. Chlorotic local lesions in 3—4 days; systemic chlorotic lesions, necrosis, twisted leaves and plant collapse during the winter.

**N. glutinosa.* Numerous brown necrotic local lesions (Fig. 1C); not systemic.

N. megalosiphon. Diffuse chlorotic, later necrotic, local lesions; systemic infection in the form of pronounced vein-clearing followed by distortion of the apex and its necrosis.

N. tabacum cvs Samsun and White Burley. Small necrotic local lesions (Fig. 1D); not systemic.

Ocymum basilicum. Necrotic local lesions characteristic of TBSV; not systemic.

Petunia hybrida. Not many necrotic local lesions in 3—6 days; not systemic.

Solanum melongena. Diffuse chlorotic local lesions in 4—6 days; systemic mottle and distortion of the leaves in 1—2 weeks, inhibition of the growth.

**Spinacia oleracea* cv. Matador. Numerous chlorotic local lesions soon turning necrotic. Severe systemic infection manifested as vein-clearing, mottle, shortening of the main veins accompanied bo twisting and curling of top leaves, by necrosis (Fig. 1A) and inhibition of growth. Plants sometimes died.

Tetragonia expansa. White necrotic local lesions; not systemic.

Zinnia elegans. Necrotic local lesions; systemic mild mottle in some plants during winter.

For more accurate comparison of TBSV-S with the tombusvirus isolates as regards the symptomatology, all were inoculated in parallel experiments to four natural hosts of these viruses and *Datura stramonium.* In these experiments TBSV-S and all the 11 reference isolates could be distinguished from each other. Table 1 gives the outline of the results.

Infectivity of TBSV-S in sap

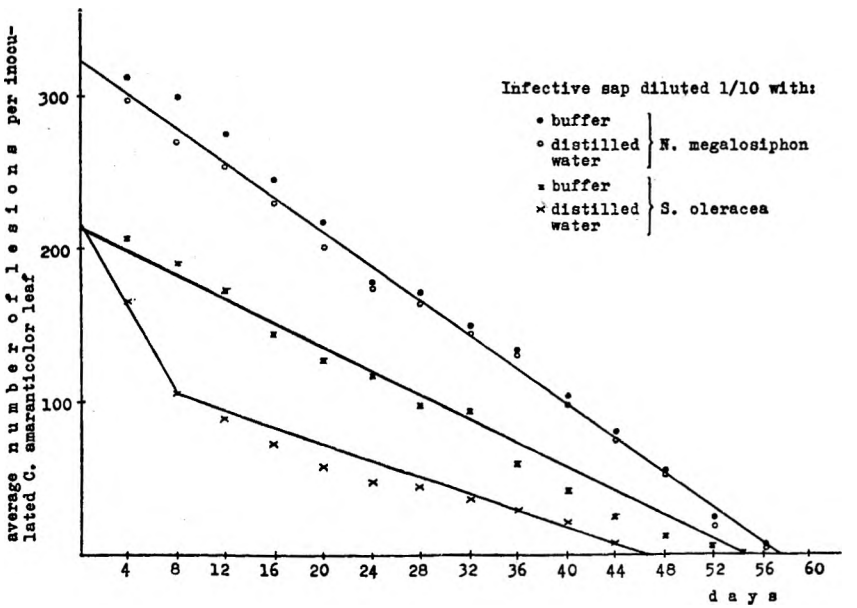
Dilution end-point. When diluted with inoculation buffer, dilution end-point of sap from infected *Nicotiana clevelandii* or *N. megalosiphon* was between 10^{-7} and 10^{-8}, the one from *C. quinoa* between 10^{-6} and 10^{-7}, and the one from *S. oleracea* between 10^{-5} and 10^{-6}.

Thermal inactivation point. In sap from inoculated leaves of *N. megalosiphon, N. clevelandii* or *C. quinoa* diluted 1/10 with distilled water infectivity survived heating for 10 min at 86 °C but not at 88 °C, and in that from *S. oleracea* 84 °C but not 86 °C.

Table 1. Types of reactions of five species to TBSV-S and the 11 reference tombusvirus isolates

Virus (isolate)	*Lycopersicum esculentum*	*Petunia hybrida*	*Solanum melongena*	*Spinacia oleracea*	*Datura stramonium*
TBSV-S	L/S	L	L/S	L/S	L/S
TBSV-Type	L/S	L	L/S	L/S ssl°	L/S
PAMV-Type	L/S	L/S	L/S sl	L/S	L/S
AMCV	L/S	L sl	L	L/S	L
CIRV	L/S ssl°	L	O	L/S ssl°	L
PLCV-456	L	L	L/S ssl	L sl	L/S
TBSV-Grape vine	L/S	L/S	L/S sl	L/S	L/S ssl
TBSV-Cherry	L/S	L	L	L	L/S
TBSV-Tulip	L	L	L/S	L	L
TBSV-Pepper	L/S	L	L/S	L/S	L
TBSV-Pb	L/S sl	L	L	L/S	L/S
EMCV	O	L	L/S	L	L

L = local infection, S = systemic infection, sl = symptomless infection, ssl = sporadic symptomless infection, ° = low virus concentration, O = plant resistant to infection.



Text-fig. 1. Effect of the buffer and distilled water on the loos of infectivity of TBVS-S in sap extracts from *Nicotiana megalosiphon* and *Spinacia oleracea* stored at 20°C.

Stability. In sap extracts from *N. megalosiphon* diluted 1/10 with inoculation buffer or distilled water infectivity survived storage at 20 °C ± 0.5 °C for 58 days, in those from *S. oleracea* diluted with buffer for 54 and those with distilled water for 46 days only.

