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# ULTRASTRUCTURAL PECULIARITIES OF TURNIP MOSAIC VIRUS (MASSIVE) INCLUSIONS IN TWO HOST SPECIES

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Massive inclusion bodies, appearing as accumulations of needles in living cells of *Brassica rapa* var. *rapa* and as granular x-bodies in those of *Petunia hybrida*, induced by isolate showing properties of the typical strain of turnip mosaic virus (TuMV) were studied in ultrathin sections. The massive inclusions from *B. rapa* consisted mainly of cylindrical inclusions and virus particles; voluminous cylindrical inclusions conformed to those of *Edwardson's* subdivision-III induced by most TuMV strains in cruciferous hosts though short curved laminated aggregates specific of subdivision-IV cylindrical inclusions were also present. The x-bodies from *P. hybrida*, in addition to relatively delicate cylindrical inclusions of the both subdivisions and different very active cell organells, contained abundant crystal-containing peroxisomes from which their light microscopic granular structure primarily derived. Observed were also some other cytophatic peculiarities. The host-specific smaller dimensions of cylindrical inclusions in *P. hybrida* in comparison to those present in *B. rapa* are discussed.

## Introduction

By light microscopy in living cells infected with turnip mosaic potyvirus (TuMV) massive inclusions appearing as granular x-bodies and bodies which look like accumulations of needle crystals (»Kristallnadeln«), including transitional forms, could be detected (Štefanac 1964, Štefanac and Miličić 1965). As known, the »needle crystals« constitute the complex cytoplasmic cylindrical inclusions which are often of specific submicroscopic configuration with particular potyvirus (*cf.* Edwardson *et al.* 1984, Francki *et al.* 1987). The granular x-bodies, found regularly in TuMV host plants in the early stages of infection, in some species (representatives of the *Solanaceae, Brassica oleracea capitata, Gomphrena globosa* and some others) are the only type of inclusions formed which could be visualised in living cells without any kind of pretreatment; however, in most studied members of the family *Brassicaceae* they gradually transform into accumulations of »needles« (= cylindrical inclusions) arranged within the former amorphous body more or less parallel to each other or being irregularly scattered (Štefanac 1964).

Here we present evidence on the submicroscopic structure of the »needles type« inclusions in cruciferous species *Brassica rapa* L. var. *rapa* and of granular x-bodies in the solanaceous plant *Petunia hybrida* Vilm., both infected with the same strain of TuMV. We show also that the size of TuMV cylindrical inclusions does not depend on the virus only but also on the host plant species.

## Materials and Methods

Samples of turnip (*Brassica rapa* var. *rapa*) and petunia plants were infected by manual inoculation with isolate from *B. oleracea* var. *capitata* (Štefanac and Miličić 1965) showing properties of the typical strain of TuMV. For electron microscopy, sections of the leaves rich in well formed aggregates of »protein needles« (Fig. 2) or granular x-bodies (Fig. 4) were used. Sections were made by razorblade, parallel to the leaf surface, and in addition to epidermal included a layer of mesophyll cells. Fixation, ultrathin sectioning and staining were done by our standard procedure (Štefanac and Wrischer 1983).

## Results and Discussion

In ultrathin sections of infected *B. rapa,* in the cytoplasm, most frequently in aggregates, cylindrical inclusions were noticed of the morphology and robust appearance earlier found with the representatives of the genus *Brassica* infected with TuMV (Hayashi et al. 1965, Edwardson and Purcifull 1970, Edwardson 1974, Christie and Edwardson 1977, Štefanac 1978, *etc.*). As shown in Figs 1 and 3, often voluminous laminated aggregates and scrolls (in longitudinal sections tubes) were evident. The presence of both long, but not always particularly straight, and short curved laminated aggregates showed the greatest similarity with TuMV subdivision-III and -IV cylindrical inclusions micrographed by Christie and Edwardson (1977; *cf.*

