

TWO NEW NATURAL HOSTS OF TURNIP MOSAIC VIRUS IN HUNGARY

By

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The present paper gives an account of the spontaneous occurrence of turnip mosaic virus (TuMV, syn.: cabbage black ring virus, */* : */* : E : E : S/Ap) in turnip (*Brassica rapa* L. var. *rapa*) and cabbage (*Brassica oleracea* L. var. *capitata*) in Hungary. The virus was identified on the basis of test plant reaction and host range, serology, electron microscopy, phid transmissibility, inclusion bodies and physical properties. The two investigated turnip mosaic virus isolates (*HS* and *K30*) seem to be similar in host reactions to TuMV-JN and TuMV-A11 reported from cauliflower and garlic mustard in Hungary (HORVÁTH *et al.* 1975). Electron microscopy revealed flexuous filamentous particles averaging 730 nm in length. The virus was readily transmitted by *Myzus persicae* Sulz. in a non-persistent manner. Both *HS* and *K30* isolates of turnip mosaic virus produced granular cytoplasmic X-bodies of oval or irregular shape, often containing crystalline needles. They had the following physical properties: thermal inactivation point 56–58 °C, dilution end point 2×10^{-3} – 2×10^{-4} , longevity *in vitro* 2–3 days. The two virus isolates were serologically related to the Yugoslav strain of turnip mosaic virus isolated from cabbage.

Introduction

The first results of experiments with turnip mosaic virus are linked with the name of SCHULTZ (1921). According to the investigations made during the past half century the turnip mosaic virus is spread all over the world and has numerous natural and artificial host plants (SMITH 1935, TOMPKINS 1937, TOMPKINS *et al.* 1938, LARSON—WALKER 1939, WALKER *et al.* 1945, BROADBENT 1957, USCHDRAWIT—VALENTIN 1957, BHARGAVA—YOSHII 1960, ARNOLD—BALD 1960, ŠTEFANAC—UDJBINAC *et al.* 1963, SHUKLA—SCHMELZER 1970, 1972, 1973, FELDMAN—GRACIA 1972, WEATHERS *et al.* 1972, PONTIS 1973, SCHMELZER—SCHMELZER 1974). In the course of studies in cruciferous plants in Hungary the occurrence of turnip mosaic virus has just recently been experimentally proved (HORVÁTH *et al.* 1975) natural hosts being cauliflower (*Brassica oleracea* L. var. *botrytis* (L.) Alef.) and garlic mustard (*Alliaria petiolata* (M. B.) Cavara et Grande). During our further investigations into the distribution of the turnip mosaic virus in Hungary we found two additional natural host plants, i.e. cabbage and turnip. In this paper identification of virus isolates from these two plants is reported.

Material and Method

In the autumn of 1973 among the turnip plants (*Brassica rapa* L. var. *rapa*) bred in the experimental field of the Research Institute for Beet Growing at Sopronhorpács (Hungary) we found some which with respect to symptoms differed from those plants from which radish mosaic virus and turnip yellow mosaic virus had earlier been isolated in Hungary (MAMULA et al. 1972, HORVÁTH et al. 1973, JURETIĆ et al. 1973). The diseased turnip plants were characterized by systemic vein clearing, vein yellowing, severe mosaic with light and dark green patches or blisters and severe distortion and stunting (Fig. 1A). In the same autumn,