By analysing the loss of infectivity as the function of time it was evidenced that it was linear except in infective sap from spinach when diluted with distilled water (Text-fig. 1). As shown in the text-fig., in the last case 50% of the infectivity was lost in the first 8 days after which time the whole process also assumed a linear course.

Purification and stability of TBSV-S

As a convenient host-plant for purification of tombusviruses the most commonly used was *N. clevelandii* and less often *C. quinoa* and *D. stramonium* (Wetter and Luisoni 1969, Hollings and Stone 1975, Albrechtova 1976, Makkouk et al. 1981, Koenig and Gibbs 1986). Since under the conditions of our glasshouse *N. clevelandii* is difficult to cultivate during a longer period of the year, the suitability of *N. megalosiphon* was examined. This species was found to be equally suitable as a source of TBSV-S for purification as *N. clevelandii.* It also proved to be a convenient propagation species for other tombusviruses. Yields of purified TBSV-S varied between 30—40 mg/100 g leaf tissue. Their absorption spectrum had a maximum at 260 nm and a minimum at 240 nm with $A_{260}/_{280}$ ratio of 1.63 (average of three separate determinations). Purified preparations contained numerous well preserved isometric virus particles c. 28 nm diameter, often with hexagonal outlines (Fig. 2A, B). Preparations appeared fairly clean with respect to the presence of host constituents, which was in agreement with the data obtained by spectrophotometry.

Following the sucrose density gradient centrifugation purified TBSV-S preparations sedimented as a single opalescent band when the sample put on the top of the gradient contained 1 mg of the virus (Fig. 2C); however, when it contained 2—3 mg of the virus an additional slower sedimenting band was also present (Fig. 2D). The faster sedimenting band contained isometric particles c. 28 nm diameter and nearly all infectivity. The slower sedimenting band contained small granules of the size corresponding to that of protein subunits and showed little, if any, infectivity. In serological tests, samples from the slower sedimenting zone reacted up to dilution 1/2 but only with undiluted antiserum to TBSV-S giving straight line typical of soluble protein.

Serological relationships

In these experiments TBSV-S was compared with the 11 reference tombusvirus isolates by determining homologous and heterologous titres of its serum (Table 2), and also with PAMV-Type, AMCV and TBSV--Tulip by using their respective antisera (Table 3). From the data quoted in the tables it follows that TBSV-S antiserum had somewhat smaller avidity against heterologous viruses than the antisera against PAMV--Type and AMCV, but greater than the one against TBSV-Tulip.

In spur formation tests by using the serum against TBSV-S no serological differences were found between TBSV-S and PAMV-Type, TBSV-Grape vine and TBSV-Cherry, respectively; however, spur was formed between TBSV-S and other isolates included in this comparison (Table 4).

Table 2. Homologous and heterologous titres (reciprocal) of antiserum to TBSV-S in agar-gel diffusion tests*

Antigen												
TBSV-S	TBSV-Type	PAMV-Type	AMCV	CIRV	PLCV-456	TBSV-Grape vine	TBSV-Cherry	TBSV-Tulip	TBSV-Pepper	TBSV-Pb	EMCV	Healthy plant sap
2048	512	256	128	512	256	256	256	64	512	256	256	—

*Presented are maximal titres obtained.
— No reaction.

Table 3. Homologous and heterologous titres (reciprocal) of antisera to TBSV-S, PAMV -Type, AMCV and TBSV-Tulip*

Antiserum	Antigen			
	TBSV-S	PAMV-Type	AMCV	TBSV--Tulip
TBSV-S	**2048**	256	128	64
PAMV-Type	128	**512**	256	256
AMCV	128	128	**128**	64
TBSV-Tulip	16	8	8	**256**

*Presented are maximal titres obtained.

Table 4. Serological relationship of TBSV-S to the 11 reference tombusvirus isolates as determined by immunodiffusion tests for spur formation

Antiserum	Antigen											
	TBSV-S	TBSV-Type	PAMV-Type	PLCV-456	CIRV	AMCV	TBSV-Grape vine	TBSV-Cherry	TBSV-Tulip	TBSV-Pepper	TBSV-Pb	EMCV
TBSV-S	L	S	L	S	S	S	L	L	S	S	S	S
PAMV-Type	L	—	L	—	—	—	—	—	—	—	—	—
AMCV	S	—	—	—	—	L	—	—	—	—	—	—
TBSV-Tulip	S	—	—	—	—	—	—	—	S	—	—	—

L = continuity of precipitin lines, no spur, S = spur formation, — = not tested.

Immunoelectrophoresis

In spite of their apparent serological identity TBSV-S, PAMV-Type and TBSV-Cherry migrated slowly towards the anode at a rate close to but different from each other; AMCV, as expected (Hollings and Stone 1975), moved slowly towards the cathode (Table 5).

Table 5. Electrophoretic movement (mm/h) of TBSV-S and three reference virus isolates of the PAMV sub-group of tombusviruses

TBSV-Type	+ 1.25
PAMV-S	+ 1.27
TBSV-Cherry	+ 1.30
AMCV	− 0.46

Cytopathology

The general cytological aspect of TBSV-S infected samples, regardless of the host, was typical of infections caused by most definitive tombusviruses. The chronological appearance of the ultrastructural changes found was as follows:

In samples taken from inoculated leaves before the appearance of external symptoms (1—2 days following inoculation), particular regions of the cytoplasm contained numerous individual vesicles (Fig. 3A) probably originating from endoplasmic reticulum, and numerous structures known as multivesicular bodies (Fig. 3B). At this stage of infection no virus particles could be seen in cells of infected plants.

In samples taken from inoculated and systemically infected leaves immediately following the appearance of symptoms, besides the two types of membranous structures mentioned, virus particles were found scattered in the cytoplasm and within the vacuole (Fig. 3C). No other ultrastructural modifications were noticed.

