CYTOPATHOLOGICAL CHARACTERISTICS OF TOMATO SPOTTED WILT VIRUS ISOLATES

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ABSTRACT

The electron microscopy study revealed that four examined virus isolates in the cells of the infected host plant produced different inclusions depending on the virus isolate and the time of passaging by mechanical transmission.

Numerous virus particle inclusions as well as viroplasm and filamentous inclusions typical for TSWV were present in the plant cells infected with TSWV isolate (PPR). This isolate was kept in *N. rustica* by 4 mechanical transmissions. A similar virus isolate but maintained for 2 years by mechanical transmission in *Nicotiana* plants (T1) produced virus particle inclusions as well as amorphous inclusions typical for defective isolates. In plant cells infected with the same isolate but maintained by mechanical transmission one year longer (T2) no virus particle inclusions were produced. In the amorphous inclusions produced by this isolate virus particles were seen, but they were not surrounded by additional membrane.

The isolate G induced only amorphous inclusions dispersed within the cytoplasm of infected cells. No virus particles were seen in the amorphous inclusions.

The mechanical transmission of TSWV isolates in *N. rustica* plants reduced the number of virus particles present in the cytoplasm. The defectiveness of the isolate cause also the appearance of a new type of inclusion – the amorphous inclusions.

KEY WORDS: tomato spotted wilt virus, defective isolates, TSWV, Bunyaviridae, *Tospovirus*.

INTRODUCTION

Tomato spotted wilt virus TSWV belongs to the genus *Tospovirus*, the only one containing viruses that infect plants among the Bunyaviridae family. The members of Bunyaviridae family have spherical, enveloped particles 70-100 nm in diameter (Elliot 1990, Ie 1982).

The *Tospovirus* genome consists of three single – stranded RNA molecules. The smallest one (S) RNA codes structural (N) protein and nonstructural (NSs) protein (de Avila et al. 1993, Pang et al. 1993). The medium (M) RNA codes glycoproteins of the virion envelope and movement protein (Kormelink et al. 1992, 1994). The mutations and deletions of the (M) RNA create defective isolates lacking particle assembly. This may occur after repeated mechanical transfer throughout the same plants (Ie 1982) or different hosts (Resende et al. 1991a and b). The largest (L) RNA codes the polymerase (de Haan et al. 1991).

Concerning the cytopathology of TSWV infections, it has been demonstrated that virus particles accumulate in sacs surrounded by membrane (Ie 1971, Kitajima et al. 1992, Milne 1970, Urban et al. 1991, Zielińska and Miciński 1987). Also several other types of cytoplasmic inclusions have been identified in TSWV infected plant cells. The inclusions designated viroplasm are composed of chains of dense masses of (N) protein (Kitajima et al. 1992, Milne 1970, Verkley and Peters

1983, Zielińska and Miciński 1987). The fibrous paracrystalline arrays of various shapes are associated with (Nss) protein. They form fibrous inclusions (Ie 1971, Kitajima et al. 1992, Milne 1970, Urban et al. 1991). Amorphous inclusions have been reported in plant cells infected with another *Tospovirus* – impatiens necrotic spot virus (INSV) (Urban et al. 1991). Also Ie (1971) reported masses of amorphous material in plant cells and Ullman et al (1995) in infected trips cells.

In this paper we describe the results of comparative cytopathological studies of some TSWV isolates varying in their properties and time of maintenance in mechanically inoculated plants.

MATERIAL AND METHODS

Virus isolates

Two isolates were collected from naturally infected tomato (T) and gerbera (G) plants (Kamińska and Korbin 1991). They were transmitted and maintained in *Nicotiana rustica* plants by 3 years – isolate T 2 and for 2.5 years – isolate G. The part of isolate from tomato was kept at -18°C from April 1993 till November 1994 and then was propagated again in *N. rustica* plants. This line was designed T1 and it was passaged in *N. rustica* plants by 2 years.

These TSWV isolates were compared with one recently obtained from a *Capsicum annuum* plant in summer 1994. The

TABLE 1. TSWV isolates and their properties

TSWV isolate	Source plant	Induced symptoms	Kept in <i>N. rustica</i>	Type of inclusions present in <i>N. rustica</i> plant cells			
				Virus particle inclusions	Viroplasm	Filamentous inclusions	Amorphous inclusions
PPR	pepper	severe	2 months	+	+	+	–
T1	tomato	severe	2 years	+	+	+	+
T2	tomato	severe	3 years	–	+	+	+
G	gerbera	mild	2.5 years	–	–	–	+

new isolate, designated PPR, was kept in *N. rustica* only by 4 mechanical transmissions before cytological examination. The systemically infected leaves of *Nicotiana rustica* with TSWV isolates were collected for cytological examination in October 1994. Soon after this collection the isolate G lost its infectivity.

