Acta Bot. Croat. 37 (1978) 21—26

TOMATO MOSAIC VIRUS INFECTION OF GLASSHOUSE TOMATO CROPS IN YUGOSLAVIA

NIKOLA JURETIĆ

(Institute of Botany, Faculty of Science, University of Zagreb)

Received February 10, 1978

Introduction

The breeding of both outdoor tomato crops and glasshouse tomato crops has an important place in the vegetable production in Yugoslavia. The cultivation of tomato crops in glasshouses is specially spread in the southern parts of the country. Both types of crops are often charged by tomato mosaic virus (ToMV), a causal agent of economic damages. The occurrence of ToMV in field tomato crops in Yugoslavia was reported earlier (Juretić 1971). However, there are no published data on the occurrence of ToMV in tomatoes cultivated in glasshouses although it is known that this virus is spread in Yugoslav glasshouse tomato crops (Panjan, personal communication).

In 1977 during a visit to glasshouse tomato crops in the southern part of Yugoslavia (Macedonia), a lot of tomato plants with virus-like symptoms were observed. In order to establish whether this disease is at least partly caused by ToMV, some identification experiments were done. The data obtained are presented here.

Material and Methods

Infected tomato plants were collected in March in glasshouses at Bogdanci (Macedonia). The identification of ToMV was done by means of differential hosts, type of virus inclusions, electron microscope analysis and serological tests.

Serological tests were carried out by immunodiffusion in agar using antisera against common tobacco and tomato mosaic virus. In these experiments purified virus preparations were tested. The purification was done by the polyethylene glycol (PEG) method (Gooding and Hebert 1967).

Results

Infected tomato plants collected in glasshouses showed four principle types of symptoms: green mosaic with deformations of younger leaves (sample M1), spotting (sample M2), "fearn leaf" symptom (sample M3) and leaf necrosis with mild distortion of younger leaves (sample M4). The last symptoms resembled the ones caused by insecticides.

1. *Symptoms on test plants*

All four types of infected samples were separately inoculated on several differential hosts, the reactions of which are shown in Table 1.

Table 1. Reactions of test plants inoculated with four types of infected samples

Inoculated plant	Symptoms*			
	Sample			
	M1	M2	M3	M4
Chenopodium amaranticolor	L, S	L	L	L, S
Chenopodium quinoa	L, S	L, S	L, S	L, S (Fig. 1b)
Datura stramonium	L	L	L	L, S
Lycopersicon esculentum	O	S	S	O
Nicotiana glutinosa	L	L	L	L
N. megalosiphon	L	L	L	L
N. sylvestris	L	L	L	L (Fig. 1a)
N. tabacum, cv. W. B.	L, S	L, S		L, S

* L local symptoms, S systemic symptoms, O symptomless

As visible in Table 1 all four isolates caused a similar type of symptoms in the majority of test plants. In a small number of test plants (*L. esculentum, C. amaranticolor)* all isolates did not provoke the same type of symptoms. However, on the basis of the symptoms on *N. sylvestris* and *C. quinoa* it could be supposed that all four isolates might contain ToMV. The samples M1 and M4 did not cause visible symptoms on inoculated tomatoes, but symptomless plants contained virus inclusions in the form of hexagonal prisms.

2. *Electron microscope analysis*

Electron microscope analysis of either purified virus preparations or infected tomato tissue revealed virus particles about 300 nm long (Fig. 1e).

3. *Serological experiments*

Four investigated isolates were serologically compared in agar diffusion tests with the common tobacco mosaic virus (TMV) using antiserum against TMV. Spur formations always occurred. However, in serological tests in which the four isolates were compared with the common strain of ToMV using antiserum against common ToMV the spurs were

never observed (Fig. 1d). In addition, the four investigated isolates showed serological reaction of identity when compared with one another using ToMV antiserum. According to the above mentioned data it can be concluded that ToMV occurred in all the four investigated samples.