At more advanced stages of infection, i.e. 2—3 days following the appearance of symptoms, at the time when the vesicles characteristic of tombusviruses containing virus detached from the cytoplasm and moved into the vacuole (Fig. 4B, C), concentric membranous structures deriving from the proliferation of endoplasmic reticulum (ER) were observed (Fig. 5). Often, virus particles were found in monolayers sandwiched between pairs of these membranes. These membranous structures were not rare, although not so abundant as the virus containing vesicles which liberated into the vacuole. At that time, the aggregates of closely packed virus particles forming one or more microcrystals were also present in the cytoplasm. In *C. quinoa* (Fig. 3D), but not in *N. megalosiphon*, the electron opaque amorphous substance was accumulated around the microcrystals. At this stage different degenerative changes of nuclei, chloroplasts and mitochondria, noticed earlier with some other tombusviruses, were also found. Concerning the development of multivesicular bodies they originated from peroxisomes (Fig. 4A).

The samples of tissue infected with each of the 11 reference tombusvirus isolates were examined for the possible presence of the proliferation of ER found in TBSV-S infected cells and illustrated in Fig. 5. However, in no case similar structures were noticed. The tissue samples

for the analysis derived from plants being at the same stage of infection in which ER proliferation in TBSV-S infected cells appeared. On the contrary, another tombusvirus isolate obtained from spinach grown in Sarajevo Plain (Bosnia), at 300 km air-line distance from Zagreb and in a different climate, produced the same proliferation of ER as the orginal TBSV-S. This isolate evidenced to be equal to the authentic TBSV-S isolate regarding the reaction of test-plants, too.

Discussion

The analysis of TBSV-S on herbaceous test-plants has shown that this tombusvirus isolate similarly to many others provokes local infection in a number of test-plants. Besides, the analysis has shown that it causes systemic infection in most of the species tested which are known as natural hosts of the tombusviruses (Martelli et al. 1977, Martelli 1981, Makkouk et al. 1981). However, TBSV-S and the 11 reference tombusvirus isolates could be distinguished in parallel experiments from each other with regard to the symptomatology on four natural hosts of these viruses (tomato, petunia, eggplant, spinach) and *Datura stramonium*. Nevertheless, the reactions obtained could have been influenced at least partially by the sort of low-molecular weight RNA similar to the one recently found associated with BS-3 strain of TBSV (Hillman et al. 1985).

The values obtained in determining infectivity of TBSV-S in sap were within those obtained for other tombusviruses. The bigger differences in stability of the virus in vitro which were established between crude sap of *Nicotiana megalosiphon* and *Spinacia oleracea* diluted with distilled water, and also between sap of spinach diluted with buffer and with distilled water, have to be accounted for by the influence of inhibitors present in spinach.

The analysis of crude sap of infected plants and of purified TBSV-S suspensions in the electron microscope has shown that the population of the virus consists of isometric particles c. 28 nm diameter, which is in agreement with the literature data for other tombusviruses. The absorption spectrum of purified TBSV-S preparations also generally corresponded to those of other tombusvirus isolates (Quacquarelli et al. 1966, Hollings et al. 1970, Martelli et al. 1971, Makkouk et al. 1981). Results of density gradient centrifugation demonstrate that TBSV-S is more stable than TBSV-Type. PLCV and CIRV (Markham 1962, Hollings and Stone 1965, Hollings et al. 1970, Pleše 1983) and in this respect similar to AMCV (Quacquarelli et al. 1966).

Results of serological tests, in which TBSV-S was compared with 11 tombusvirus isolates, show that TBSV-S by its serological characteristics belongs to the PAMV sub-group of Hollings and Stone (1975) in which in addition to PAMV-Type, TBSV-Cherry, TBSV-GCRI and AMCV also TBSV-Grape vine, TBSV-Pb, PAMV obtained from cherry by Koenig and Kunze (1982) and some others could be placed. In our experiments TBSV-S showed to be serologically identical to PAMV-Type, TBSV-Grape vine and TBSV-Cherry and related but not identical to AMCV.

In comparative immunoelectrophoretic experiments, TBSV-S showed also to be similar to the members of the PAMV sub-group by its faint electrophoretic movement in the direction of anode (cf. Hollings and

Stone 1975). However, TBSV-S and the members of the sub-group included in comparison expressed significant although not big differences in their electrophoretic movement.

The ultrastructural changes provoked by TBSV-S were generally similar to those caused by most other tombusviruses (Martelli et al. 1977, Martelli 1981, Makkouk et al. 1981, Di Franco et al. 1984). The accumulation of electron dense amorphous substance around the virus crystals in cells of *C. quinoa*, and its absence in *N. megalosiphon* suggest that this phenomenon is a consequence of interaction between the virus and the host and cannot be ascribed to the influence of one or the other factor alone. Similar substance was earlier found in artichoke cells infected by AMCV (Russo et al. 1967, 1968) while in the cells of *C. quinoa* infected by the same virus it was not observed (Martelli and Russo 1973). Concerning the multivesicular bodies we have found that they originate in TBSV-S infected cells from peroxisomes as they do in other tombusvirus infections excluding CIRV (Di Franco et al. 1984). However, the appearance of the concentric membranous structures in the cytoplasm with virus particles between the membranes was not registered up to now in other tombusvirus infections. Such ER proliferation is certainly connected, similarly to the displacement of virus containing vesicles into the vacuole, with mechanism of the protection of the cell from harmful influence of the virus. We note, however, that morphologically similar structures have been found in cells infected by soil-borne wheat mosaic (Hibino et al. 1974), tobacco ringspot (Francki and Hatta 1977), radish mosaic (Milne et al. 1980) and some other viruses. This ultrastructural characteristic of TBSV-S also points to the great variability among tombusviruses, which is expressed in overlapping of their various properties (Galitelli et al. 1985, Koenig and Gibbs 1986 — the lack of correlation between relationships assessed by serological, nucleic acid homology and electrophoretic migration tests) and which makes their classification difficult. The last characteristic of TBSV-S together with its other properties and the fact of the repeated isolation of exactly identical virus from the plant collected by chance from another location show that TBSV-S is a distinct tombusvirus apparently specific of spinach.

