

IDENTIFICATION OF TURNIP MOSAIC VIRUS IN *TROPAEOLUM MAJUS* L.

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Introduction

Turnip mosaic virus (TuMV; cabbage black ringspot virus, cabbage virus A) is a virus with a world-wide distribution. It attacks under natural conditions many cruciferous and non-cruciferous plants. Among the latter plants infection with TuMV has been recorded on *Tropaeolum majus* L. (Smith 1950, 1953; Kristensen 1959).

T. majus with virus-like symptoms on the leaves was found and investigated. It was established that the plant was infected with an isolate of TuMV.

Material and Methods

Among over 50 fully developed *T. majus* specimens, which grew in a public park at Vela Luka (Island of Korčula, Yugoslavia), only one appeared to be virus-infected. On somewhat malformed and dwarfed leaves of that exemplar a strong variegation and mosaic symptoms were present (Fig. 1 A, B). A virus isolate (designated TK) was drawn from that plant and investigated with the aim of identification.

Isolate TK was examined for test plants reactions, serological relationship, properties of virus particles, inclusion bodies and thermal inactivation point. In serological experiments (microprecipitin test in free liquid on microscope slide) and in determination of thermal inactivation point, infective leaf extracts were first centrifuged at 3500 g for 10 min. Antiserum against a Yugoslav isolate of TuMV from *Brassica oleracea* L. cv. capitata (cf. Miličić et al. 1963) was used in serological experiments. Homologous antiserum titre was 1/16.

Analysis of Isolate TK

Reaction of test plants

Transmission of the TK isolate was successful to *Nicotiana tabacum*, *N. glutinosa*, *Chenopodium amaranticolor*, *C. quinoa*, *Brassica rapa* var. *rapa* and *Tetragonia expansa* with the first inoculation from spontane-

ously infected *T. majus*. Afterwards, a few other species were infected, too. The results of inoculation tests are shown in Table 1.

Table 1. Symptoms in test plants infected with isolate TK of turnip mosaic virus. 0 = No symptoms

Tablica 1. Simptomi na pokusnim biljkama koje su bile inficirane izolatom TK virusa mozaika postrne repe. 0 = Bez simptoma

Test plant	Primary symptoms	Secondary symptoms
<i>Brassica oleracea</i> L. cv. <i>capitata</i>	0	Mild mosaic and line pattern
<i>Brassica rapa</i> L. var. <i>rapa</i>	0	Faint to moderate vein-clearing; mosaic, often blistered leaves, other leaf malformations, stunting (Fig. 2 B)
<i>Chenopodium amaranticolor</i> Coste et Reyn.	Light green lesions with minute necrotic centre which later enlarges (Fig. 2 D)	Sporadically chlorotic and necrotic patches along bigger veins on leaves
<i>Chenopodium quinoa</i> Willd.	Chlorotic lesions	0
<i>Nicotiana glutinosa</i> L.	Sometimes chlorotic lesions	Light green mosaic and variegation, necrotic patches, blistered leaves, other leaf malformations, stunting
<i>Nicotiana megalosiphon</i> Heurck et Muell.	Sporadic lesions	Mild mosaic, necrotic patches, leaf malformations, stunting
<i>Nicotiana tabacum</i> L. cv. "White Burley" and "Xanthi"-nc	Dark brown necrotic lesions often with brighter centre (Fig. 1 D)	0
<i>Physalis peruviana</i> L.	Sporadic small necrotic lesions	Bright mosaic (Fig. 2 C)
<i>Tetragonia expansa</i> Thunb.	Chlorotic lesions (Fig. 2 A)	0

Serological relationship

Isolate TK gave positive serological reaction with the antiserum against TuMV. Weak precipitation was obtained in antiserum dilution 1/16, too. A rather similar result was achieved with an isolate of TuMV from *B. rapa* var. *rapa* which was involved in experiments for comparison. Healthy leaf extracts gave a weak precipitation only with the undiluted antiserum. Leaf extracts of *B. rapa* var. *rapa* were used in all cases of experiments.