4. *Virus cell inclusions*

In the tobacco tissue infected with four isolates crystalline cell inclusions were always found. The inclusions were in the form of regular and/or irregular hexagonal prisms (Fig. 1c). Also, virus crystalls were found in ultrathin sections of tomatoes (Fig. 1e).

Discussion

Yugoslav tomato breeders have often virus infections in their glasshouses. Our check up of tomatoes at Bogdanci revealed that about 60% tomato plants showed visible symptoms of virus infection. Regardless of the type of symptoms in all investigated samples tomato mosaic virus (ToMV) was found. It was not investigated whether some virus other than ToMV occurred in infected plants (see Lisa and Lovisolo 1973). Because of considerable differences in the symptoms it is probable that the symptoms described here are caused by complex infections in which ToMV was always present. It must be pointed out that about 2—3% of plants which were symptomless or with mild symptoms concerning leaves, stems and petiolus, often had fruits with more or less severe necroses. Such fruits were not tested and it remains to be seen whether the necroses are an effect of ToMV infection. However, fruit symptoms such as external and/or internal browning is a frequent symptom in tomatoes provoked by ToMV. Therefore it is possible that fruit necroses observed in tomatoes at Bogdanci were caused by ToMV. In contrast to the observations of Paludan (1973) that internal browning appeared only in cold greenhouses we noticed them in glasshouses with high temperature. In our case the virus symptoms were rather mild in the majority of tomato plants. It could be a result of high temperature (up to 30° C) or/and infections with mild ToMV strains.

Further investigations of virus diseases of tomatoes cultivated under glass should be concentrated on the identification of ToMV strains and on the usage of virus free tomato seeds (Hollings and Huttinga 1976). ToMV can be transmissible through tomato seed up to 94% (Nitzany 1960, Broadbent 1965, Hollings and Huttinga 1976, Gooding and Suggs (1976) and, therefore, infected seed can be a dangerous source of tomato infections. According to Gooding (1975), a treatment of TMV infected tomato seeds by 1% aqueous solution of trisodium orthophosphate for 15 minutes and then in 0.52% sodium hypochlorite for 30 minutes was very effective. In addition, the seeds can be disinfected by heat treatment. The cross immunization of tomatoes with weak ToMV strains which can protect plants against severe strains, can also be useful (Paludan 1973, Schmelzer and Wolf 1975, Cassels and Herrick 1977).

It is necessary to point out that ToMV is a separate virus from the tobamovirus group (tobacco mosaic virus group), and not a strain of common TMV (Harrison et al. 1971). In spite of that many researchers still denote this virus under the name of "tomato strain of TMV« (Wang and Knight 1967, Broadbent and Winsor 1964, Steepy

1968). It is true that most strains of ToMV are more closely related to the common strain of TMV than to other tobamoviruses (Hollings and Huttinga 1976). Nevertheless, ToMV can be differentiated from all other tobamoviruses on the basis of differential hosts, serological properties and amino acid composition of the virus protein (Wetter 1968, Hollings and Huttinga 1976).

Tobamoviruses from tomato most often belong to ToMV whereas the strains of TMV were seldom found in tomato (Hollings and Huttinga 1976, Broadbent 1962, Broadbent and Fletcher 1966). Similar data were noted in Yugoslavia as well. Panjan (personal communication) found in 1964 during an ivestigation into tomato viruses from glashouses that from 24 virus isolated 20 belong to ToMV and 4 to common TMV only.

Breeders in Yugoslavia ought to know that ToMV is readily spread by mechanical handling in agricultural operations. Moreover this virus is transmissible not only by seed but also by soil (Broadbent and Winsor 1964). The symptoms of ToMV in tomato can differ greatly depeding on the virus strain, tomato type, temperature, age of plant and other external factors (Hollings and Huttinga 1976).

Summary

From glasshouse tomato crops in Yugoslavia (Macedonia) which showed a virus disease of differential feature and intensity, tomato mosaic virus (ToMV) has been isolated. The identification was based on the symptoms in test plants, type of virus cell inclusions, serological investigations, and the presence of virus particles in situ and in vitro. Some practical measures are discussed to help Yugoslav farmers.