By analogy with some other tombusviruses (Tomlinson and Faithfull 1984), TBSV-S probably disseminates by the seed of spinach. However, in a pilot experiment to prove that, we did not establish any transmission. On the contrary, the results of our experiments to establish transmission of TBSV-S during cultivation are consistent with its soil-borne nature.

*

We are indebted to Drs O. Lovisolo, M. Hollings, G. P. Martelli, R. Bercks, W. R. Allen, W. P. Mowat, H. U. Fischer, M. I. Henriques and R. Koenig for their isolates of the tombusviruses, Dr B. Kassanis for TBSV-Type, and Drs O. Lovisolo, A. Quacquarelli and W. P. Mowat for supplying antisera. This study was done in partial fulfilment of the requirements for the M. Sc. degree of Ž. Erić at the University of Zagreb in 1982.

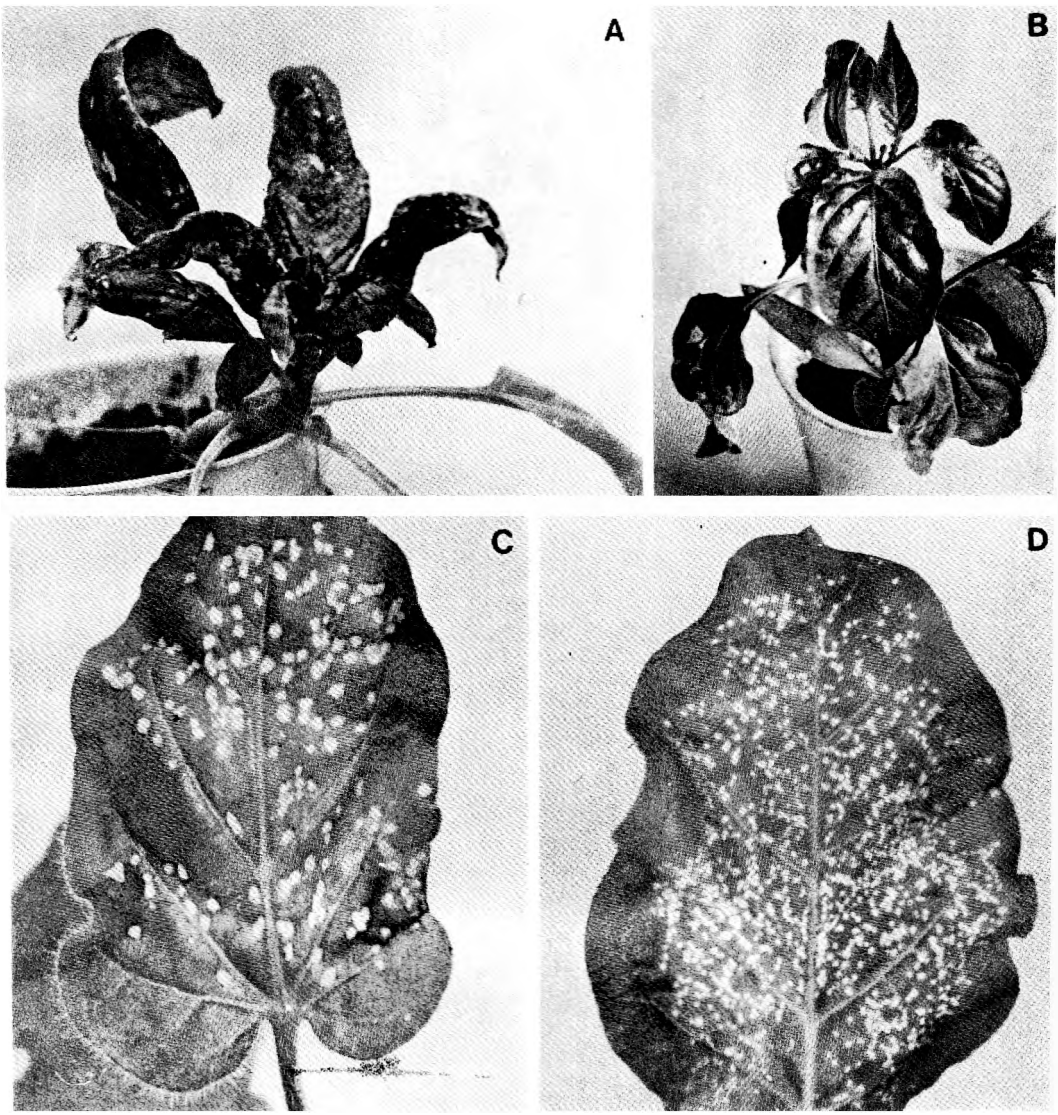


Fig. 1. Effect of the spinach strain of TBSV (TBSV-S) on experimentally infected plants. **A, B** Systemic symptoms in a plant of spinach *(Spinacia oleracea)* cv. Matador and a pepper (*Capsicum annuum*), respectively. **C** Necrotic lesions in inoculated *Nicotiana glutinosa* leaf. **D** Local necrotic lesions in *N. tabacum* cv. Samsun.