Electron microscope examinations

Systemically infected leaves of *B. rapa* var. *rapa* and *N. glutinosa* were subjected to these examinations. Dipping method and negative staining with phosphotungstate were applied. The preparations of both plants contained a few elongated flexuous virus particles (Fig. 3 A, B). Measurements of 5 particles from *B. rapa* var. *rapa* showed that the average length was 726 nm and the average width less than 20 nm. Similarly, in *N. glutinosa* the average length of 4 particles was 730 nm and the width between 10 and 20 nm, too. The length of all 9 particles averaged 728 nm.

It is interesting to note that at certain stages of infection (cca 6 weeks after inoculation) tubular structures about 40 nm wide and over 700 nm, and perhaps considerably longer, could be observed in infective sap of *N. glutinosa* (Fig. 3 C). The electron microscope apparatus used was a Siemens Elmiskop I.

Hystological examinations

Cytoplasmic inclusion bodies were often observed in systemically infected leaves of *B. rapa* var. *rapa* twenty or more days after inoculation. The inclusions were built up of cca 10—20 or more needle-like crystals linked together in an amorphous base mass (Fig. 2 F).

Thermal inactivation point

Experiments were performed to establish thermal inactivation point of isolate TK. Systemically infected *B. rapa* var. *rapa* leaves served as the source of the virus, while *C. amaranticolor* was the test plant. The results are shown in Table 2.

Table 2. Examination of the thermal inactivation point of isolate TK of TuMV, the Numerator indicates total number of lesions and the denominator shows the number of *C. amaranticolor* leaves inoculated.

Tablica 2. Ispitivanje terminalne inaktivacije izolata TK. Brojnik označuje ukupan broj lezija a nazivnik broj inokuliranih listova vrste *C. amaranticolor*

Infective sap unheated	Infective sap heated at temperature of °C						
	50	52	54	55	56	60	65
65/6	6/6	5/6	0/6	0/6	0/6	0/6	0/6

The results suggested that the virus was inactivated in the temperature region between 52° and 54° C.

The spread of various viruses in *Tropaeolum majus*

Several viruses have been found infecting spontaneously *Tropaeolum* spp. It seems a question was always of *T. majus*. An outline of these data is given in Table 3. (cf. Schmelzer 1960).

Table 3. List of virus isolates found in *Tropaeolum* spp. under natural conditions

Tablica 3. Popis virusnih izolata koji su nađeni na vrstama *Tropaeolum* u prirodi

Plant	Virus	Country	Author
<i>Tropaeolum majus</i>	Mosaic	Australia	Pittman 1929
"	Mosaic	China	Yu 1939
"	Mosaic	U. Kingdom	Smith 1950, 1953
"	Mosaic	USA	Jensen 1950
"	Mosaic	Brasil	Silberschmidt 1953
"	Mosaic	Germany	Mischke 1957
"	Mosaic	Italy	Moriondo 1958
"	Mosaic	Denmark	Kristensen 1959
"	Tomato spotted wilt	Australia	Bald and Samuel 1931
"	Tomato spotted wilt	U. Kingdom	Pittman 1934 Holmes Smith 1933
"	Tomato spotted wilt	USA	Gardner and Whipple 1934
<i>Tropaeolum</i> sp.	Tomato spotted wilt	U. Kingdom	Smith 1936
<i>Tropaeolum majus</i>	Spotted wilt	USA	Harris 1939
"	Spotted wilt	South Africa	Hopkins 1940
"	Curly top	USA	Severin and Freitag 1933
"	Broad bean virus	U. Kingdom	Smith 1949
"	Nasturtium ringspot	U. Kingdom	Smith 1950, 1953
"	Nasturtium ringspot	India	Bhargava and Joshi 1959
"	Nasturtium ring mosaic	Germany	Schmelzer 1960
"	Nasturtium ringspot	Belgium	Verhoyen and Autrique 1968
"	Nasturtium ringspot	Jugoslavia	Juretić et al 1970
"	Nasturtium ringspot	Germany	Sahambi et al. 1973

Table 3 shows that the viruses have been repeatedly found in *Tropaeolum majus*. The following viruses were found to be present: nasturtium mosaic (Silberschmidt 1953; cf. Smith 1972), turnip mosaic (Smith 1950, 1953; Kristensen 1959), tomato spotted wilt, beet curly-top and broad bean wilt virus (nasturtium ringspot). Some of the isolates included in Table 3 under "Mosaic" might belong to nasturtium mosaic whereas the others might be grouped with turnip mosaic virus.