References

Broadbent, L., 1962: The epidemiology of tomato mosaic. II Smoking tobacco as source of virus. Ann. appl. Biol. *50*, 461—466.

Broadbent, L., 1965: The epidemiology of tomato mosaic. XI Seed-transmission of TMV. Ann. appl. Biol. *56*, 177—205.

Broadbent, L. and *J. T. Fletcher*, 1966: The epidemiology of tomato mosaic. XII Sources of TMV in commercial tomato crops under glass. Ann. appl. Biol. *57*, 113—120.

Broadbent, L. and *G. W. Winsor*, 1964: The epidemiology of tomato mosaic. V The effect on TMV infected plants of nutrient foliar sprays and of steaming the soil. Ann. appl. Biol. *54*, 23—30.

Cassels, A. C. and *C. C. Herrick*, 1977: The identification of mild and severe strains of tobacco mosaic virus in double inoculated tomato plants. Ann. appl. Biol. *86*, 37—46.

Gooding, G. V., Jr., 1975: Inactivation of tobacco mosaic virus on tomato seed with trisodium orthophosphate and sodium hypochlorite. Plant Dis. Reptr. *59*, 770—772.

Gooding, G. V., Jr. and *T. T. Hebert*, 1967: A simple technique for purification of tobacco mosaic virus in large quantities. Phytopathology *57*, 1285.

Gooding, G. V., Jr. and *E. G. Suggs*, 1976: Seed-borne tobacco mosaic virus in commercial sources of tomato seed. Plant Dis. Reptr. *60*, 441—442.

Harrison, B. D., J. T. Finch, M. Gibbs, R. J. Shepherd, V. Valenta and *C. Wetter*, 1971: Sixteen groups of plant viruses. Virology *45*, 356—363.

Hollings, M. and *H. Huttinga*, 1976: Tomato mosaic virus. Descriptions of plant viruses C. M. I./A. A. B. No. *156*.

Juretić, N. 1971: Serološka istraživanja virusa mozaika rajčice iz Jugoslavije. Acta Bot. Croat. *30*, 23—31 (with summary in English).

Lisa, V. and *O. Lovisolo*, 1973: Viruses of tomato in North and Central Italy. 2nd Internat. Congr. Plant Pathology, Minneapolis 1973.

Nitzany, F. E., 1960: Transmission of tobacco mosaic through tomato seed and virus inactivation by methods of seed extraction and seed treatments. Ktavim *10*, 63—67.

Paludan, N., 1973: Tobacco mosaic virus (TMV): infection, cross protection, time of infection and yield in tomato as influenced by tomato strains of TMV. Tidsskrift for Planteavl 77, 495—515.

Schmelzer, K. and *P. Wolf*, 1975: Possibilities of control of virus diseases in cucumber and tomato under glass and plastics. Nachr. Blatt Pflanzensch, der D.D.R. *29*, 21—26.

Steepy, T. L., 1968: Studies involving two tomato virus strains of tobacco mosaic virus (TMV) in tomato *Lycopersicum esculentum* L. »Revermun«. Tidsskrift for Planteavl *72*, 81—85.

Wang, A. L. and *C. A. Knight*, 1967: Analysis of protein components of tomato strains of tobacco mosaic virus. Virology *31*, 101—106.

Wetter, C., 1968: Serological similarities among viruses of the tobacco mosaic virus group. 1st Internat. Congr. Plant Pathology, London 1968.

SADRŽAJ

U Jugoslaviji se, osobito u južnim dijelovima, sve više širi uzgoj rajčice u staklenicima. Stakleničke kulture rajčice podložne su raznovrsnim oboljenjima među kojima značajno mjesto pripada bolestima koje uzrokuju virusi. Rajčice koje se gaje u našim poljima, tj. na otvorenom, napada često virus mozaika rajčice (tomato mosaic virus, ToMV) (Juretić 1971). Međutim od tog virusa nisu pošteđene u nas ni stakleničke kulture rajčice (Panjan, usmeno saopćenje). Na žalost o dolaženju ToMV na kulturama rajčice u našim staklenicima nema podataka u literaturi.