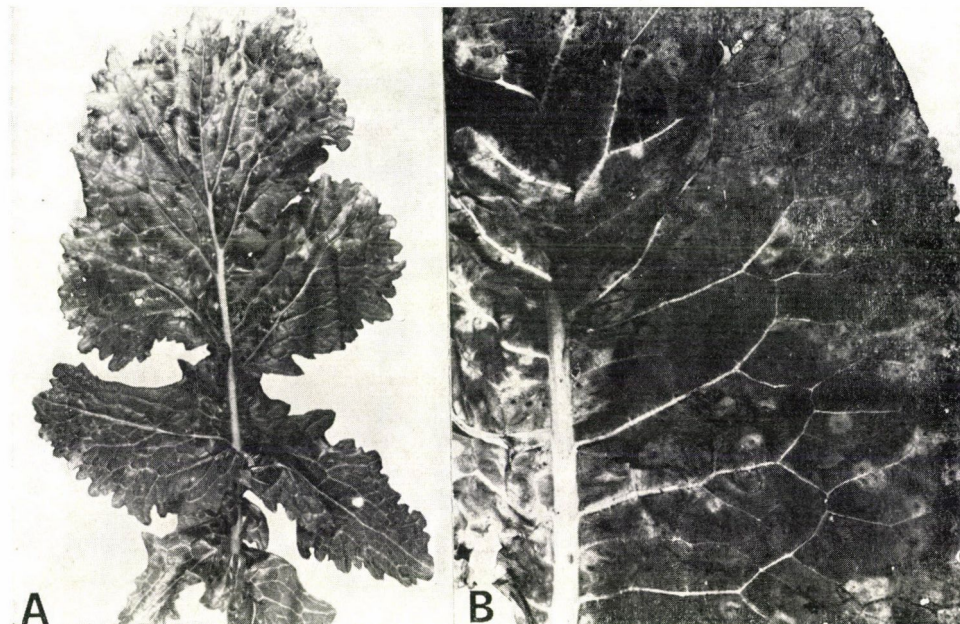


Fig. 1. Symptoms of the spontaneously infected *Brassica rapa* L. var. *rapa* (A) and *Brassica oleracea* L. var. *capitata* plants (B) with turnip mosaic virus

at the Variety Testing Station at Tordas (Hungary) our attention was attracted by cabbage plants (*Brassica oleracea* L. var. *capitata*) showing typical greyish brown necrotic ring-shaped spots. These spots were particularly conspicuous when looked at from the abaxial part of the leaf (Fig. 1B). From the leaves of the diseased turnip and cabbage plants separate samples were collected. The sample collected from turnip plants was marked with the symbol *HS*, while that taken from cabbage plants with *K30*.

The two isolates (*HS* and *K30*) were transmitted from turnip and cabbage, respectively, to *Brassica rapa* var. *rapa* and then to other test plants by the conventional technique of grinding young leaves in 0.15 M phosphate buffer at pH 7.0. In serological experiments immune sera were used against radish mosaic virus, turnip yellow mosaic virus and turnip mosaic virus. The tests performed by the first two sera were done by means of the double diffusion technique (VAN REGENMORTEL 1966, WETTER-LUISONI 1969, MATTHEWS 1970). Slide precipitin tests were used in case of turnip mosaic virus antiserum application. All sera were kindly supplied by the Institute of Botany, University of Zagreb (Yugoslavia). Infected plant sap of turnip was examined by the dipping-method with a Siemens Elmiskop I.

For the aphid transmission studies of *HS* and *K30* isolates, fasted, non-viruliferous aphids were used. *Myzus persicae* Sulz. aphids, starved for 3 hours in glass tubes, were allowed to feed on diseased turnip leaves for 5–10 minutes, and then ten of them were transferred to each single healthy turnip plant. In the transmission studies 30 turnip plants were used for each of the *HS* and *K30* isolates.

The light microscope examinations of inclusion bodies were only performed with living cells of turnip plants. The inclusion bodies were studied in tissue sections taken from the midribs of the turnip leaf. Only epidermis cells were investigated. The physical properties (thermal inactivation point, longevity *in vitro*, dilution end point) of the two isolates were determined in the sap of the infected leaf material of the turnip as source. *Nicotiana tabacum* L. cv. *Xanthi-nc* and *Chenopodium amaranticolor* Coste et Reyn. were used as test plants (HORVÁTH et al. 1975).

Results

The experiments aimed at establishing the host range of the *HS* and *K30* isolates included 35 species of eight plant families. Generally, test plant reactions to isolates *HS* and *K30* corresponded to those provoked by turnip mosaic virus (Table 1, Figs 2 and 3). These data suggested that our isolates could belong to turnip mosaic virus. The symptoms produced by isolates *HS* and *K30* were similar to each other in all plants with the exception of *Brassica*

