

SOME PROPERTIES OF TOMATO SPOTTED WILT VIRUS ISOLATED IN YUGOSLAVIA

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Introduction

Recently Bužančić and Juretić (1977) and Buturović et al. (1977) have found that tomato spotted wilt virus (TSWV) is widespread in various regions of Yugoslavia. The identification of the virus was performed on the basis of electron microscope analyses. The data in the above mentioned papers are in agreement with investigations of Todorovski and Mickovski (1970) and Bužančić and Pannjan (1973) who have based the virus identification mainly on test plant analysis.

Over the last couple of years TSWV has become a very common virus in tobacco in the northern part of Yugoslavia causing considerable damage. Two years ago TSWV was isolated from tobacco in a field near Podravska Slatina (northern part of Croatia). In this region TSWV occurred in tobacco either as single virus or in a mixture together with some potyviruses (Bužančić and Juretić 1977).

In this paper some properties concerning primarily the symptomatology, serology and purification of TSWV isolate S1 found by Bužančić and Juretić (1977) will be presented.

Material and Methods

The object of this paper was the isolate S1 of tomato spotted wilt virus derived from tobacco collected near Podravska Slatina. (cf. Bužančić and Juretić 1977).

The virus was purified by means of sucrose density gradient centrifugation following the method of Best (1968). Serological tests were carried out by agar gel immunodiffusion method using antiserum against TSWV kindly supplied by Dr J. P. Tsakiridis (Tobacco Institute of Greece, Drama). The antiserum had the titre 1:8.

Results

1. Test plant reactions

At the beginning of the investigations the isolate was cleaned by local lesion transmissions. To determine symptomatological properties of the isolate SI some test plants were used (Table 1).

As shown in Table 1, the isolate SI caused symptoms rather characteristic of TSWV (cf. Tsakiridis and Gooding 1972, Best 1968). On some test plants it provoked severe necrotic and ringspot types of symptoms. It seems, therefore, that the isolate SI resembles the necrotic and chlorotic isolates of TSWV described by Tsakiridis and Gooding (1972) in Greece.

Table 1. Reactions of test plants infected with isolate SI of tomato spotted wilt virus

Plant	Symptoms	
	Local	Systemic
<i>Capsicum annuum</i>	chlorotic lesions	vein clearing, ring-spotting
<i>Chenopodium amaranticolor</i>	chlorotic lesions	O*
<i>C. murale</i>	chlorotic lesions	O
<i>C. quinoa</i>	chlorotic lesions	O
<i>Cucumis sativus</i>	chlorotic lesions	O
<i>Datura stramonium</i>	O	vein clearing, ring-spotting
<i>Lycopersicon esculentum</i>	necrotic lesions	vein clearing, spotting, wilting, stunting (cf. Fig. 1d, e)
<i>Nicotiana glutinosa</i>	necrotic lesions	chlorosis, stunting, leaf deformations
<i>N. debneyi</i>	necrotic lesions	vein clearing, chlorosis, leaf deformations, stunting
<i>N. megalosiphon</i>	necrotic lesions	chlorosis, necrosis, deformations
<i>N. tabacum</i> , cv. W. B.	necrotic lesions	vein clearing, chlorosis, decline of plant (cf. Fig. 1a, b)
<i>N. tabacum</i> , cv. Samsun	necrotic lesions	vein clearing, chlorosis, necrosis, decline
<i>N. tabacum</i> , cv. H. R.	necrotic lesions	vein clearing, chlorosis, necrosis, decline
<i>N. sylvestris</i>	necrotic lesions	vein clearing, chlorosis, necrosis, decline
<i>Petunia hybrida</i>	necrotic lesions (Fig. 1c)	O
<i>Tropaeolum majus</i>	O	mild mosaic

* O symptomless

2. Infectivity in vitro

The infectivity in vitro of the isolate S1 was estimated on the basis of thermal inactivation point, longevity in vitro and dilution end point. The results are presented in Table 2.