U toku 1977. godine posjetio sam Bogdance u Makedoniji s namjerom da pregledam stakleničke rajčice s obzirom na virusnu zarazu s ToMV. Tom sam prilikom ustanovio da preko 60% biljaka pokazuje vidljive simptome virusne zaraze. Iako su simptomi podosta varirali, prevladavala su četiri osnovna tipa: zeleni mozaik s deformacijama na mlađim listovima (uzorak M1), pjegavost (uzorak M2), papratolikost lista (uzorak M3), nekroze s blagim deformacijama mlađih listova (uzorak M4).

Sva četiri uzorka ispitao sam s pomoću pokusnih biljaka, seroloških pokusa, elektronsko mikroskopskih analiza te istraživanja tipa virusnih staničnih inkluzija (vidi tab. 1, sl. 1). Istraživanja su pokazala da su sva četiri uzorka sadržavala ToMV. Da li je taj virus bio jedini uzročnik opisanih simptoma, zasad se ne može određeno reći. Nisam, naime, istraživao da li zajedno s ToMV, tj. u smjesnoj infekciji, dolaze i neki drugi virusi. U staklenicima smo opazili da na plodovima oko 2 do 3% biljaka, koje su bile besimptomne ili s blagim simptomima na listovima,

dolaze simptomi u obliku većih ili manjih nekrotičnih područja. Iako nisam ispitivao da li su te nekroze posljedica infekcije s ToMV, može se pretpostaviti da je te nekroze uzrokovao upravo ToMV. Naime, jedan od čestih simptoma zaraze rajčice s ToMV jesu nekrotične zone na plodovima (Hollings i Huttinga 1976, Paludan 1973).

ToMV se prenosi sjemenom i do 94%. Prema tome inficirano sjeme može biti važan izvor zaraza. Gooding (1975) preporučuje da se sjeme rajčice prije sijanja drži 15 minuta u 1% vodenoj otopini tri-natrijeva ortofosfata, a zatim 30 minuta u 0,52% otopini natrijeva hipoklorita. Sjeme se može također prije sijanja držati na povišenoj temperaturi. Štete od ToMV danas se još suzbijaju na taj način da se mlade biljke namjerno zaraze blagim sojevima ToMV koji ne uzrokuju veće štete. Ti blaži sojevi zaštićuju biljke od zaraza agresivnijim sojevima ToMV (Schmelzer and Wolf 1975).

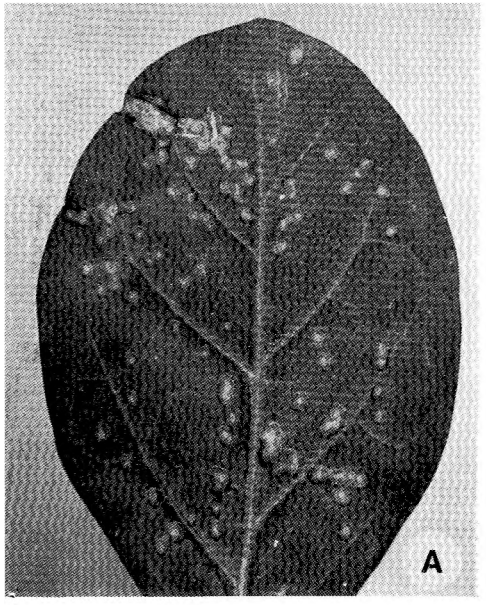
Potrebno je naglasiti da je ToMV poseban virus iz skupine tobamovirusa (skupina virusa mozaika duhana), a ne soj običnog virusa mozaika duhana (TMV). Istina je da je većina sojeva ToMV srodnija običnom TMV nego drugim tobamovirusima. Međutim ToMV se razlikuje od svih drugih tobamovirusa ne samo po simptomima na diferencijalnim pokusnim biljkama nego i po serološkim osobinama i aminokiselinskom sastavu (Wetter 1968, Hollings i Huttinga 1976).