In addition, some mycoplasma diseases have been recorded on *T. majus*. These are: witches' broom (Bos 1957, Bos and Grancini 1965), tomato big bud (Rept. N. S. W. Dept. Agriculture 1958; cf. Rev. appl. Mycol. 38, 563) and a disease similar to clover dwarf (Lehmann 1969) (cf. Maramorosch et al. 1970, Matthews 1971, Smith 1972).

Fig. 1. Symptoms of isolate TK on plants. A and B: Variegation and mosaic patterns on leaves of spontaneously infected *Tropaeolum majus* plant; leaf malformations are present, too. C: Leaf of healthy *T. majus* plant. D: Necrotic lesions on inoculated leaf of *Nicotiana tabacum* cv. Xanthi-nc. E. Systemic symptoms in *Nicotiana glutinosa*; blistering of leaves and other malformations are visible.

Sl. 1. Simptomi izolata TK na biljkama. A i B: Različiti uzorci šarenila i mozaika na listovima prirodno inficirane vrste *T. majus*; listovi su deformirani. C: List zdrave biljke *T. majus*. D: Nekrotične lezije na inokuliranom listu duhana Xanthi-nc. E: Sistemski simptomi na vrsti *N. glutinosa*. Vide se mjehurasta ispupčenja i udubljenja, te druge deformacije listova.

Fig. 2. Symptoms (A-E) and cell inclusion (F) of isolate TK. A: Inoculated leaf of *Tetragonia expansa* displaying chlorotic lesions. B: Systemically infected leaf of *Brassica rapa* var. *rapa*; blished mosaic is present. C: Leaves of systemically infected *Physalis peruviana* showing mild mosaic. D: Inoculated leaf of *Chenopodium amaranticolor* with necrotic lesions. E: Leaf of healthy *B. rapa* var. *rapa* plant. F: Cytoplasmic inclusion body in leaf lower epidermis cell (mid-rib region) of *B. rapa* var. *rapa*. Needle-shaped crystals in the body can be recognised; nucleus is marked n.

Sl. 2. Simptomi (A-E) i stanična uklopina (F) izolata TK. A: Inokulirani list vrste *T. expansa* na kojemu se vide klorotične lezije. B: Sistemski inficirani list vrste *B. rapa* var. *rapa*; uočavaju se mozaik i mjehurasta ispupčenja i udubljenja. C: Sistemski inficirana vrsta *Physalis peruviana*; na listovima se vidi blagi mozaik. D: Nekrotične lezije na inokuliranom listu biljke *C. amaranticolor*. E: List zdrave biljke *B. rapa* var. *rapa*. F: Citoplazmatska uklopina u donjoj epidermi lista (područje glavne žile) kod vrste *B. rapa* var. *rapa*. Unutar uklopine mogu se uočiti kristali igličastog oblika; stanična jezgra označena je s n.

Fig. 3. Electron micrographs of structures due to infection with isolate TK. A: Particles of isolate TK from infected *B. rapa* var. *rapa*. B: Similar as in A but from *N. glutinosa*. Bar in A is valid for B, too. C: Tubular structures in leaf sap of infected *N. glutinosa*.

Sl. 3. Elektronskomikroskopske snimke struktura koje su u vezi s infekcijom izolatom TK. A: Elementarne čestice izolata TK iz inficirane biljke *B. rapa* var. *rapa*. B: Virusne čestice u vrsti *N. glutinosa*. Mjerilo iz A vrijedi i za B. C: Cjevaste strukture u soku lista od inficirane *N. glutinosa*.