Electron microscopy preparation

Leaf specimens were fixed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer pH = 7.2, for 4 h. Postfixation in 1% osmium tetroxide for 2h. at 4°C was applied. The material was dehydrated in concentration gradients of ethanol (10-70%), acetone (70-100%) and embedded in Epon 812. The ultrathin sections on copper grids were stained with uranyl acetate and lead citrate. Observations were taken by JEM 100C electron microscopy in the Laboratory of Electron Microscopy, Warsaw Academy of Agriculture.

RESULTS

The electron microscopy studies revealed that the cytopathological properties of four TSWV isolates maintained in *Nicotiana rustica* plants during periods from 2 months to 3 years were differentiated depending on the virus isolate and time of maintenance.

Inclusions composed of sacs containing TSWV particles (Fig. 1) were present in mesophyll parenchyma cells of *N. rustica* plants infected with the PPR isolate. Between the sacs there were some cisterns of endoplasmatic reticulum and Golgi apparatuses. The inclusions of virus particles were usually situated near the nucleus of the cell. The viroplasm (Fig. 8) composed of dark compact substances was present in the cells of infected plants as well as filamentous inclusions (Fig. 9) composed of bundles of dark rods. There were changes in the chloroplast of infected plants and cell walls, but no other inclusions in the cytoplasm were seen.

In the mesophyll parenchyma cells infected with severe isolate T1 maintained in test plants for 2 years were present virus particle inclusions (Fig. 2) and the viroplasm (Fig. 10). Both types of inclusions were similar to those of PPR isolate. The filamentous inclusions were present in the cytoplasm as well (Fig. 11), but they were different in shape from those produced by the PPR isolate. In the infected plant cells cytoplasm appears regions filled with dark homogenous masses (Fig. 2, 3, 4). These regions were described as amorphous inclusions and they were rather small in plants infected with T1 isolate.

Isolate T2 maintained in test plants for 3 years did not form any virus particle inclusions but only some amorphous inclu-

sions (Fig. 5, 7) – the regions of cytoplasm with convoluted membranes and droplets of osmophilic substances. In the regions of those formations the virus particles could be observed but they were not surrounded by additional membrane (Fig. 7). The amorphous inclusions had many membranes surrounding different shaped spaces. In the cytoplasm of infected plants there were also regions of viroplasm and fibrous inclusions resembling those produced by T1 isolate, but much less frequent.

The isolate G was propagated in *N. rustica* for 2.5 years in the cells of infected plants. Neither virus particle inclusions nor viroplasm and fibrous inclusions were seen in the cells. There were observed only the amorphous inclusions (Fig. 6) described for long transmitted isolates T1 and T2. However they consist of shorter fragments of membranes. No virus particles were observed in the amorphous inclusions appearing in plant cells infected with this virus isolate.

All the examined isolates caused changes in the chloroplasts connected with the fibrous material in the stroma and starch accumulations. In the infected cells there were also seen the tonoplast breakages and local outgrowing of the cell wall and thickening of the plasmodesmata.

DISCUSSION

Numerous virus particle inclusions composed of sacs with virus particles as well as viroplasm and fibrous inclusions typical for several TSWV isolates (Ie 1971, Kitajima et al. 1992, Milne 1970, Urban et al. 1991, Verkley and Peters 1983, Zielińska and Miciński 1987) were present in the cells of plants infected with recently obtained severe virus isolate (PPR). A similar virus isolate (T1) but maintained 2 years by mechanical transmission in *Nicotiana* plants produced virus particle inclusions as well as amorphous inclusions typical for defective isolates. In the infected cells the same isolate but passaged in *N. rustica* one year longer (T2) became defective and no virus particle inclusions were produced.

The isolate G became defective much quicker than isolate T2 and induced only amorphous inclusions in the cytoplasm of infected plants.

Kitajima et al. (1992) describe amorphous inclusions in plants infected with defective isolates of TSWV. Those regions were marked with antibodies for (N) gene and not with G-protein sera. Ullman et al. (1995) describe amorphous inclusions in trips cells infected with TSWV. The inclusions from trips cells react with antibodies against TSWV membrane glycoproteins. Also Ie (1971) reported "dark diffused masses" in some plant species cells infected with TSWV. Amorphous inclusions have been reported in plant cells in-

Fig. 1. *N. rustica* infected with PPR isolate. Virus particle inclusion composed of sacs containing TSWV particles (V) situated near the nucleus (NU) of the cell. Magn. 17 000x, bar = 500 nm.

Fig. 2. *N. rustica* infected with T1 isolate. Inclusion composed of sacs containing TSWV particles (V) and amorphous inclusion (AI) composed of amorphous material. Magn. 40 000x, bar = 500 nm.

Fig. 3. *N. rustica* infected with T1 isolate. Amorphous inclusion in the cytoplasm composed of convoluted membranes (arrow) and amorphous material. Magn. 40 000x, bar = 500 nm.

Fig. 4. *N. rustica* infected with T1 isolate. Amorphous inclusion composed of convoluted membranes (arrow) and amorphous material (star). Magn. 45 000x, bar = 500 nm.