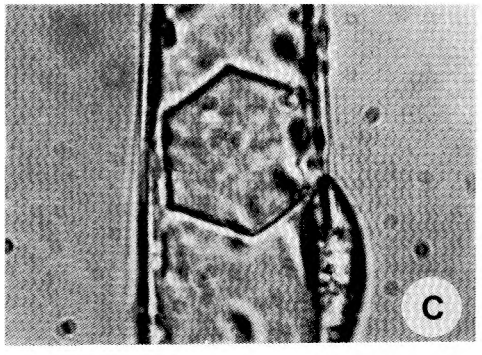
Valja uvijek imati na umu da se ToMV lako prenosi dodirom za vrijeme agrotehničkih zahvata. Osim toga ToMV prenosi se i zemljom pa zaštitna mjera protiv toga virusa je i sterilizacija zemlje koja se upotrebljava u staklenicima. Simptomi koje na stakleničkim kulturama rajčice uzrokuje ToMV mogu jako varirati ovisno o temperaturi, dužini dana, jakosti svjetla, starosti biljke, virusnom soju i sorti rajčice (vidi Hollings i Huttinga 1976, Paludan 1968).

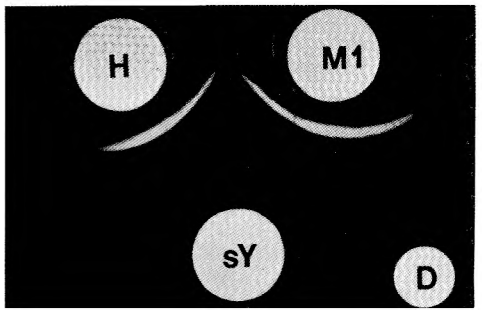
Prof. dr Nikola Juretić
Botanički zavod PMF
Maruličev trg 20/II
Yu-41000 Zagreb (Jugoslavija)

Fig. 1. *A, B* symptoms of samples M1: necrotic local lesions in *Nicotiana sylvestris (A)* and systemic symptoms in the form of yellow bands along the veins in *Chenopodium quinoa (B); C* virus hexagonal prisms in infected tobacco hair caused by isolate M3; *D* serological reaction between investigated sample M1 and ToMV from Hungary (H) with sY antiserum against ToMV isolated in Yugoslavia earlier; *E* ultrathin section of a part of cell infected with sample M1: elongated virus particles (VP), cytoplasm (CP), chloroplast (C), vacuole (V) can be seen.

Sl. 1. *A, B* simptomi uzorka M1: nekrotične lokalne lezije na vrsti *Nicotiana sylvestris (A)* i sistemični simptomi u obliku žutih vrpca uz nerve na vrsti *Chenopodium quinoa (B); C* virusne heksagonalne prizme u dlaci duhana inficiranog s uzorkom M3; *D* serološka reakcija između istraživanog uzorka M1 i ToMV iz Mađarske (H) s imunim serumom sY priređenim protiv ToMV ranije nađenim u Jugoslaviji; *E* ultratanki presjek kroz dio stanice inficirane s uzorkom M1: vide se nakupine produženih virusnih čestica (VP), citoplazma (CP), kloroplast (C) i vakuola (V).







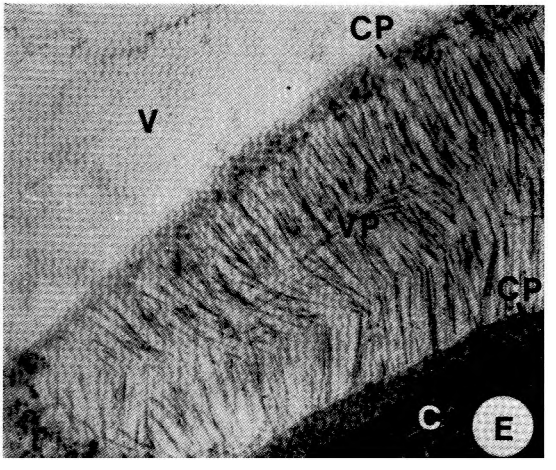


Fig. 1. — Sl, 1.