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ELECTRON MICROSCOPE EVIDENCE OF TOMATO SPOTTED WILT VIRUS IN YUGOSLAVIA

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Introduction

Tomato spotted wilt virus (TSWV; R/* : */* : S/S : S/Th) represents a common plant virus in all temperate and subtropical regions. It has a wide host range in nature and attacks many ornamental and crop plants causing severe diseases, especially, in tobacco and tomato (I e 1970; L u c a s 1975). Consequently TSWV is a permanent subject of phytopathological research. On the other hand this virus arouses great interest because of its virion. The isometric virus particle of TSWV (70—90 nm in diameter) is bounded by two concentric membranes (M i l n e 1970). Particles of TSWV resemble in shape the virion of influenza virus (a myxovirus). There is also a similarity between TSWV and myxoviruses in respect of chemical composition; in addition to protein, TSWV particle contains also lipid and carbohydrate (I e 1970). However, TSWV differs from myxoviruses in many properties (M i l n e 1970). This virus is very unstable. TSWV is interesting also because of its vector specificity; it is transmissible in nature by *Thrips* spp. only (B e s t 1968). Since this vector is present in the Balkans, TSWV is widely spread in Balkan countries (I v a n c h e v a - G a b r o v s k a 1974; T s a k i r i d i s and G o o d i n g 1972). The occurrence of this virus in Yugoslavia has also been reported (M i c k o v s k i 1969; T o d o r o v s k i and M i c k o v s k i 1970; B u ž a n č i ć and P a n j a n 1973). However, these findings were founded on test plant identification only, but not on the basis of data obtained by some direct methods. This is the first report based on electron microscope evidence on the occurrence of TSWV in Yugoslavia.

Material and Methods

Spontaneously infected tobacco specimens (*Nicotiana tabacum* cv. Virginia) were collected in June 1976 in a field near Podravska Slatina. The presence of *Thrips tabaci* Lind. on tobacco plants was observed. Among tabacco seedlings about 35% of them showed symptoms fairly characteristic of TSWV. Two specimens of infected tobacco were selected for further investigations. The virus isolate found in one tobacco plant was denoted S1, and the isolate from the second specimen S2. Both virus isolates were separately transmitted by mechanical inoculation to *Petunia hybrida, N. tabacum* cv. White Burley and *Lycopersicum esculentum*. To reveal virus particles in the infected tissue spontaneously infected tobacco plant containing isolate S1 was analysed by an electron microscope. The isolate S2 was also investigated by the electron microscope. In this case artificially infected tissue of *Lycopersicum esculentum* was used.

For electron microscopy strips of tobacco and tomato leaf tissue were fixed for 30 min in 1% (v/v) glutaraldehyde in cacodylate buffer pH 7.2 and postfixed for 2 hr in 1% (w/v) osmium tetraoxide. Then, the samples of tissue were dehydrated in ethanol series and embedded in Araldite. Sections were analysed by Siemens Elmiskop I.

Results and Discussion

Electron microscope analyses of tobacco tissue spontaneously infected with S1 isolate revealed characteristic isometric particles (Fig. 1). Their diameter was about 80 nm on an average. The virus particles occurring usually in clusters consisted of three to four particles. As can be seen in Fig. 1 each cluster was situated in the membrane-bound interconnecting cisternae (c). The particles themselves were enveloped by a membrane (m). Such particles were observed only in the cytoplasm and could not be found in other parts of infected cells. This type of virus particles surrounded by enveloping membranes is characteristic of TSWV (cf. I e 1965; M i l n e 1970). In the ultrathin sections of tobacco tissue, in addition to TSWV particles the minute virus aggregates made up of elongated flexuous virus particles were also present. These particles indicate the presence of an elongated virus.

In ultramicrotome sections of tomato leaf infected with S2 isolate, more or less isometric virus particles about 80 nm in diameter were found. These particles were also present in clusters surrounded by enveloping membranes, and individual particles had a membrane as

Fig. 1. Ultrathin section through leaf tissue of spontaneously infected tobacco plant with isolate S1 of TSWV: clusters of virus particles (V) placed in the cisternae (C) of the endoplasmic reticulum; the individual particles are surrounded by a membrane (M). Above right, infected tissue with virus particles under higher magnification.

Sl. 1. Ultratanki presjek kroz lisno tkivo duhana spontano zaraženog sa S1 izolatom TSWV-a: vide se nakupine virusnih čestica (V) u cisternama (C) endoplazmatskog retikuluma; svaka pojedina čestica obavijena je membranom (M). Gore desno inficirano tkivo s virusnim česticama pod većim povećanjem.