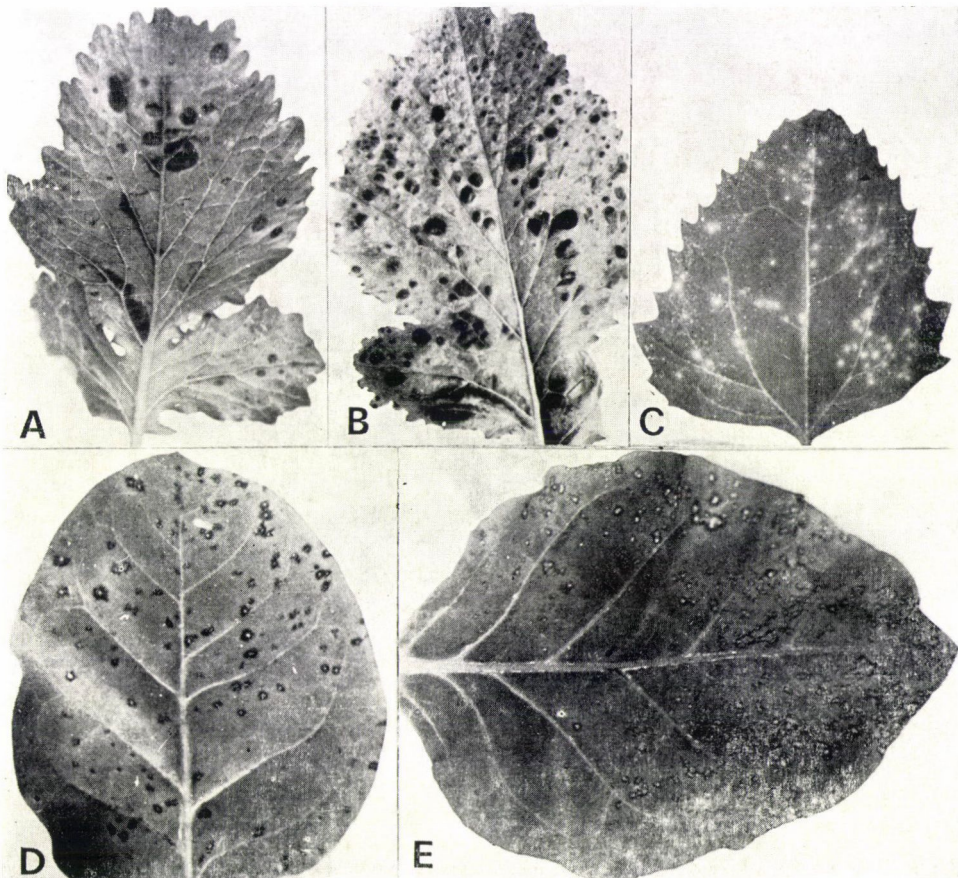


Fig. 2. Symptoms of turnip mosaic virus on various test plants. A and B: Systemic symptoms of the *K30* isolate; C, D and E: Local symptoms of the *K30* isolate (C and D), and *HS* isolate (E). A and B: *Brassica rapa* L. var. *rapa*, C: *Chenopodium amaranticolor* Coste et Reyn., D: *Nicotiana tabacum* L. cv. *Samsun*, E: *Nicotiana tabacum* L. cv. *Xanthi-nc*

oleracea varieties and *Nicotiana glutinosa* L. (Table 1). In the last two plants isolate *K30* provoked much stronger symptoms than isolate *HS*. The plants that were resistant to the two isolates during the host range investigations had been also resistant to two isolates of the turnip mosaic virus described by HORVÁTH *et al.* (1975). Our supposition based on test plant reactions that the *HS* and *K30* isolates belong to the turnip mosaic virus was later unam-

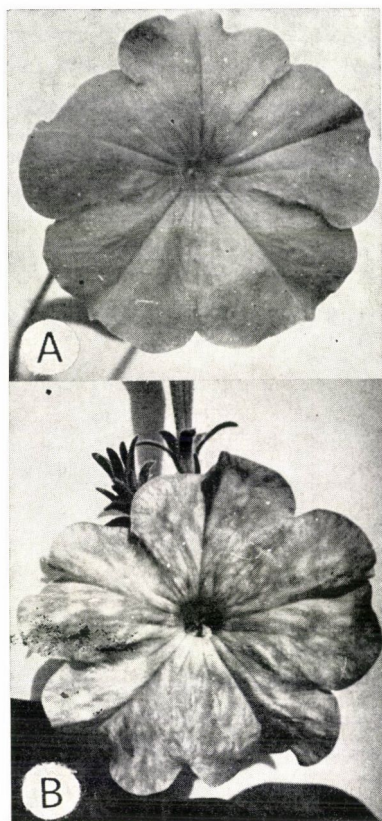


Fig. 3. Healthy flower of *Petunia hybrida* hort. ex Vilm. (A) and colour flower breaking (B) of the diseased plant inoculated with the *K30* isolate of turnip mosaic virus