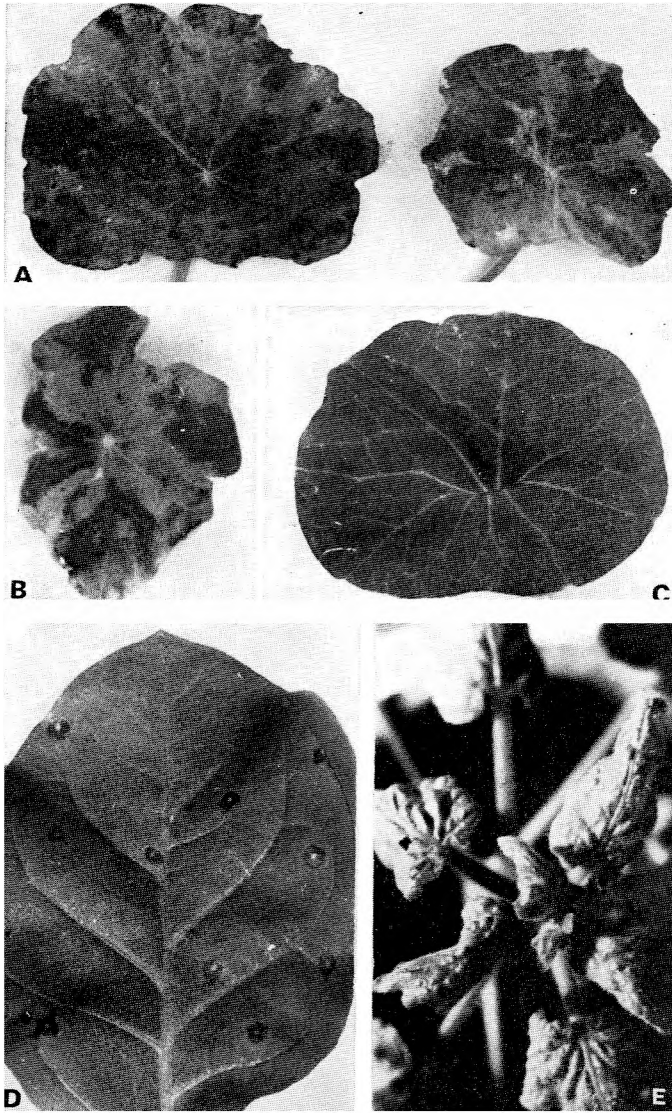


Fig. 1. — Sl. 1.