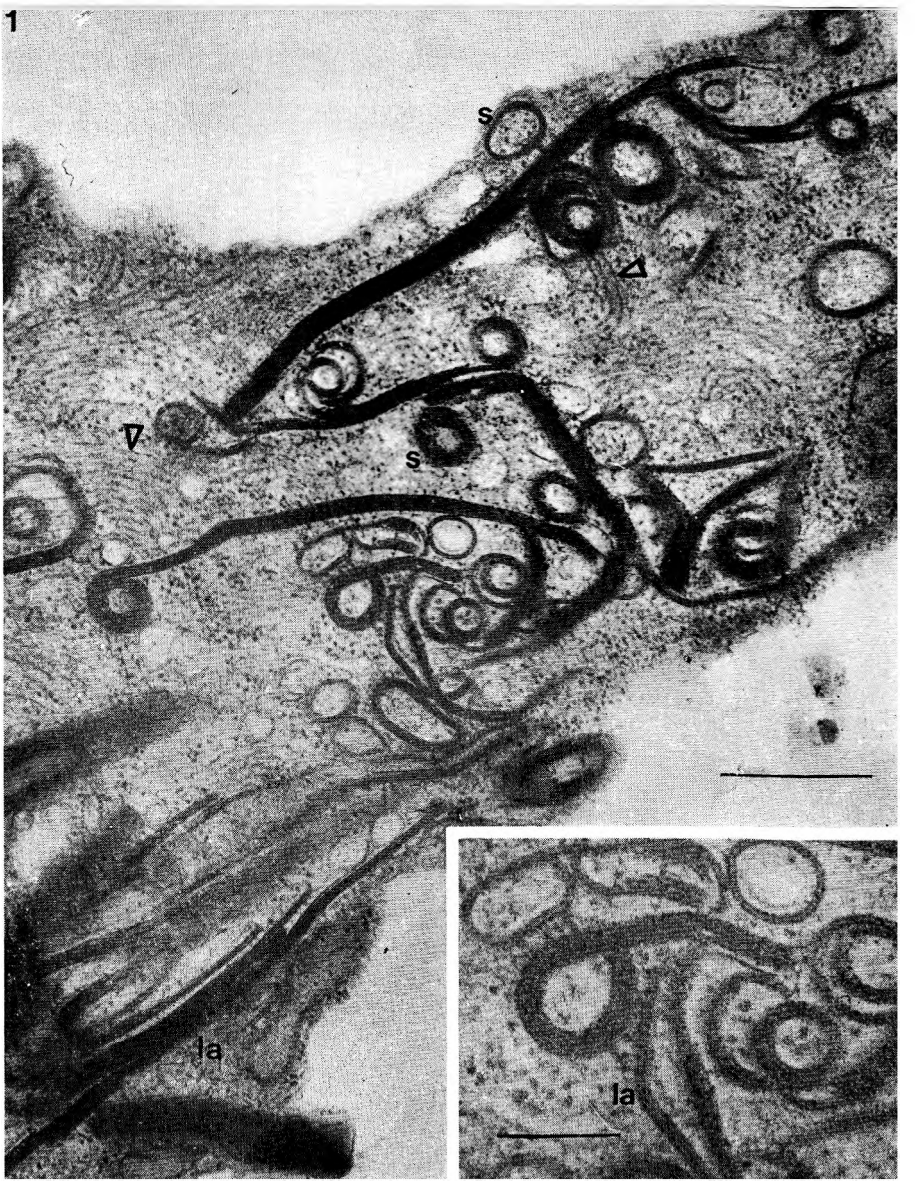



Fig. 1. Electron micrograph of an accumulation of cytoplasmic cylindrical inclusions with attached virus particles (arrowheads) in *Brassica rapa* var. *rapa* infected leaf cell. Inclusions appear as scrolls (s), and both long and short curved laminated aggregates (la). Insert shows higher magnification of the inclusions with discernible layers. Bars = 500 nm and 200 nm (insert).


Fig. 2. Massive inclusions in alive *Brassica rapa* var. *rapa* epidermal leaf cells. Bar = 10 μm.

Fig. 3. Electron micrograph of tube (t) and of laminated aggregate (la) cylindrical inclusions in turnip, in one place with attached virus particles (arrowhead). Bar = 500 nm.