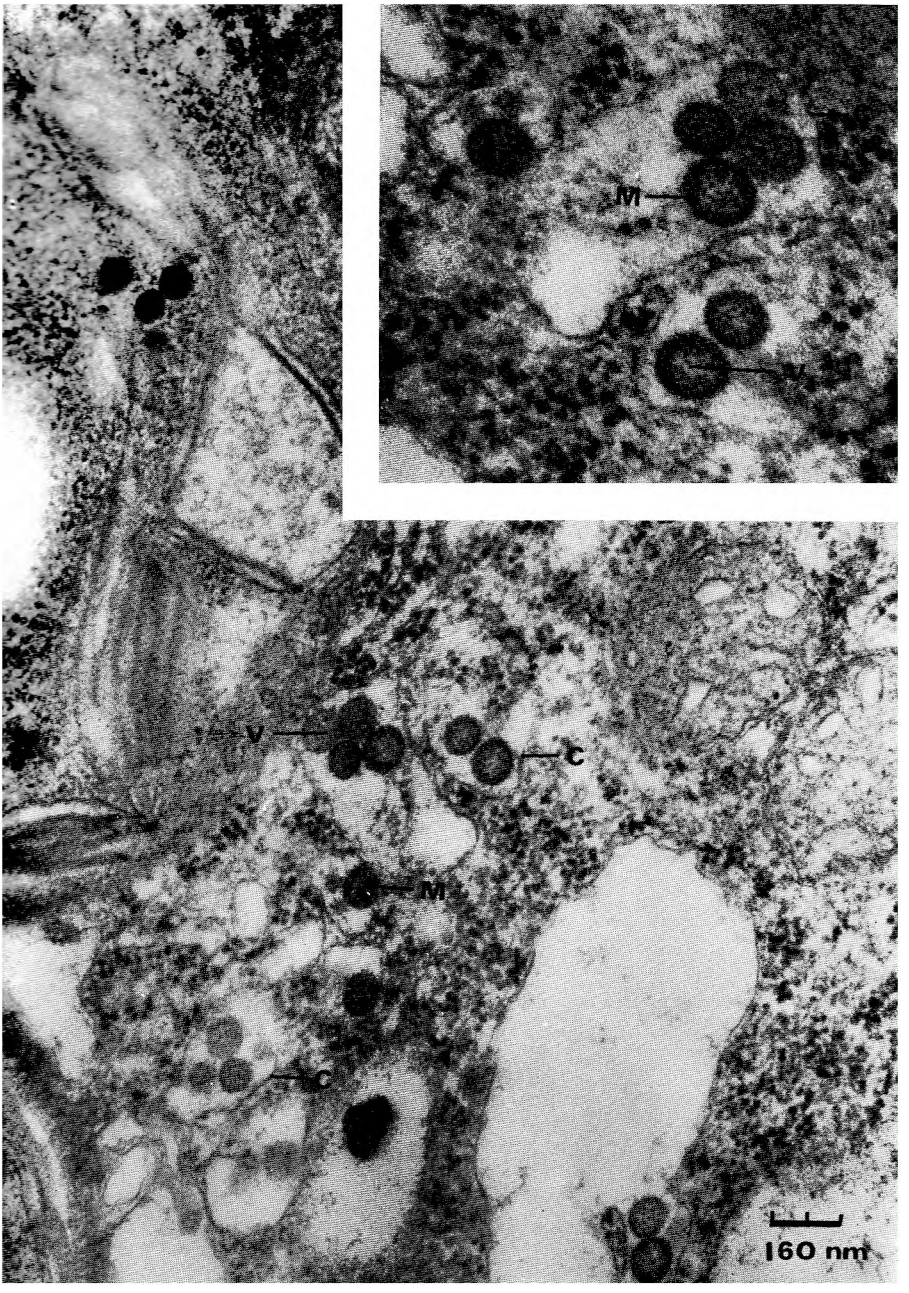

Fig. 1. — Sl. 1.

well. Isolate S2 does not seem to have been contaminated with other viruses. Therefore, this isolate will be the subject of our further studies.

The data presented here are a proof that TSWV is indeed spread in Yugoslavia. Earlier findings of this virus in Yugoslavia (Mickovski 1969; Todorovski and Mickovski 1970; Bužančić and Panjan 1973) were based on the identification performed by test plants, and therefore those findings required a confirmation obtained by some more exact methods.

Summary

In ultrathin sections of spontaneously infected tobacco plants which schowed symptoms characteristic of tomato spotted wilt virus (TSWV) the characteristic particles of about 80 nm in diameter were found (Fig. 1). The particles were enveloped with a membrane and were in clusters placed in the cisternae of the endoplasmic reticulum. The characteristic form of virus particles confirms that TSWV is indeed spread in Yugoslavia. Earlier reports on the occurrence of this virus in Yugoslavia were based mainly on symptomatological investigations only.

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SADRŽAJ

ELEKTRONSKOMIKROSKOPSKI DOKAZ VIRUSA PJEGAVOSTI I VENUĆA RAJČICE U JUGOSLAVIJI

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Na ultratankim presjecima kroz tkivo duhana koji je pokazivao simptome karakteristične za virus pjegavosti i venuća rajčice (tomato spotted wilt virus; TSWV) našli smo izometrične virusne čestice promjera oko 80 nm. Obično su po tri do četiri čestice činile nakupine koje su bile smještene u cisternama endoplazmatskog retikuluma (vidi sliku). Svaka pojedina čestica bila je obavijena membranom. Budući da je takav tip čestica karakterističan za TSWV, zaključili smo da je taj virus stvarno raširen u Jugoslaviji. O raširenosti TSWV u našoj zemlji saopćili su već ranije drugi autori (Mickovski 1969; Todorovski i Mickovski 1970; Bužančić and Panjan 1973). Međutim, njihova se identifikacija zasniva gotovo isključivo na simptomatološkim istraživanjima.

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