biguously confirmed by serological reactions; in all serological tests both *HS* and *K30* isolates reacted positively with immune serum of turnip mosaic virus (homologous titer 1/256). These experiments showed, moreover, that isolates *HS* and *K30* were serologically related to a Yugoslavian isolate of this virus which was isolated from cabbage (MILIČIĆ *et al.* 1958, 1963). The lack of positive reaction between the two isolates and antisera against radish mosaic and turnip yellow mosaic viruses showed that isolates *HS* and *K30* were free from these viruses.

Table 1

Reaction of several plants to the HS and K30 isolates of turnip mosaic virus*

AIZOACEAE	
<i>Tetragonia echinata</i> Ait.	I: Chlorotic spots II: Not infected
<i>T. tetragonoides</i> (Pall.) O. Ktze	I: Chlorotic spots II: Not infected (sometimes secondary spots on the non-inoculated leaves)
AMARANTHACEAE	
<i>Gomphrena globosa</i> L.	I: Gray local lesions with pink border II: Not infected
CHENOPODIACEAE	
<i>Atriplex nitens</i> Schk.	I: Local chlorotic or necrotic spots II: Sometimes secondary spots on the non-inoculated leaves
<i>Chenopodium amaranticolor</i> Coste et Reyn.	I: Chlorotic local lesions that turn into necrotic lesions (Fig. 2C) II: Not infected
<i>Ch. foliosum</i> Aschers.	I: Chlorotic and necrotic lesions II: Not infected
<i>Ch. murale</i> L.	I: Minute necrotic lesions II: Not infected
<i>Ch. quinoa</i> Willd.	I: Chlorotic local lesions II: Not infected
<i>Obione sibirica</i> (L.) Fisch.	I: Chlorotic local lesions II: Not infected
CRUCIFERAE	
<i>Brassica campestris</i> L.	I: Not infected II: Systemic vein clearing, mosaic with light and dark green patches or blisters
<i>Br. carinata</i> A. Br.	I: Not infected II: Systemic vein clearing and mosaic
<i>Br. oleracea</i> L. var. <i>capitata</i>	I: Not infected II: Isolate K30; pale green rings, mottling, black necrotic rings Isolate HS; weak pale green rings and mottling
<i>Br. oleracea</i> L. var. <i>botrytis</i>	I: Not infected II: Isolate K30; pale green rings, mottling, black necrotic rings Isolate HS; weak pale green rings and mottling
<i>Br. rapa</i> L. var. <i>rapa</i>	I: Chlorotic and sometimes necrotic local lesions II: Systemic vein clearing and veinal flecking, developing into severe mosaic with light and dark islands Severe distortion and stunting (Fig. 2A and B)
<i>Bunias orientalis</i> L.	I: Not infected II: Systemic mosaic spots
<i>Cherianthus cheiri</i> L.	I: Not infected II: Systemic leaf distortion, mottling and colour breaking of flower
<i>Lunaria annua</i> L.	I: Not infected II: Mosaic and leaf deformations
<i>Matthiola incana</i> (L.) R. Br.	I: Not infected II: Mild mottling

* I, denotes rubbed leaves (local symptoms); II, denotes leaves developed after inoculation (systemic symptoms)

Table 1
(continued)