Fig. 5. *N. rustica* infected with T2 isolate. Amorphous inclusion composed of convoluted membranes (arrow) and amorphous material (star). Magn. 24 000x, bar = 500 nm.

Fig. 6. *N. rustica* infected with G isolate. Amorphous masses in the cytoplasm of infected parenchyma cells. Magn. 17 000x, bar = 500 nm.

Fig. 7. *N. rustica* infected with T2 isolate. Amorphous masses in the cytoplasm of infected parenchyma cells. In the region the virus particles not surrounded by additional membrane are present (arrows). Magn. 40 000x, bar = 500 nm.

Fig. 8. *N. rustica* infected with PPR isolate. Viroplasm in cytoplasm of infected cell. Magn. 39 000x, bar = 500 nm.

Fig. 9. *N. rustica* infected with PPR isolate. The fibrous inclusion (arrows) in the cytoplasm. Magn. 45 000x, bar = 500 nm.

Fig. 10. *N. rustica* infected with T1 isolate. Viroplasm in cytoplasm of infected cell. Magn. 35 000x, bar = 500 nm.

Fig. 11. *N. rustica* infected with T1 isolate. The fibrous inclusion (arrow) in the cytoplasm. Magn. 30 000x, bar = 500 nm.

fectured with INSV (impatiens necrotic spot virus) *Tospovirus* (Urban et al. 1991). The amorphous inclusions described by other authors were similar in structure to the inclusions observed by us in *N. rustica* plants, but were not associated with membranes.

The absence of virions was observed in the INSV naturally infected tissue (Law and Moyer, 1990). It can be also experimentally maintained by repeated mechanical transmission (Milne 1970, Ie 1982) and it has been associated with a reduction in the amount of G protein (Law and Moyer 1990, Resende et al. 1991a and b).

The mechanical transmission of our TSWV isolates in *N. rustica* plants reduced the number of virus particles present in the cytoplasm. The defectiveness of the isolate cause also the appearance of a new type of inclusion – the amorphous inclusions. There were intermediate stages of that process – the isolate T1 produced virus particle inclusions and amorphous inclusions.

There were numerous TSWV particles in amorphous inclusions produced by T2 isolate. They were not surrounded by additional membrane as it always happened in normal isolates producing virions. In the infection of plants with nondefective isolates TSWV particles accumulate in sacs surrounded by membrane (Ie 1971, Kitajima et al. 1992, Milne 1970, Urban et al. 1991, Zielińska and Miciński 1987). No virions without this additional membrane were seen in plants infected with nondefective isolates. This is connected with the possible maturation pathways of the virus particles. Tospoviruses use a plant cell membrane enriched by glycoproteins coded by their genome for producing their virions coat. The membranes may be derived from endoplasmatic reticulum or Golgi apparatus as were suggested by Kitajima et al. (1992). The virions mature by budding into the cistern of endoplasmatic reticulum. The outer membrane is a membrane of endoplasmatic reticulum. Rudzińska-Langwald and Kamińska (1996) show a process of virion maturation by its formation from cisterns of dictyosome. In this case the outer membrane was also a part of the cistern of dictyosome. The lack of additional membrane suggests that the particles seen in amorphous inclusions in *N. rustica* infected with T2 isolate of TSWV are not a product of normal assembly of the particle.

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ZMIANY CYTOLOGICZNE WYWOŁYWANE
PRZEZ IZOLATY WIRUSA BRĄZOWEJ PLAMISTOŚCI POMIDORA

STRESZCZENIE

Podjęte badania cytologiczne wykazały, że cztery badane izolaty TSWV różniły się rodzajem powstających w komórkach *Nicotiana rustica* inkluzji.

W komórkach roślin porażonych izolatem (PPR), który był krótko pasażowany w roślinach *N. rustica* występowały typowe dla TSWV, liczne inkluzje cząstek wirusa, wiropłazma i inkluzje włókniste. Podobny izolat (T1), lecz pasażowany za pomocą mechanicznych inokulacji w roślinach *N. rustica* przez dwa lata wytwarzał inkluzje cząstek wirusa na równi z inkluzjami amorficznymi. W komórkach roślin zainfekowanych tym samym izolatem, ale pasażowanym rok dłużej nie występowały inkluzje cząstek wirusa, występowały natomiast inkluzje amorficzne, na terenie których obserwowano pojedyncze cząstki TSWV. Izolat G TSWV wywoływał w cytoplazmie komórek porażonych jedynie inkluzje amorficzne. Nie zaobserwowano cząstek wirusa na terenie tych inkluzji. Tak więc pasażowanie TSWV w roślinach z rodzaju *Nicotiana* powoduje powstawanie inkluzji amorficznych przy jednoczesnym zahamowaniu tworzenia się inkluzji cząstek wirusa i stopniowym zaniku powstawania gotowych cząstek.

SŁOWA KLUCZOWE: wirus brązowej plamistości pomidora, izolat ułomny, TSWV, Bunyaviridae, *Tospovirus*.