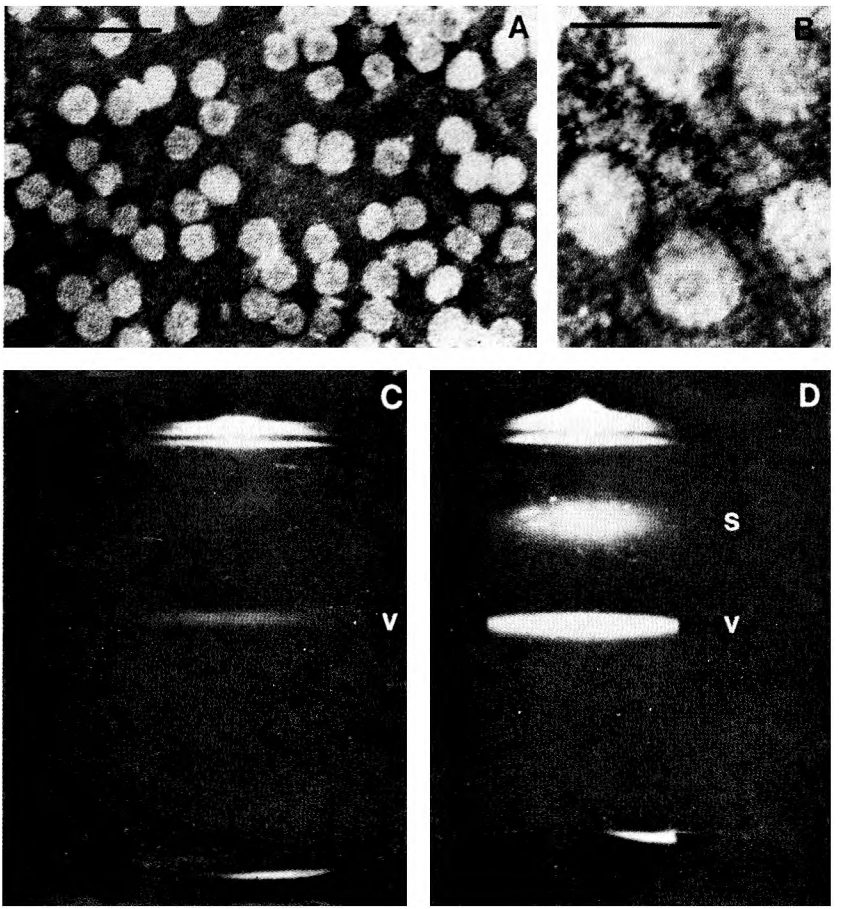


Fig. 2. **A, B** Purified preparation of TBSV-S mounted in phosphotungstate; notable are hexagonal outlines and rough granular surface of virus particles. Bars = 100 nm(A), 50 nm(B). **C, D** Separation of virus particles (v, virus zone) and protein subunits (s, soluble protein zone) from TBSV-S purified preparation by density gradient centrifugation. In **C** preparation contained 1 mg, in **D** 3 mg virus.