CUCURBITACEAE	
<i>Bryonia alba</i> L.	I: Not infected II: Not infected
<i>B. dioica</i> Jacq.	I: Not infected II: Not infected
<i>Cucumis sativus</i> L.	I: Not infected II: Not infected
<i>Cucurbita pepo</i> L. convar. <i>patissonina</i> Greb. f. <i>radiata</i> Nois.	I: Not infected II: Not infected
LEGUMINOSAE	
<i>Phaseolus vulgaris</i> L. cv. Red Kidney	I: Not infected II: Not infected
SOLANACEAE	
<i>Capsicum annuum</i> L.	I: Not infected II: Not infected
<i>Datura stramonium</i> L.	I: Not infected II: Not infected
<i>Nicotiana chinensis</i> Fisch.	I: Local chlorotic spots II: Not infected
<i>N. clevelandi</i> A. Gray	I: Necrotic local lesions II: Necrosis on top leaves
<i>N. glutinosa</i> L.	I: Not infected II: Isolate <i>K30</i> ; conspicuous systemic mottling and leaf distortion Isolate <i>HS</i> ; systemic mottling and leaf distortion (sometimes erratic reaction)
<i>N. megalosiphon</i> Heurck. Muellj.	I: Not infected II: Systemic mottling
<i>N. occidentalis</i> Wheeler	I: Local necrotic lesions II: Systemic mosaic, sometimes necrotic spots
<i>N. tabacum</i> L. cv. Bel 61-10, Samusn, White Burley, and Xanthi-nc	I: Local necrotic lesions (Fig. 2D-E) II: Not infected
<i>Petunai hybrida</i> hort. ex Vilm.	I: Black necrotic local lesions II: Systemic vein clearing, mottling and colour breaking of flower (Fig. 3)
<i>Solanum capsicastrum</i> Lk.	I: Not infected II: Not infected
<i>S. ochroleucum</i> Bast.	I: Not infected II: Not infected
UMBELLIFERAE	
<i>Ammi majus</i> L.	I: Not infected II: Not infected
<i>A. visnaga</i> (L.) Lam.	I: Not infected II: Not infected

Electron microscope analysis of the sap of infected turnip plants revealed that our isolates had flexuous filamentous particles of about 730 nm length. It must be pointed out that only several particles were measured. The *HS* and *K30* isolates were easily transmitted by *Myzus persicae* aphids from one turnip plant to another in a non-persistent manner. Investigations concerning the

cell inclusion bodies showed that *K30* and *HS* isolates produced *X*-bodies in the cytoplasm of infected plants. The bodies were oval or of irregular shape, with a granular structure (Fig. 4). At a later stage of the infection numerous

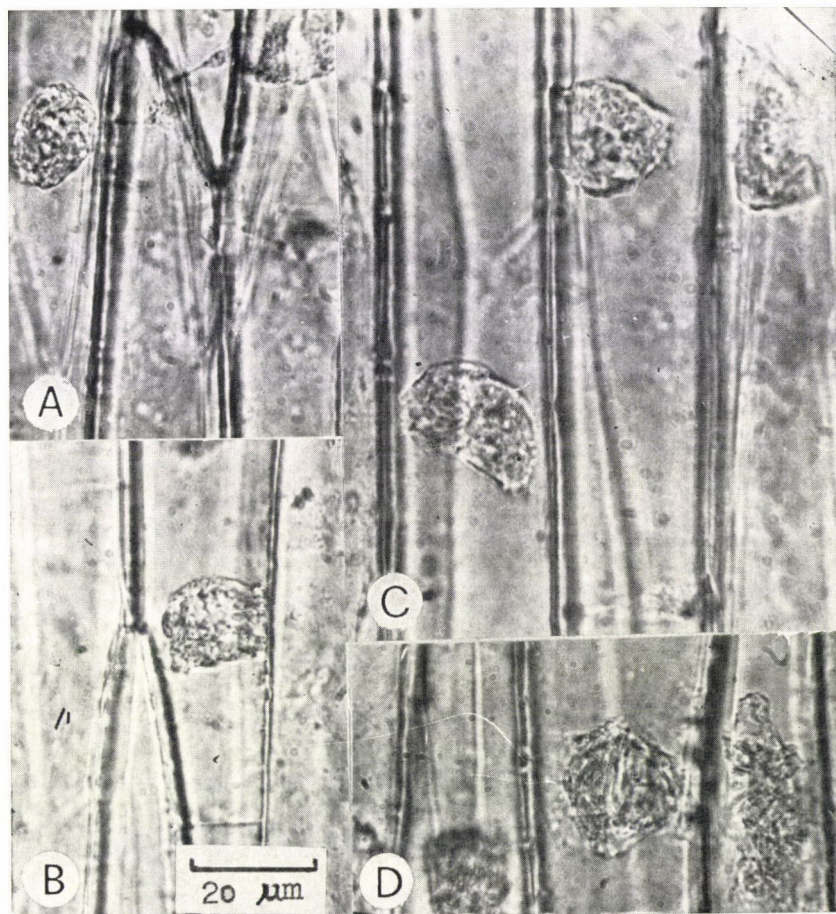


Fig. 4. Inclusion bodies (*X*-bodies) in epidermal cells of *Brassica rapa* L. var. *rapa* leaf (midrib region) inoculated with the *K30* isolate of turnip mosaic virus