Table 2. Results of investigations of infectivity in vitro of TSWV isolate S1

Source of infective sap	Test plant		
		<i>N. glutinosa</i>	<i>P. hybrida</i>
<i>N. tabacum</i> , cv. W. B.	Sap heated at °C		
	untreated inf. sap.	176/20*	102/20
	40	64/20	36/20
	42	18/20	21/20
	44	6/20	2/20
	46	0/20	0/20
<i>N. tabacum</i> , cv. W. B.	Sap diluted		
	1·2	161/20	153/20
	10 ⁻¹	109/20	111/20
	10 ⁻²	12/20	9/20
	10 ⁻³	0/20	2/20
	10 ⁻⁴	0/20	0/20
<i>N. tabacum</i> , cv. W. B.	Sap longevity at room temperature (hours)		
	0	66/20	72/20
	2	7/20	5/20
	3	2/20	0/20
	4	0/20	0/20

* Average number of lesions on 20 leaves

Data in Table 2 show that the isolate S1 has the thermal inactivation point between 44 and 46 °C, dilution end point between 10⁻³ and 10⁻⁴ and longevity in vitro at room temperature between 3 and 4 hr. Therefore, the isolate S1 does not differ essentially in the same properties from most TSWV isolates described in the literature (cf. Bald and Samuel 1931, Ie 1970, Kobatake et al. 1976).

3. Serological properties

In agar gel diffusion tests our isolate reacted positively with the antiserum against one TSWV isolate from Greece (Tsakiridis and Gooding 1972). Positive reactions were obtained with both crude infective sap and purified virus preparations of our isolate (Fig. 2a, b, d). Usually, besides the virus precipitin line, an additional precipitin line appeared. This nonspecific line was probably a result of a reaction of normal plant constituents. Nonspecific reaction was not observed when purified samples of our isolate were tested (Fig. 2a).

4. Purification

The purification of the isolate S1 by centrifugation in sucrose density gradient after the method of Best (1968) yielded only one virus

centrifugal component. (Fig. 2c). Using the same method Tsakiridis and Gooding (1972) also obtained one component in gradient. Our analyses of virus particles from the gradient band showed that numerous virus particles were destroyed. In spite of that, infectivity test gave positive results, i. e. the purified preparations caused local necrotic lesions in *Petunia hybrida*. In addition, the purified samples reacted specifically with TSWV antiserum (Fig. 2a).

Discussion

The investigated isolate provoked necrotic type of symptoms experimentally in *Lycopersicon esculentum* and *N. glutinosa*, and necrotic and ringspot type of symptoms in differential tobacco species. Tsakiridis and Gooding (1972) reported that three strains of TSWV occurred simultaneously in tobacco in Greece. One of them had the characteristics of mild strain, one of necrotic strain and the third one had characteristics of ringspot strain. These authors also confirmed the finding of Best (1968) who established that TSWV usually occurred in tobacco in a mixture of several TSWV strains. We did not analyse the isolate Sl in order to state whether or not it represented a mixture consisting of more strains. However, since our isolate caused two main types of symptoms, i. e. the necrotic and the ringspot types, it seems that our "strain" of TSWV consists at least of two TSWV strains. On the basis of the data presented it is difficult to say whether isolate Sl includes any strains described by Best (1968).

In spite of the low titre of the antiserum used, the precipitin lines in serological reactions were rather intensive. Tas et al. (1977) have found that the precipitin lines of TSWV can partly depend on host polysaccharides covalently associated with the virus proteins.

The presence of TSWV in Yugoslavia was first observed in tobacco in 1969 (Mickovski 1969). However, investigations of this virus began only about ten years later. It is necessary to point out that until now TSWV infections of tomato and other vegetable plants in Yugoslavia are not yet in the focus of research. The climatic conditions in the northern as well as in the southern parts of Yugoslavia facilitate a constant appearance of thrips species. Therefore, the extent and intensity of TSWV infections of tobacco in Yugoslavia are often of an epidemic character.

It is known that TSWV is transmissible by at least three species of thrips, i. e. *Thrips tabaci*, *Frankliniella fusca* and *F. occidentalis*. Paliwal (1974) found that *T. tabaci* fails to transmit TSWV in Canada. In practice our attention is usually concentrated on the species *T. tabaci*. However, Paliwal's (ib.) data show that the same attention should be focussed on all thrips species as TSWV vectors.

