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# The evidence of *Tobacco rattle virus* impact on host plant organelles ultrastructure

Katarzyna Otulak[a,*], Marcin Chouda[a], Józef Bujarski[b,c], Grażyna Garbaczewska[a]

[a] *Department of Botany, Faculty of Agriculture and Biology, Warsaw University of Life Sciences, WULS-SGGW, Nowoursynowska Str. 159, 02-776 Warsaw, Poland*
[b] *Plant Molecular Biology Center and the Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, USA*
[c] *Institute of Bioorganic Chemistry, Polish Academy of Sciences, 61-704 Poznan, Poland*

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ABSTRACT

Tobraviruses, like other (+) stranded RNA viruses of plants, replicate their genome in cytoplasm and use such usual membranous structures like endoplasmic reticulum. Based on the ultrastructural examination of *Tobacco rattle virus* (TRV)-infected potato and tobacco leaf tissues, in this work we provide evidence of the participation of not only the membranous and vesicular ER structures but also other cell organelles during the viral infection cycle. Non-capsidated TRV PSG particles (potato isolate from the Netherlands) (long and short forms) were observed inside the nucleus while the presence of TRV capsid protein (CP) was detected in the nucleus caryolymph and within the nucleolus area. Both capsidated and non-capsidated viral particles were localized inside the strongly disorganized chloroplasts and mitochondria. The electron-dense TRV particles were connected with vesicular structures of mitochondria as well as with chloroplasts in both potato and tobacco tissues. At 15–30 days after infection, vesicles filled with TRV short particles were visible in mitochondria revealing the expanded cristae structures. Immunodetection analysis revealed the TRV PSG CP epitope inside chloroplast with disorganized thylakoids structure as well as in mitochondria of different tobacco and potato tissues. The ultrastructural analysis demonstrated high dynamics of the main cell organelles during the TRV PSG–*Solanaceous* plants interactions. Moreover, our results suggest a relationship between organelle changes and different stages of virus infection cycle and/or particle formation.



## 1. Introduction

Tobacco rattle virus (TRV) belongs to the Tobravirus genus. The tobraviruses are positive-strand RNA viruses with rod-shaped virus particles that are transmitted between plants by trichodorid nematodes. The TRV genome is divided into two single stranded (ss) RNAs of positive (+) polarity. The genetic material undergoes separate encapsidation into simple, rod-shaped, helical capsids, with identical diameters of 22.5 nm. The particles of TRV vary in terms of length: longer particles (L) range from 180 to 197 nm and shorter particles (S) from 55 to 114 nm. Both types of particles contain about 5% RNA and 95% of protein. The two types of virions play different functions during viral infection and multiplication. The longer particle, containing RNA1, induces infection, whereas the shorter one, containing RNA2, is responsible for the synthesis of coat protein (CP) (Bergh et al., 1985) as well as two nonstructural proteins that are involved in the nematode-vector spread (Hernandez et al., 1997). When the plant is infected with L particles, only RNA1 multiplies (Frost et al., 1967). The complete capsidated forms (virions) are present in TRV-infected plants (Harrison and Robinson, 1978; MacFarlane, 1999, 2010). Tobraviruses display a wide host range, infecting more than 600 plant species. In addition to being plant pathogens, these viruses can be used as vectors for expression in plants of non-viral proteins and also as initiators of virus-induced gene silencing (VIGS).

As pathogens, plant viruses use and subvert host cell functions to support their replication assembly and various forms were identified during translocation among cells, within the tissue and also during plant-to-plant-host or plant-vector spread. Eucaryotic (+) ssRNA viruses replicate their genomes on intracellular membranes dubbed replication "factories" that are composed of viral replication protein and RNA templates, usually in association

* Corresponding author. Tel.: +48 22 5932 657.
*E-mail address:* katarzyna_otulak@sggw.pl (K. Otulak).

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with the vesicles or other host membrane rearrangements (Kopek et al., 2007; Diaz et al., 2010). In view of the fact that cellular organelles such as lysosomes, chloroplasts, peroxisomes, endosomes were identified as the replication sites for tymoviruses and tombusviruses, this might also be the case for tobraviruses (McCartney et al., 2005; Prodhomme et al., 2001).

The ultrastructural research exploring cytopathological changes in plant cells and tissues caused by TRV is very limited. Our previous ultrastructural studies have demonstrated the presence of both the capsidated and non-capsidated forms of TRV virions in either *Nicotiana tabacum* cv. Samsun or in *Solanum tuberosum* cv. Glada hosts that were mechanically infected with PSG strain of TRV (Cornelissen et al., 1986). Both forms could be transported between cells through plasmodesmata as well as systemically via tracheary elements and the phloem (Garbaczewska et al., 2012). The following investigations have documented additional ultrastructural effects of infection caused by TRV transmitted by *Trichodorus primitivus* in potato and tobacco plants (Otulak et al., 2012).

The aim of the present paper concerns the ultrastructural evidence of whether and how the *Solanaceous* cellular organelles like nucleus, mitochondria, chloroplasts and ER can participate and facilitate TRV infection cycle in both tobacco and potato plants.