minute crystalline needles were usually seen in the *X*-bodies. The *X*-bodies of our isolates were in every respect similar to those described for turnip mosaic virus by RUBIO (1956), MILIČIĆ (1956), MILIČIĆ *et al.* (1963) and STEFANAĆ—MILIČIĆ (1965). Inclusion bodies were observed in the cells of *Brassica rapa* var. *rapa*, *Nicotiana glutinosa*, *N. megalosiphon* Heurck. et Muell. and *Petunia hybrida* hort. ex Villm. When examining the physical properties of the two isolates we found that they were slightly different from each other (see Table

2). Additionally, *HS* and *K30* isolates differed also slightly in physical properties from two isolates of turnip mosaic virus found earlier in Hungary (HORVÁTH *et al.* 1975).

Table 2

Physical properties of the HS and K30 isolates of turnip mosaic virus

Isolates of turnip mosaic virus	Physical properties*		
	TIP (in °C)	DEP	Liv (in days)
<i>HS</i>	58	$10^{-3} - 2 \times 10^{-4}$	3
<i>K30</i>	56	2×10^{-3}	2

* TIP, thermal inactivation point; DEP, dilution end point; Liv, longevity *in vitro*

On the basis of differences in physical properties, and especially with respect to the different reaction of *Brassica oleracea* varieties and *Nicotiana glutinosa* to isolate *HS* and *K30*, it is likely that the two isolates belong to different strains of turnip mosaic virus. Accordingly, isolate *HS* should be attached to the *ordinary strain*, and *K30* isolate to the *cabbage strain* of this virus (comp. YOSHII 1963). In this respect isolate *HS* is similar to the TuMV-All isolate, and *K30* to the TuMV-JN isolate of turnip mosaic virus found earlier in Hungary (HORVÁTH *et al.* 1975).

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References

- ARNOLD, W. N.—BALD, J. G. (1960): Turnip mosaic virus from two weed hosts. *Phytopathology*, **50**, 578—589.
- BHARGAVA, K. S.—JOSHI, R. D. (1960): A distinctive strain of cabbage black ring virus from some ornamental plants. *Phytopath. Z.*, **40**, 109—116.
- BROADBENT, L. (1957): *Investigations of virus diseases of Brassica crops*. Cambridge Univ. Press, Cambridge.
- FELDMAN, J. M.—GRACIA, O. (1972): Studies of weed plants as sources of viruses. II. *Eruca sativa*, *Rapistrum rugosum* and *Sisymbrium irio*, new natural hosts for turnip mosaic virus. *Phytopath. Z.*, **73**, 115—122.
- HORVÁTH, J.—JURETIĆ, N.—MILIČIĆ, D. (1973): *Crambe abyssinica* Hochst. ex R. E. Frees as a new host plant for turnip yellow mosaic virus and radish mosaic virus. *Phytopath. Z.*, **78**, 69—74.
- HORVÁTH, J.—JURETIĆ, N.—BESADA, W. H.—MAMULA, D. (1975): Natural occurrence of turnip mosaic virus in Hungary. *Acta Phytopath. Acad. Sci. Hung.*, **10**, 77—88.
- JURETIĆ, N.—HORVÁTH, J.—MAMULA, D.—MILIČIĆ, D. (1973): Natural occurrence of turnip yellow mosaic virus in Hungary. *Acta Phytopath. Acad. Sci. Hung.*, **8**, 175—183.