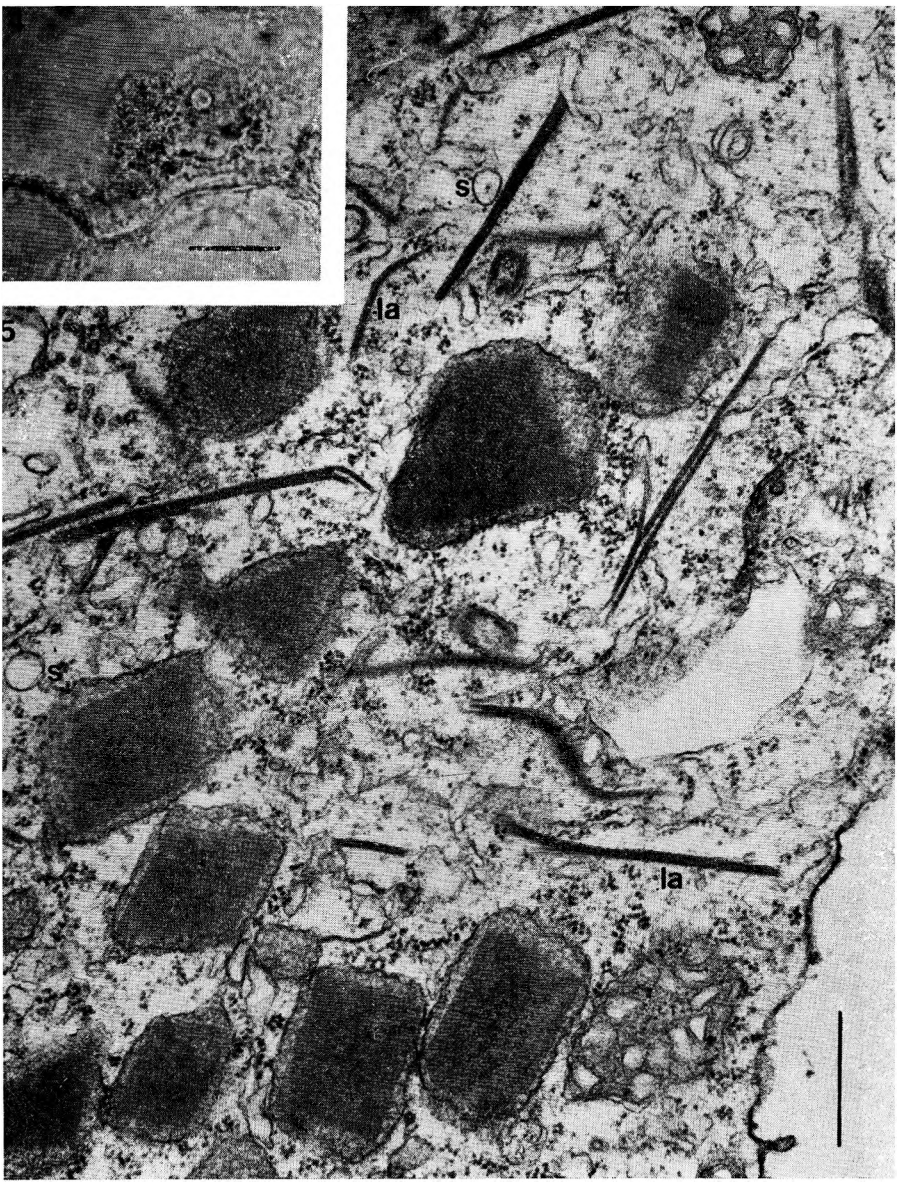
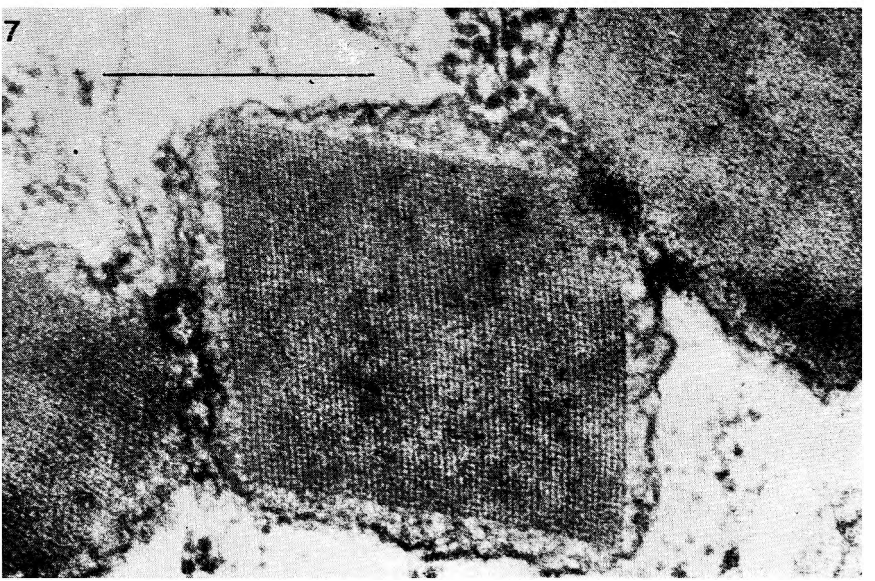



Fig. 4. Massive inclusion (granular x-body) near the nucleus (right) of an alive *Petunia hybrida* epidermal leaf cell. Bar = 10 μm.

Fig. 5. Electron micrograph of a part of the massive inclusion in section of infected petunia leaf cell, showing agglomeration of crystal-containing peroxisomes, the aspect of scroll (s) and laminated aggregate (la) cylindrical inclusions, mitochondria and strong activity of endomembranes. Bar = 500 nm.


Figs 6, 7. Laminated aggregate (la) with gaps in construction close to active dictyosomes (Fig. 6), and joined crystal-containing peroxisomes (Fig. 7), all inside infected *Petunia hybrida* cells. Scroll (s) and tube (t) cytoplasmic inclusions. Bars = 500 nm (Fig. 6) and 300 nm (Fig. 7).