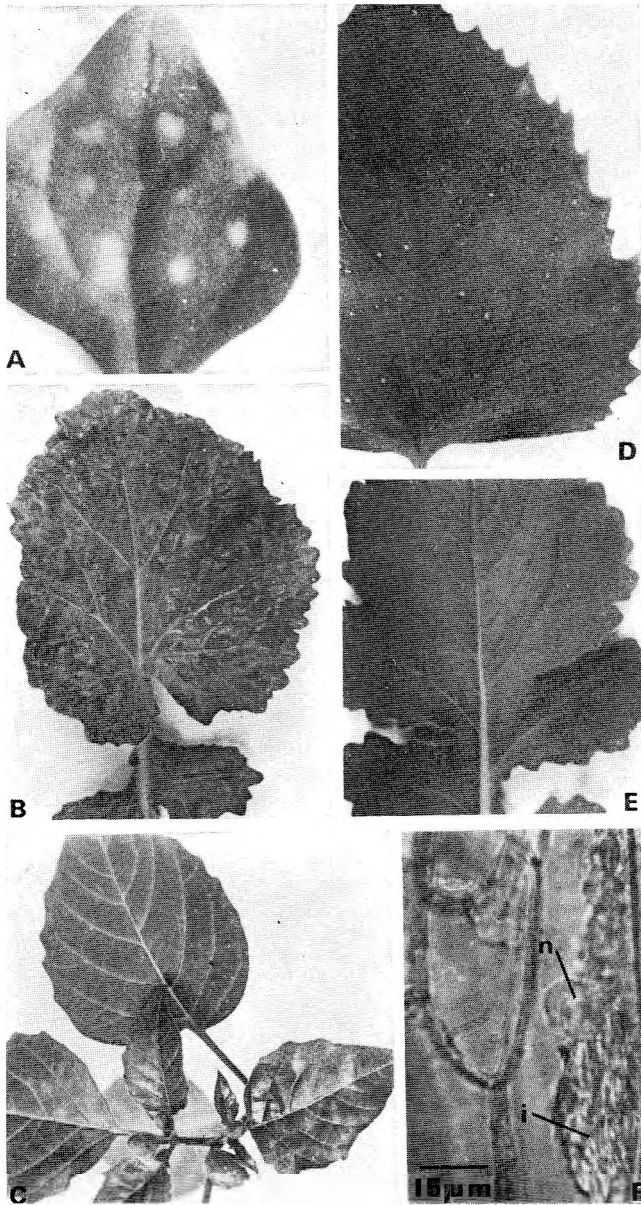


Fig. 2. — Sl. 2.

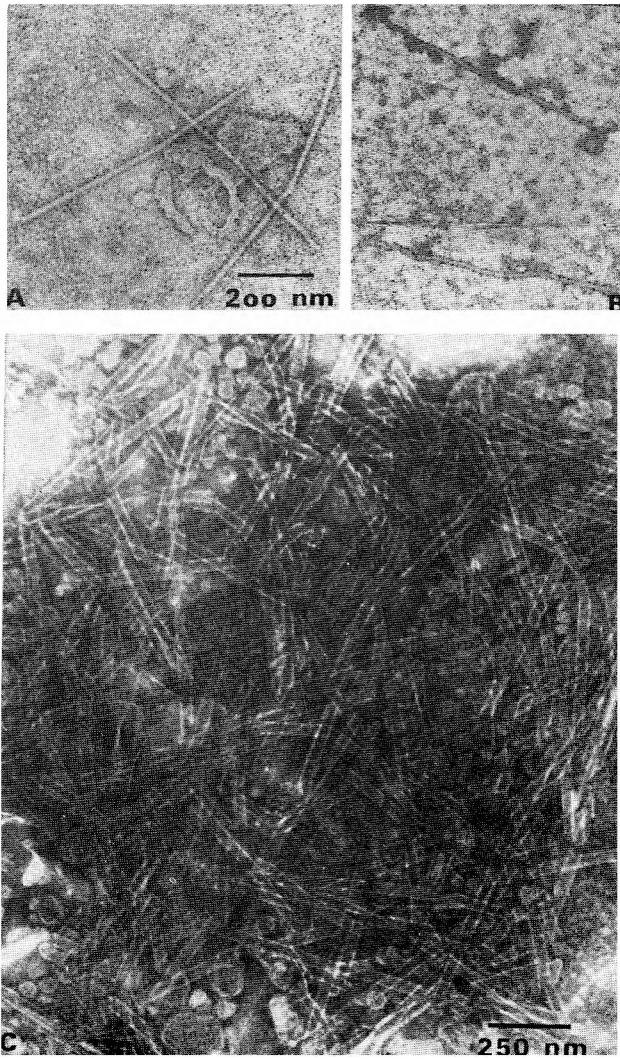


Fig. 3. — Sl. 3.

Table 4. Spontaneous hosts of TuMV among non-cruciferous species

Tablica 4. Prirodni domaćini virusa mozaika postrne repe izvan porodice krstašica

Plant	Family	Country	Author
<i>Impatiens parviflora</i>	Balsaminaceae	Czechoslovakia	Polák 1967
<i>Anchusa capensis</i>	Boraginaceae	South Africa	Dyer 1949
<i>Cichorium endivia</i>	Compositae	South Africa	McClellan and Cowin 1952/53
<i>Helipterum (Acroclinium) roseum</i>	"	India	Vashisth 1963
<i>Lactuca sativa</i>	"	USA	Zink and Duffus 1969
<i>Senecio vulgaris</i>	"	U. Kingdom	Hollings 1957
<i>Papaver nudicaule</i>	Papaverraceae	South Africa	Dyer 1949
<i>P. somniferum</i>	"	"	" "
<i>P. rhoeas</i>	"	"	McClellan and Cowin 1952/53
<i>Plantago depressa</i>	Plantaginaceae	China	Wang et al. 1965
<i>Limonium perezii</i>	Plumbaginaceae	USA	Niblett et al. 1969
<i>Rheum rhaponticum</i>	Polygonaceae	Germany	Schade 1959
<i>Rheum rhaponticum</i>	"	U. Kingdom	Tomlinson and Walkey 1967
<i>Anemone coronaria</i>	Ranunculaceae	"	Walkey and Cooper 1972
<i>Ranunculus</i> sp. (Claremont hybrids)	"	"	Hollings 1957
<i>Verbascum thlaspi-forme</i>	Scrophulariaceae	Czechoslovakia	Polák and Neubauer 1967
<i>Hyoscyamus niger</i>	Solanaceae	England	Smith 1945a
<i>Tropaeolum majus</i>	Tropaeolaceae	"	Smith 1950, 1953
<i>Tropaeolum majus</i>	"	Denmark	Kristensen 1959