- LARSON, R. H.—WALKER, J. C. (1939): A mosaic disease of cabbage. *J. Agr. Res.*, **59**, 367—392.
- MAMULA, D.—MILIČIĆ, D.—ŠTEFANAC, Z.—HORVÁTH, J. (1972): Neue Angaben über Verbreitung und Wirtspflanzen des Rettichmosaik-Virus (radish mosaic virus). *Acta Phytopath. Acad. Sci. Hung.*, **7**, 369—375.
- MATTHEWS, R. E. F. (1970): *Plant Virology*. Academic Press, New York—London.
- MILIČIĆ, D. (1956): Virus-Zelleinschlüsse in *Alliaria officinalis*. *Protoplasma*, **47**, 341—346.
- MILIČIĆ, D.—PANJAN, M.—BILANOVIĆ, D.—KATIĆ, B. (1958): Viruskrankheit von *Alliaria officinalis*. *Acta Bot. Croatica*, **17**, 159—176.
- MILIČIĆ, D.—ŠTEFANAC-UDJBINAC, Z.—MAMULA, D. (1963): Rasprostranjenost nekih virusa krucifera u Jugoslaviji. *Agronomski Glasnik*, **13**, 92—100.
- PONTIS, R. E. (1973): Turnip mosaic virus on annual stock in Argentina. *Plant Dis. Rep.*, **57**, 379—382.
- RUBIO, M. (1956): Origin and composition of cell inclusions associated with certain tobacco and crucifer viruses. *Phytopathology*, **46**, 553—556.
- SCHMELZER, K.—SCHMELZER, A. (1974): *Euphorbia peplus* L., ein mögliches Reservoir des Kohlschwarzring-Virus (cabbage black ring virus). *Arch. Phytopathol. u. Pflanzenschutz*, **10**, 217—220.
- SCHULTZ, E. S. (1921): A transmissible mosaic disease of Chinese cabbage, mustard and turnip. *J. Agr. Res.*, **22**, 173—177.
- SHUKLA, D. D.—SCHMELZER, K. (1970): Studies on viruses and virus diseases of cruciferous plants. I. Viruses in some ornamentals. *Acta Phytopath. Acad. Sci. Hung.*, **5**, 137—144.
- SHUKLA, D. D.—SCHMELZER, K. (1972): Studies on viruses and virus diseases of cruciferous plants. VII. Occurrence and effect of cabbage black ring and cauliflower mosaic viruses on *Brassica* crops. *Acta Phytopath. Acad. Sci. Hung.*, **7**, 325—342.
- SHUKLA, D. D.—SCHMELZER, K. (1973): Studies on viruses and virus diseases of cruciferous plants. XIII. Cabbage black ring, nasturtium ringspot and alfalfa mosaic viruses in ornamental and wild species. *Acta Phytopath. Acad. Sci. Hung.*, **8**, 139—148.
- SMITH, K. M. (1935): A virus disease of cultivated crucifers. *Ann. Appl. Biol.*, **22**, 239—242.
- ŠTEFANAC, Z.—MILIČIĆ, D. (1965): Zelleinschlüsse des Kohlrübenmosaikvirus. *Phytopath. Z.*, **52**, 349—362.
- ŠTEFANAC-UDJBINAC, Z.—MILIČIĆ, D.—ZELJKO, M. (1963): Virus mozaika postrne repe (turnip mosaic virus) u Jugoslaviji. *Acta Bot. Croatica*, **22**, 107—117.
- TOMPKINS, C. M. (1937): A transmissible mosaic disease of cauliflower. *J. Agr. Res.*, **55**, 33—46.
- TOMPKINS, C. M.—GARDNER, M. W.—THOMAS, H. R. (1938): Black ring, a virus disease of cabbage and other crucifers. *J. Agr. Res.*, **57**, 929—943.
- USCHDRAWAIT, H. A.—VALENTIN, H. (1957): Untersuchungen über ein Kruziferen-Virus. *Phytopath. Z.*, **31**, 139—148.
- VAN REGENMORTEL, M. H. V. (1966): Plant virus serology. *Adv. Virus Res.*, **12**, 207—271.
- VAN REGENMORTEL, M. H. V. (1967): Serological studies on naturally occurring strains and chemically induced mutants of tobacco mosaic virus. *Virology*, **31**, 467—480.
- WALKER, J. C.—LEBEAU, F. J.—POUND, G. S. (1945): Virus associated with cabbage mosaic. *J. Agr., Res.*, **70**, 379—404.
- WEATHERS, L. G.—GUMPF, D. J.—ENDO, R. M. ((1972): A disease of "Green Ball" cauliflower caused by turnip mosaic virus. *Plant Dis. Rep.*, **56**, 441—442.
- WETTER, C.—LUISONI, E. (1969): Precipitin, agar gel diffusion, and intragel absorption tests with three strains of tomato bushy stunt virus. *Phytopath. Z.*, **65**, 231—242.
- YOSHII, H. (1963): On the strain distribution of turnip mosaic virus. *Ann. Phytopath. Soc. Japan*, **28**, 221—227.