Edwardson *et al.* 1984). Pinwheels could not be clearly distinguished. The width of approximately 0.25 μm and length of more than 4 μm of the tube structures (*cf.* Fig. 3) including portions of laminated aggregates agreed with dimensions of needle-like crystals visualised in agglomerations by light microscopy. In close proximity of, or attached to, the cylindrical inclusions there were often numerous virus particles (Figs 1,3), earlier also detected nearby cylindrical inclusions of different potyviruses including TuMV isolates (Hayashi *et al.* 1965, Francki *et al.* 1987. *etc.*). In some places between cylindrical inclusions mitochondria of normal appearance were present.

Referring to infected *P. hybrida* cells, in distinct cytoplasmic aggregates, which corresponded to the light microscopic granular x-bodies, cylindrical inclusions, numerous peroxisomes, some mitochondria and commonly very active dictyosomes and endoplasmic reticulum were present (Figs 5,6,7). In petunia the virus also induced *Edwardson's* subdivision--III and -IV laminated aggregates. But, laminated aggregates and especially scrolls (tubes) were of an appreciably smaller size than in *B. rapa* and agreed in dimensions and appearance with those of R isolate of TuMV micrographed by Hayashi *et al.* (1965) in the same species. In certain places some layers of laminated aggregates were interrupted (Fig. 6), perhaps as a result of close contact of the laminated aggregates during their formation with dictyosomal vesicles. Similar gaps in the construction of cylindrical inclusions which contributed to their incoherency were sometimes present also in *B. rapa*. Similarly to *B. rapa,* typical pinwheels were not found. Their absence was probably a consequence of relatively old infection (Francki *et al.* 1987). Numerous peroxisomes included large protein crystals with dimensions of about 0.6 μm (Figs 5,7) and sometimes more. Some peroxisomes also showed the pathological phenomenon of being closely connected to one another (Figs. 5,7).

From our ultrastructural analysis of TuMV inclusions in the two hosts it also follows that the granular appearance of the massive light microscopic inclusions in *P. hybrida* cells derived primarily from abundant crystal-containing peroxisomes, which have been earlier observed to increase in solanaceous plants in response to certain potyvirus infections (Edwardson 1974, Christie and Edwardson 1977). Cylindrical inclusions inside TuMV infected *P. hybrida* cells could not be noticed as needles not only because of their rather small size (particularly in the case of scrolls, i.e. the delicacy of tubes) and the presence of crystal-containing peroxisomes, but also because of the abundance of other cell organelles in their close proximity, i.e. in the area of x-bodies. It is not excluded that the cylindrical inclusions in *P. hybrida* were of reduced dimensions owing to the expense of constructive materials in formation of big peroxisomal crystals, but their size was certainly dependent on the specific physiology of the host-cells. It can be presumed that in solanaceous plants the fine structure of TuMV x-bodies including reduced dimensions of cylindrical inclusions is generally similar to the one established, in the present study, in infected petunia. Congruent with this conclusion are also the data on the increased number of crystal-containing peroxisomes in several well--known solanaceous test-plants following infections with certain other potyviruses (Francki et al. 1987).

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## SAŽETAK

### ULTRASTRUKTURNE OSOBITOSTI (MASIVNIH) UKLOPINA VIRUSA MOZAIKA POSTRNE REPE U DVIJE DOMAĆINSKE VRSTE

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U ultratankim prerezima studirali smo finu građu masivnih uklopina prouzročenih zarazom virusom mozaika postrne repe (TuMV). Te su uklopine u živim stanicama vrste *Brassica rapa* var. *rapa* podsjećale na nakupine iglica, a u vrste *Petunia hybrida* zaražene istim sojem virusa imale su izgled zrnatih x-tijela. Masivne uklopine u stanicama biljke *B. rapa* sastojale su se većinom od cilindričnih uklopina i virusnih čestica; velike cilindrične inkluzije nalikovale su onima iz *Edwardsonove* III podskupine koje u kruciferskim domaćinima inducira većina sojeva TuMV, premda su bili prisutni i kratki zakrivljeni lamelarni agregati svojstveni za IV podskupinu cilindričnih uklopina. X-tijela u vrste *P. hybrida* sadržavala su, pored cilindričnih uklopina iz obiju podskupina razmjerno fine građe te različitih vrlo aktivnih staničnih organela, također i brojne peroksisome s kristalima od kojih je prvenstveno potjecala njihova svjetlosnomikroskopska zrnata struktura. Zapažene su i neke druge citopatološke osobitosti te raspravljene za domaćina specifične manje dimenzije cilindričnih uklopina u vrste *P. hybrida* u usporedbi s onima u vrste *B. rapa.*

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