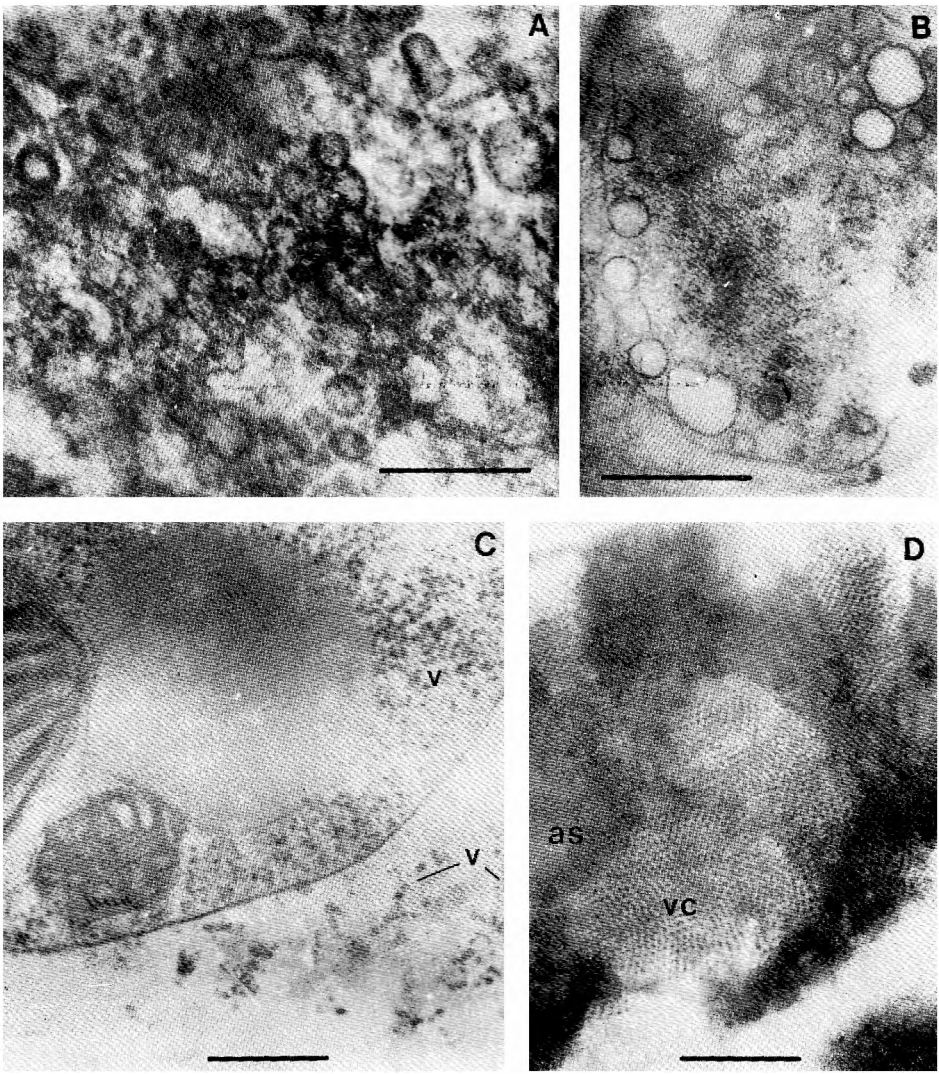


Fig. 3. Portions of *Chenopodium quinoa* mesophyll cells infected with TBVS-S. **A** Numerous individual vesicles and **B** multivesicular body in the cytoplasm of cells deriving from inoculated leaf one day following inoculation. **C** A large number of virus particles (v) in the cytoplasm and some in the vacuole, detected immediately following appearance of symptoms. **D** Virus microcrystals (vc) surrounded by the electron opaque amorphous substance (as), few days after appearance of symptoms. Bars = 500 nm (A. B. C), 400 nm (D).

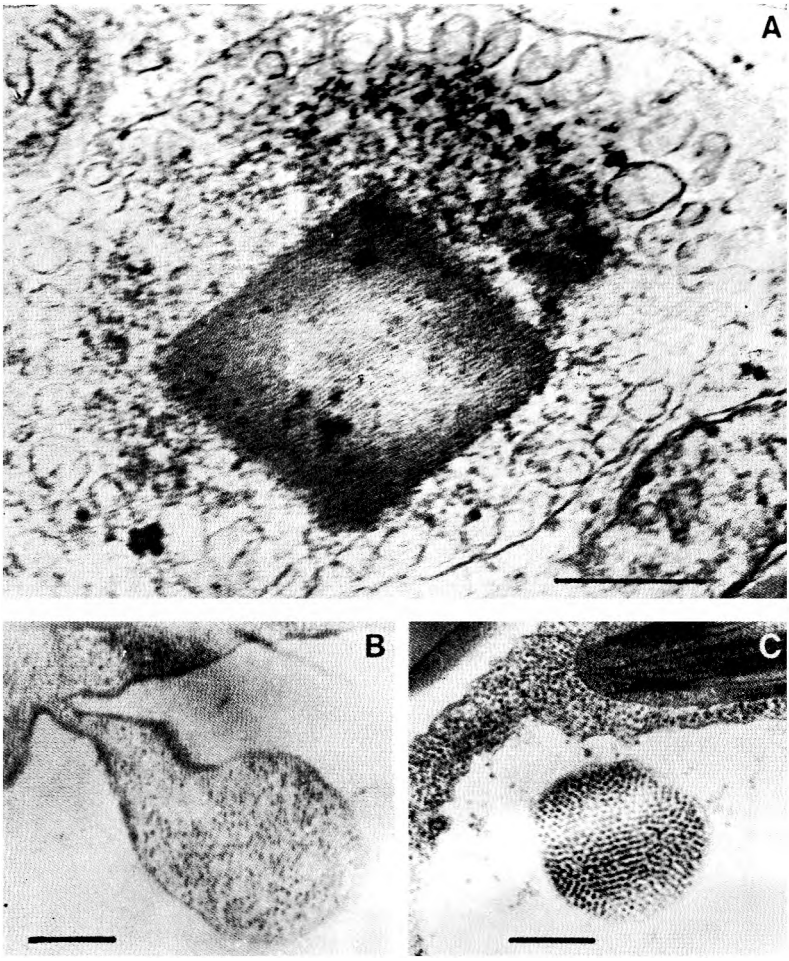


Fig. 4. Sections of **A** *Nicotiana glutinosa* and **B**, **C** *N. megalosiphon* TBVS-S infected cells few days following appearance of symptoms. In **A** multivesicular body with a large crystalline inclusion shows its origin from peroxisome, in **B** an elongated vesicle with virus particles connected to the cytoplasm, in **C** vesicle detached from the cytoplasm into the vacuole. Bars = 500 nm.