Viruses experimentally transmitted to *Tropaeolum majus*

Tropaeolum spp., predominantly or exclusively *T. majus* as it seems, have been infected artificially with a number of viruses. These include: tomato spotted wilt (Ainsworth 1933, Whipple 1936, Smith 1945 b, Delle Coste and Zabala 1946, Jones 1959, Ie 1971) and a strain of it, i. e. tomato tip-blight (Milbrath 1939; cf. Smith 1972), chrysanthemum mosaic (Smith 1952), tomato mosaic (Cesaroni 1953), raspberry leaf-curl (Chambers and Fiske 1954; probably raspberry ringspot, cf. Smith 1972), aster ringspot (Anderson 1954), tomato ringspot (Brierley 1954), cherry rasp leaf (Pfaeltzer 1959; probably raspberry ringspot or arabis mosaic, cf. Smith 1972), cucumber mosaic (Roland 1959), pea early browning (Rept. Inst. Phytopath. Res. Wageningen 1961; cf. Rev. appl. Mycol. 41, 272), papaw mosaic (Rept. Indian Counc. agric. Res. 1954; cf. Rev. appl. Mycol. 34, 770; corresponds to papaya (mild) mosaic, cf. Purcifil and Hiebert 1971), white clover virus 1 (Bancroft et al.

1960; cf. Smith 1972), hop mosaic (Schmidt 1965), apple stem pitting (Twenty-seventh Ann. Meet. of the Potomac Div. of the Am. Phytopath. Soc., cf. Abs. in Phytopathology 60, 1013—1019), radish mosaic virus (Štefanac and Mamula 1971) and sesame phyllody (Rept. Indian Agr. Res. Inst. 1962; cf. Rev. appl. Mycol. 42, 727; probably mycoplasma disease, cf. Smith 1972).

Non-cruciferous plant as spontaneous hosts to turnip mosaic virus

Under natural conditions TuMV has most frequently been found in cruciferous plants (cf. Broadbent 1957, Tomlinson 1970). However, there is a comparatively large number of non-cruciferous species which have been recorded as natural hosts to the virus but have been reviewed rather rarely. The list of those plants is given in Table 4.

Table 4 lists only the authors who found a given host for the first time. Authors who have confirmed the finding of the host or have studied the host-virus relationship in more detail are listed only exceptionally.

Discussion

Some crucifer viruses are known to be spread in Yugoslavia. Hitherto they have been found in this country in cruciferous plants only (cf. Mamula and Miličić 1971, Mamula et al 1972). The present finding of TuMV in *T. majus* seems to be the first finding of that or any other crucifer virus in a non-cruciferous plant in Yugoslavia. A few years ago *T. majus* was detected in Yugoslavia as a spontaneous host to broad bean wilt virus (Juretić et al. 1970).

T. majus has first been found as a natural host of TuMV by Smith (1950, 1953) and Kristensen (1959). These authors identified the virus on the basis of experiments with test plants. Serological, electron microscope and other examinations carried out with TK isolate in the course of the present work supported the findings of the authors cited above.

TK isolate caused quite severe symptoms in *N. glutinosa*. Despite this fact it could probably be assigned to ordinary strain of TuMV as it induced mild symptoms in *Brassica oleracea* cv. *capitata* (cf. Yoshii 1963). It is interesting to mention that several attempts to infect *T. majus* and *Petunia hybrida* Vilm. artificially (by mechanical inoculation) were unsuccessful.