## 2. Materials and methods

### 2.1. Plant material and TRV passages

*S. tuberosum* cv. *Glada* and *N. tabacum* cv. *Samsun* plants were maintained in growth room at 18 °C, with 16 h light of intensity 400 μmol $m^{-2}$ $s^{-1}$ PAR (photosynthetically active radiation). Seedlings of tobacco and potato cv. *Glada* were susceptible to TRV PSG infection at the level 2 in a 9-stage resistance scale. Fourleaf seedlings were infected with PSG strain of *TRV* (TRV PSG), obtained from Plant Breeding and Acclimatization Institute, Młochów Research Center. This TRV isolate was previously passaged three times a year for 3 years, 10 plants per passage via mechanical inoculation by using carborundum. Leaves were inoculated with TRV leaf sap (one drop, ca. 50 μl), obtained by grinding the tobacco cv. *Samsun* leaves 15 days post infection in a 0.1 M phosphate buffer (pH 7.4). Control plants were mock inoculated with the phosphate buffer. The tobacco plants were tested by using a DAS-ELISA. In addition, the leaf material was harvested 3, 7, 10 and 15, 30, 35 days after TRV PSG infection for the compatibility interaction tests. Tissue samples were collected from five plants, from either directly infected or non-infected seedlings, as well as separately from the necrotic and surrounding tissues.

### 2.2. Ultrastructural analysis with the transmission electron microscopy

Leaf fragments were fixed in 2% (w/v) paraformaldehyde and 2% (v/v) glutaraldehyde in 0.05 M the cacodylate buffer (pH 7.2–7.4) (Karnovsky, 1965) for 2 h at room temperature. Then, the samples were contrasted and fixed in 2% (w/v) $OsO_4$ in cacodylate buffer for 2 h at 4 °C. The material was rinsed with sodium cacodylate and then dehydrated by transferring through a series of ethanol soakings (10–100%). The specimens were gradually saturated with resin Epon 812 (Fluka) and polymerized for 24 h at 60 °C. Ultrathin sections were stained with 1.2% ethanolic uranyl acetate and 2.5% lead citrate. Observations were conducted under a Morgagni 268D transmission electron microscope (FEI, The Netherlands). Photographic documentation was done with the use of a "Morada" (SIS) digital camera and the iTEM (SIS) computer program.

### 2.3. immunolocalization of the TRV PSG CP

The immunolocalization of TRV CPs in the TRV PSG-infected potato or tobacco tissues was performed according to van Lent and Verduin (1986). For immunolocalization, we used primary rabbit polyclonal antibodies directed to CLKSYYRRNFEKNF-amino acids sequence of CP, that was specific to TRV PSG strain (New England Peptide, USA). The specificity of interaction between antibodies and the virus suspension was tested with DAS-ELISA test by measuring the extinction a wavelength of 405 nm. The following results were obtained: >1700 after 30 min and >3450 after 60 min, demonstrating a strong reaction between antibodies and the virus. A control spectrophotometric measurement of the sap from healthy plants (both tobacco and potato) gave the following reads: 0.075 after 30 min, and 0.080 after 60 min.

The formwar-coated nickel grids with the ultra-thin tissue sections were placed in drops of 10% solution of hydrogen peroxide for 10 min in order to remove the epoxide resin (an alternative treatment was in 15% water solution of sodium periodate for 30 min). Next, the samples were rinsed three times with re-distilled water. The sections were incubated for 1 h in a blocking buffer, NGS (ICN) containing 3% BSA in 0.1 M PBS, pH 7.6. Then the grids were rinsed three times in PBS with 0.05% Tween 20 for 10 min and incubated for 1 h with a primary rabbit antibody anti CP-TRV 1:50 in PBS with 2% BSA. Then, the sections were washed in PBS with 0.05% Tween 20 followed by incubation with the secondary anti-rabbit IgG antibody conjugated with colloidal gold (15 nm, Sigma–Aldrich) diluted 1:50 in PBS buffer for 1 h. As before, the sections were rinsed first in buffer, and then in distilled water twice for 5 min. The specificity of labeling was determined by incubation of the above treated sections from healthy plant tissues and replacing the antibody with the rabbit pre-immune serum. The material was contrasted by counterstaining with 2% uranyl acetate solution for 7 min and rinsed with distilled water. The sections were examined under the Morgagni 268D TEM and photographed with the "Morada" camera.

## 3. Results

### 3.1. Ultrastructural analysis before the TRV infection symptoms occurrence

Fifteen days after TRV PSG inoculation the susceptible host plants: tobacco cv. Samsun and potato cv. Glada revealed systemic necrosis on the leaflets and petioles (Fig. 1A). Browning and external stem necroses were observed 30 days post inoculation on both tobacco and potato plants (Fig. 1B). Our ultrastructural analyses of this compatible interaction focused on the contribution of organelles during TRV PSG infection cycle starting from 3 days after virus inoculation, when the infection symptoms did not yet occur. Moreover, no ultrastructural alterations between two susceptible hosts: potato cv. Glada and tobacco cv. Samsun were observed. Three days after infection we did not notice plant cell organelles abnormalities in either potato or tobacco tissues, despite the fact that single virus particle or small inclusion of TRV PSG particles was visible in the mesophyll cell (Fig. 1C and D). No virus particles were observed in vascular tissues. Starting from 7 days after infection short and long TRV particles were observed in vacuole as well as in the cytoplasm in phloem cells (especially phloem parenchyma, but not in xylem, Fig. 1E–G). Moreover, 7 days after infection high activity of endoplasmic reticulum was noted in mesophyll and phloem cells (Figs. 1F and 2A and B) often in connection with virus particles and vesicular structures. ER cisterns were very often characterized by a swollen structure and were filled with fibril material (Fig. 1F). The observation demonstrated distinct virus particles connection to such cisterns