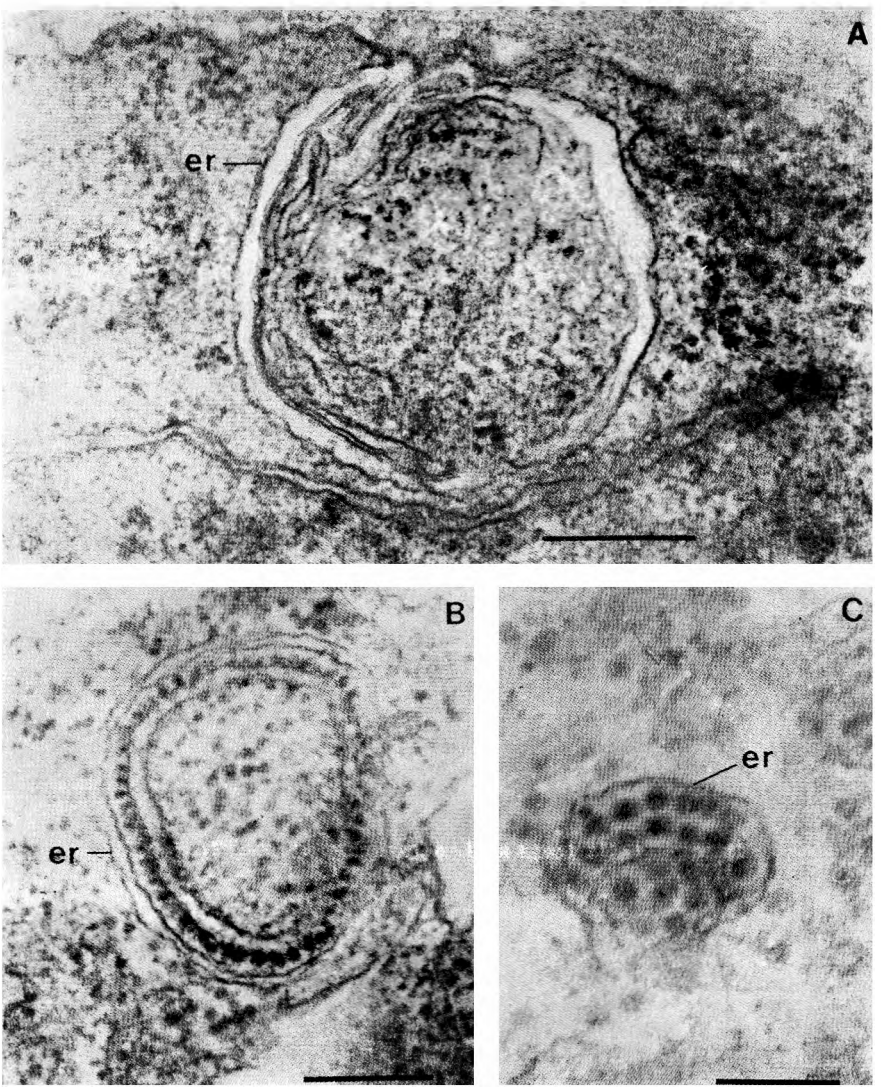


Fig. 5. Proliferation of ER membranes (er) in **A** *Nicotiana glutinosa*, **B** *Chenopodium quinoa and* **C** *N. megalosiphon* TBVS-S infected cells with virus particles in the cytoplasm between the membranes in **B** and **C**. In **A** and **B** the structures are sectioned transversely, in **C** the appearance of two layers of virus particles between ER membranes in tangential section. Bars = 300 nm (A, B), 150 nm (C).

References

Albrechtová, L., 1976: Příprava antiséra vuči viru zakrslosti rajčete z třešně. Ochr. Rostl. *12*, 9—12.

Allen, W. R., T. R. Davidson, 1967: Tomato bushy stunt virus from *Prunus avium* L. I. Field studies and virus characterisation. Can. J. Bot. *45*, 2375—2383.

Bercks, R., 1967: Über den Nachweis des Tomatenzwergbusch-Virus (tomato bushy stunt virus) in Reben. Phytopath. Z. *60*, 273—277.

Di Franco, A., M. Russo, G. P. Martelli, 1984: Ultrastructure and origin of cytoplasmic multivesicular bodies induced by carnation Italian ringspot virus. J. gen. Virol. *65*, 1233—1237.

Erić, Ž., Z. Štefanac, B. Plavšić, 1985: Ultrastructures characteristic of infection with a spinach strain of petunia asteroid mosaic virus. Int. Conf. New Developments in Techniques for Virus Detection, University of Cambridge (Abstracts), p. 62.

Fischer, H. U., B. E. L. Lockhart, 1977: Identification and comparison of two isolates of tomato bushy stunt virus from pepper and tomato in Morocco. Phytopatholoy *67*, 1352—1355.

Francki, R. I. B., T. Hatta, 1977: Nepovirus (tobacco ringspot virus) group. In The Atlas of Insect and Plant Viruses, pp. 221—235. Edited by K. Maramorosch. Academic Press, New York, San Francisco and London.

Gallitelli, D., R. Hull, R. Koenig, 1985: Relationship among viruses in the tombusvirus group: nucelic acid hibridization studies. J. gen. Virol. *66*, 1523—1531.

Henriques, M. I., D. E. Schlegel, 1978: Tomato bushy stunt virus isolated from piggiback plants. Third Int. Congr. Pl. Path., München (Abstracts of Papers), p. 43. Paul Parey, Berlin and Hamburg.

Hibino, H., T. Tsuchizaki, Y. Saito, 1974: Electron microscopy of inclusion development in rye leaf cells infected with soil-borne wheat mosaic virus. Virology *57*, 522—530.

Hillman, B. I., T. J. Morris, D. E. Schlegel, 1985: Effects of low-molecular--weight RNA and temperature on tomato bushy stunt virus symptom expression. Phytopathology *75*, 361—365.

Hollings, M., O. M. Stone, 1965: Studies of pelargonium leaf curl virus. II Relationships to tomato bushy stunt and other viruses. Ann. appl. Biol. *56*, 87—98.

Hollings, M., O. M. Stone, 1975: Serological and immunoelectrophoretic relationships among viruses in the tombusvirus group. Ann. appl. Biol. *80*, 37—48.

Hollings, M., O. M. Stone, G. C. Bouttell, 1970: Carnation Italian ringspot virus. Ann. appl. Biol. *65*, 299—309.

Koenig, R., A. Gibbs, 1986: Serological relationships among tombusviruses. J. gen. Virol. *67*, 75—82.

Koenig, R., L. Kunze, 1982: Identification of tombusvirus isolates from cherry in southern Germany as petunia asteroid mosaic virus. Phytopath. Z. *103*, 361—368.

Lovisolo, O., 1957: Petunia: nuovo ospite naturale del virus del rachitisimo cespuglioso del Pomodoro. Boll. Staz. Pat. veg. Roma, Serie III, *14*, 103—119.

Makkouk, K. M., R. Koeng, D. E. Lesemann, 1981: Characterisation of a tombusvirus from eggplant. Phytopathology *71*, 572—577.

Markham, R., 1962: The analytic centrifuge as a tool for the investigation of plant viruses. Adv. Vir. Res. *9*, 241—272.

Martelli, G. P., 1965: L'arricciamento maculato del Carciofo (*Cynara scolymus* L.). Phytopath. medit. *4*, 58—60.

Martelli, G. P., 1981: Tombusviruses. In Handbook of Plant Virus Infections: Comparative Diagnosis, pp. 61—90. Elsevier/North-Holland Biomedical Press. Amsterdam, New York, Oxford.