There was no reliable evidence in our experiments of the presence of any other virus in the investigated *T. majus* specimen but only of TK isolate. In this connection it might be useful to point out the presence of submicroscopic tubular structures in the leaf tissue of the infected *N. glutinosa*. Structures of similar shape have been detected in virus infected plants, notably with cherry leaf roll virus (cf. Cropley and Tomlinson 1971). Those tubules, however, contained virus particles in the halo, the virus being the one with isometric particles. A further isometric virus which provokes tubular structures of similar in shape to TK isolate is turnip yellow mosaic virus (Hitchborn and Hills 1967; cf. Matthews 1970). The tubes caused by that virus are supposed to consist of viral protein. However, these tubes were larger in diameter than those of TK isolate. The nature of tubes induced by TK isolate was not examined. They, too, may consist of viral protein.

Summary

An isolate (TK) from a virus-infected *Tropaeolum majus* specimen found on the island of Korčula (Yugoslavia) was investigated for purpose of identification. The virus was identified as an isolate of turnip mosaic virus (TuMV) on the basis on the following results:

Isolate TK caused only local lesions in *N. tabacum* cvs. "White Burley" and "Xanthi"-nc and *C. quinoa*, local lesions and sporadic systemic symptoms in *C. amaranticolor*, as well as systemic symptoms in *N. glutinosa* and *B. rapa* var. *rapa*. Infected *N. glutinosa* and *B. rapa* var. *rapa* plants contained elongated flexuous particles with an average length of 728 nm. Isolate TK gave a positive serological reaction with the antiserum to TuMV. It provoked the building of cytoplasmic inclusion bodies composed of a great number of needle-shaped crystals which are characteristic of TuMV. Thermal inactivation point of isolate TK was between 52° and 54° C. Isolate TK may belong to the ordinary strain of TuMV. This is the first finding of TuMV in a non-cruciferous species in Yugoslavia.

Infective sap of *N. glutinosa* contained occasionally tubular structures of submicroscopic dimensions.

The paper contains literature data on occurrence of TuMV in non-cruciferous plants, on spontaneous infections of *T. majus* with viruses, and data on the viruses which have been experimentally transmitted to *T. majus*.

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(The abbreviation RAM designates Review of applied Mycology)

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SADRŽAJ

IDENTIFIKACIJA VIRUSNOG IZOLATA IZ VRSTE *TROPAEOLUM MAJUS* L.

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Istraživanja, koja smo iznijeli u ovom radu, vršena su na virusnom izolatu (TK) iz vrste *Tropaeolum majus* radi njegove identifikacije. Spontano inficirani primjerak *T. majus* potjecao je iz Jugoslavije (Vela Luka, otok Korčula). Na listovima prirodno inficirane biljke bili su uočljivi jako šarenilo, mozaik i deformacije. Na temelju istraživanja utvrdili smo da izolat TK predstavlja virus mozaika postrne repe (TuMV). To su, čini se, ujedno prvi podaci o nalazu TuMV u Jugoslaviji na biljci izvan porodice krstašica.

Rad predstavlja rijetku, ako ne i jedinu, identifikaciju TuMV iz vrste *T. majus*, koja osim simptoma na pokusnim biljkama (*N. tabacum*, *N. glutinosa*, *C. amaranticolor*, *C. quinoa*, *B. rapa* var. *rapa*) uzima u obzir i rezultate seroloških, elektronskomikroskopskih i drugih istraživanja.

Zanimljivo je da smo u soku inficirane *N. glutinosa* mogli ponekad opaziti cjevaste strukture submikroskopskih dimenzija koje se mogu dovesti u vezu s virusnom infekcijom.

U radu je iznesen veći broj podataka iz literature o prirodnoj raširenosti TuMV na biljkama iz više biljnih porodica, o virusima koji su prirodno rašireni na vrsti *T. majus*, te o virusima koji su na tu vrstu preneseni eksperimentalno.

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