Summary

The identification of tomato spotted wilt virus (TSWV) isolate found in the northern part of Yugoslavia is described. On the basis of symptomatological properties it seems that the investigated isolate represents a mixture of several TSWV strains. The virus reacted positively in agar gel diffusion tests with TSWV antiserum. The purification by sucrose density gradient centrifugation performed after Best (1968) yielded

only one virus centrifugal component. Electron microscope analysis showed that during the purification procedure numerous virus particles were destroyed. The transmission by vectors is also discussed.

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SADRŽAJ

NEKE OSOBINE VIRUSA PJEGAVOSTI I VENUĆA RAJČICE IZOLIRANOG U JUGOSLAVIJI

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Nedavno su Bužančić i Juretić (1977) na osnovi elektron-skromikroskopskih istraživanja zaraženog tkiva duhana dokazali da je na duhanu u sjevernom dijelu Hrvatske raširen virus pjegavosti i venuća rajčice (tomato spotted wilt virus; TSWV). Izolat Sl opisan u tom radu istraživali smo dalje s obzirom na simptomatološka i serološka svojstva i s obzirom na njegovo vladanje in vitro. Osim toga virus smo pokušali purificirati.

Na osnovi simptoma na pokusnim biljkama zaključili smo da naš izolat najvjerojatnije predstavlja smjesu od barem dvaju sojeva TSWV. Naime, na nekim pokusnim biljkama izolat je uzrokovao osim izrazitih nekrotičnih simptoma još i simptome u obliku prstenaste pjegavosti (usp. sl. 1a, b, c; tab. 1). U literaturi je već otprije poznato da TSWV dolazi često kao smjesa od više sojeva (Tsakiridis i Gooding 1972).

Izolat Sl odlikovao se ovim svojstvima in vitro: termalna točka inaktivacije mu je bila oko 46 °C, postojanost in vitro oko 3 sata, a krajnja točka razrjeđivanja između 10^{-3} i 10^{-4} (vidi tab. 2). U pokusima izvedenim u agarskom gelu virus je pozitivno serološki reagirao s imunim serumom protiv TSWV (sl. 2a, b, d).

Virus smo purificirali koristeći se Bestovom (1968) metodom centrifugiranja u gradijentu gustoće. Dobivena je bila samo jedna vidljiva centrifugalna zona (sl. 2c). Elektronskomikroskopska analiza virusnih čestica iz te zone pokazala je da su mnogo čestice bile raspadnute. Unatoč tome inokulum uzet iz te zone bio je infektivan, što se vidjelo po karakterističnim lokalnim lezijama koje su se pojavile na inokuliranim listovima vrste *Petunia hybrida*. Širi pregled svojstava i nalaza toga virusa u Jugoslaviji može se naći u radu Bužančića (1978).

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Fig. 1. A, B, C symptoms caused by isolate Sl of TSWV. A, B natural infection of *Nicotiana tabacum*, cv. Virginia, C local lesions on inoculated leaf of *Petunia hybrida*; D, E ultrathin sections through tissue of experimentally infected tomato plants with isolate Sl: clusters of virus particles (V) placed in the cisternae (M) of the endoplasmic reticulum.

Sl. 1. A, B, C simptomi koje uzrokuje izolat Sl. A, B prirodna infekcija vrste *Nicotiana tabacum*, cv. Virginia, C lokalne lezije na inokuliranom listu vrste *Petunia hybrida*; D, E ultratanki presjeci kroz tkivo rajčice umjetno zaražene s izolatom Sl: nakupine virusnih čestica (V) smještene u cisternama (M) endoplazmatskog retikuluma.

Fig. 2. A, B, D serological reactions between isolate Sl and TSWV antiserum: Vp purified virus, Vl-f sap of naturally infected tobacco, Vl-e sap of experimentally infected tobacco, S TSWV antiserum; C centrifugal tube at the end of isolate Sl centrifugation upon sucrose density gradient, the arrow shows the virus band.

Sl. 2. A, B, D serološke reakcije između izolata Sl i imunog seruma protiv TSWV: Vp purificirani virus, Vl-f sok iz prirodno zaraženog duhana, Vl-e sok iz umjetno zaraženog duhana, S imuni serum protiv TSWV; C epruveta nakon centrifugiranja izolata Sl u gradijentu gustoće šećera, strelica pokazuje virusnu vrpcu.