Martelli, G. P., A. Quacquarelli, M. Russo, 1971: Tomato bushy stunt virus. Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses, no. 69.

Martelli, G. P., M. Russo, 1973: Electron microscopy of artichoke mottled crinkle virus in leaves of *Chenopodium quinoa* Willd. J. Ultrastruct. Res. *42*, 93—107.

Martelli, G. P., M. Russo, A. Quacquarelli, 1977: Tombusvirus (tomato bushy stunt virus) group. In The Atlas of Insect and Plant Viruses, pp. 257—280.

Matthews, R. E. F., 1982: Classification and nomenclature of viruses. Intervirology *17*, 1—199.

Milne, R. G., V. Masenga, R. Lenzi, O. Lovisolo, 1980: Radish mosaic virus in *Eruca sativa* Miller. Phytopath. medit. *19*, 145—149.

Mowat, W. P., 1972: A necrotic disease of tulip caused by tomato bushy stunt virus. Pl. Path. *21*, 171—174.

Pleše, N., 1983: Detection of pelargonium leaf curl virus in Yugoslavia. Acta Bot. Croat. *42*, 15—20.

Quacquarelli, A., G. P. Martelli, M. Russo, 1966: Ricerche sull'agente dell'arricciamento maculato del Carciofo. II. Purificazione, microscopia elettronica e caratteristiche fisico-chimiche. Atti 1° Congr. Un. Fitopat. Medit., 178—194.

Russo, M., G. P. Martelli, A. Quacquarelli, 1967: Occurrence of artichoke mottled crinkle virus in leaf vein xylem. Virology *33*, 555—558.

Russo, M., G. P. Martelli, A. Quacquarelli, 1968: Studies on the agent of artichoke mottled crinkle. IV. Intracellular localization of the virus. Virology *34*, 679—693.

Smith, K. M., 1935: A new virus disease of the tomato. Ann. appl. Biol. *22*, 731—741.

Štefanac, Z., 1978a: Disease of spinach caused by tomato bushy stunt virus. Third Int. Congr. Pl. Path., München (Abstracts of Papers), p. 37. Paul Parey, Berlin and Hamburg.

Štefanac, Z., 1978b: Investigation of viruses and virus diseases of spinach in Croatia. Acta Bot. Croat. *37*, 39—46.

Tomlinson, J. A., E. M. Faithfull, 1984: Studies on the occurrence of tomato bushy stunt virus in English rivers. Ann. appl. Biol. *104*, 485—495.

Wetter, C., E. Luisoni, 1969: Precipitin, agar gel diffusion, and intragel absorption tests with three strains of tomato bushy stunt virus. Phytopath. Z. *65*, 231—242.

SAŽETAK

SVOJSTVA TOMBUSVIRUSA IZ ŠPINATA (*SPINACIA OLERACEA*)

Živojin Erić, Zlata Štefanac i Biljana Plavšić*

(Biološki odsjek Prirodno-matematičkog fakulteta Univerziteta u Sarajevu i *Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Istražena su biološka, serološka i neka biofizička svojstva tombusvirusa, uzročnika vrlo štetne bolesti špinata (*S. oleracea* L.), koji u radu označavamo kraticom TBSV-S. Virus je uspoređen s 11 izolata, predstavnika šest serološki međusobno srodnih tombusvirusa.

U serološkim pokusima dvostruke difuzije TBSV-S pokazao se identičnim s tombusvirusima PAMV-Type, TBSV-Grape vine i TBSV-Cherry is podskupine zvjezdolikog mozaika petunije (PAMV sub-group) i stvarao ostrugu s AMCV iz iste podskupine te s ostalim izolatima s kojima je bio uspoređivan. Virus se, međutim, razlikovao od članova navedene podskupine tombusvirusa u simptomima na pokusnim biljkama i elektroforetskoj pokretljivosti. Elektronskomikroskopski pregled purificiranih preparata i centrifugiranje u gradijentu gustoće pokazali su da je TBSV-S razmjerno stabilan i po tome sličan AMCV. Ovisno o biljci domaćinu, čiji je infektivni sok bio korišten, krajnja točka razrjeđenja virusa kretala se između 10^{-5} i 10^{-8}, termalna točka inaktivacije između 84 °C i 88 °C a postojanost u soku pri 20 °C iznosila 46 i 58 dana. Osim ultrastrukturnih promjena koje su ranije zapažane pri zarazama drugim tombusvirusima, TBSV-S je uzrokovao u citoplazmi pojavu posebnih membranskih struktura koje su bile posljedica proliferacije endoplazmatskog retikuluma. Na nekim mjestima između parova tih zakrivljenih i međusobno paralelno postavljenih endoplazmatskih membrana bile su mjestimično u obliku jednog sloja smještene virusne čestice. Takve strukture nismo mogli naći u tkivima koja su bila zaražena drugim izolatima koji su bili u usporedbi. Na osnovi istraženih svojstava i sadašnjeg poznavanja tombusvirusa, tombusvirus iz špinata smatramo, šire uzevši, posebnim špinatskim sojem virusa grmolike kržljavosti rajčice (tomato bushy stunt virus). Ovaj rad također pokazuje izuzetnu varijabilnost tombusvirusa, izraženu u preklapanju mnogih njihovih svojstava, zbog koje je teško izvršiti klasifikaciju tih virusa.

Mr. Živojin Erić
Prof. dr. Biljana Plavšić
Prirodno-matematički fakultet
Vojvode Putnika 43
YU-71000 Sarajevo (Jugoslavija)

Prof. dr. Zlata Štefanac
Prirodoslovno-matematički fakultet
Maruličev trg 20/II
YU-41000 Zagreb (Jugoslavija)