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ULTRASTRUCTURAL CHANGES IN LEAF CELLS INFECTED WITH *ARABIS MOSAIC NEPOVIRUS* I. IN BEAN PLANTS (*PHASEOLUS VULGARIS* L.)

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Abstract. Two experimental methods were used in the study. The aim of the first one was focused on a detection of *Arabis mosaic nepovirus* (ArMV) particles and tubules with viruses in extracts obtained from crushed leaves of bean. Second one consisted on investigation of ultrastructural changes occuring in the bean leaf tissues with symptoms caused by ArMV. Characteristic membranous inclusions in the cytoplasm were observed and described. ArMV occured either as irregularly scattered particles in the cytoplasm, crystal-like aggregates or semiconcentric and concentric layers.
Not numerous but sometimes very long tubules with viruses were mainly observed near the cell wall. Protrusion of the cell wall into the protoplast very often containing viruses in the plasmodesmata were observed many times. Plasmalemmasomes were frequently situated near the cell wall.

Key words: Arabis mosaic nepovirus, cytopathology

I. INTRODUCTION

This work is the result of the further studies on an identification of *a* virus that occurs in a natural infection on *Laburnum vulgare* (Pospieszny et al. 1999). *Arabis mosaic virus* (ArMV), representative of the nepoviruses group, was identified. Typical feature of this group is ability to infect all plant tissues and merystems, and appearance of virus particles in tubules, and generating specific cytoplasmic vesiculated inclusion (Francki et al. 1985). Gerola *et al.* (1964; 1965; 1966) described ultrastructural changes in leaf cells of *Chenopodium amaranticolor* and *Petunia hybrida* showing systemic symptoms of ArMV infection. They didn't notice viruses located in tubules. However, clear presence of a few tubules with viruses in an extract of studied leaves with ArMV was observed in our previous studies.

Besides the cognitive values this study will become a documentation of ultrastructure of leaf cells of plants infected with ArMV due to requirements for proper identification of virus (Francki 1981; Hamilton et al. 1981).

II. MATERIALS AND METHODS

Phaseolus vulgaris L. plants mechanically infected with ArMV in a greenhouse were the object of the research. Studies on the identification of virus particles were performed on

specimens prepared using negative staining of an extract of crushed fresh leaves and young roots. Lower leaves of beans were three times inoculated mechanically with ArMV virus to achieve good material for this study. 10 days after inoculation specimens were prepared from all leaves. The procedure started with inoculated lower leaves and ended up with top ones that already showed first systemic symptoms. Parts of young roots were also included in this experiment. Phosphotungstic acid (PTA) with pH 6.8 and 7.2 and ammonium molybdate were used as negative stain (Brenner and Horne 1959). Specimens for ultrastructural examinations were taken from *Phaseolus vulgaris* control leaves and from leaves with systemic symptoms, 14 days after infection. The samples were fixed in 0.025 M phosphate-buffered 3% glutaraldehyde pH 7.0 for 2 h and then postfixed in 0.025 M phosphate-buffered 2% osmium tetroxide for 2 h. After the dehydration in ethanol and acetone gradients the material was embedded in epoxy resin Epon 812. The ultrathin sections were stained with uranyl acetate and lead citrate. All preparations were observed in a Philips EM 201 electron microscope.

III. RESULTS

Examinations of the extract plants 10 days after inoculation showed the presence of individually scattered polyhedral virus particles (about 30 nm in diameter). It was detected in both leaves and roots samples. The most abundant aggregates of virus particles were present in the youngest leaves. The presence of very few, singular tubules containing virus particles was determined in the inoculated leaves and the youngest top leaves as well. The tubules contained from several to a dozen or so virus particles in a single strand.

Ultrastructure studies of leaf cells of *Phaseoulus vulgaris* L. revealed the presence of virus particles in both palisade parenchyma cells and spongy parenchyma cells, also in sieve elements, companion cells and parenchyma phloem cells. The virus particles were not found in xylem cells of vascular bundles. The highest concentration of the virus particles was found in the very young differentiating phloem cells. The most changed area in examined cells is cytoplasm. There are placed specific inclusions without membrane but surrounded by endoplasmic reticulum (Fig. 1). Inclusions are the most often located near cell nucleus. At times they take up a large area of a cell therefore they often invaginate deeply inside central vacuole in leaf mesophyll. Those inclusions are a complex of slightly stained microfibrous and membranous structures. Some membranous structures in form of vesicles contain fibrils similar to RNA fibrils infected with the comovirus. Membranous structures resemble a distended and washed away endoplasmic reticulum. Most inclusions contain a smaller or larger area of amorphous electron dense material, dark contrasted of homogenous consistency or very fine-grained (Fig. 2). The consistency might be compared with viroplasm due to numerous, irregularly scattered ArMV virus particles that are placed within its region or nearby. Larger concentrations of loosely located viruses occasionally might be found at the periphery of the inclusions. Both nucleoprotein viruses particles and empty capsids were observed within inclusions and among concentric layers of aggregated virus particles (Figs. 3, 7).

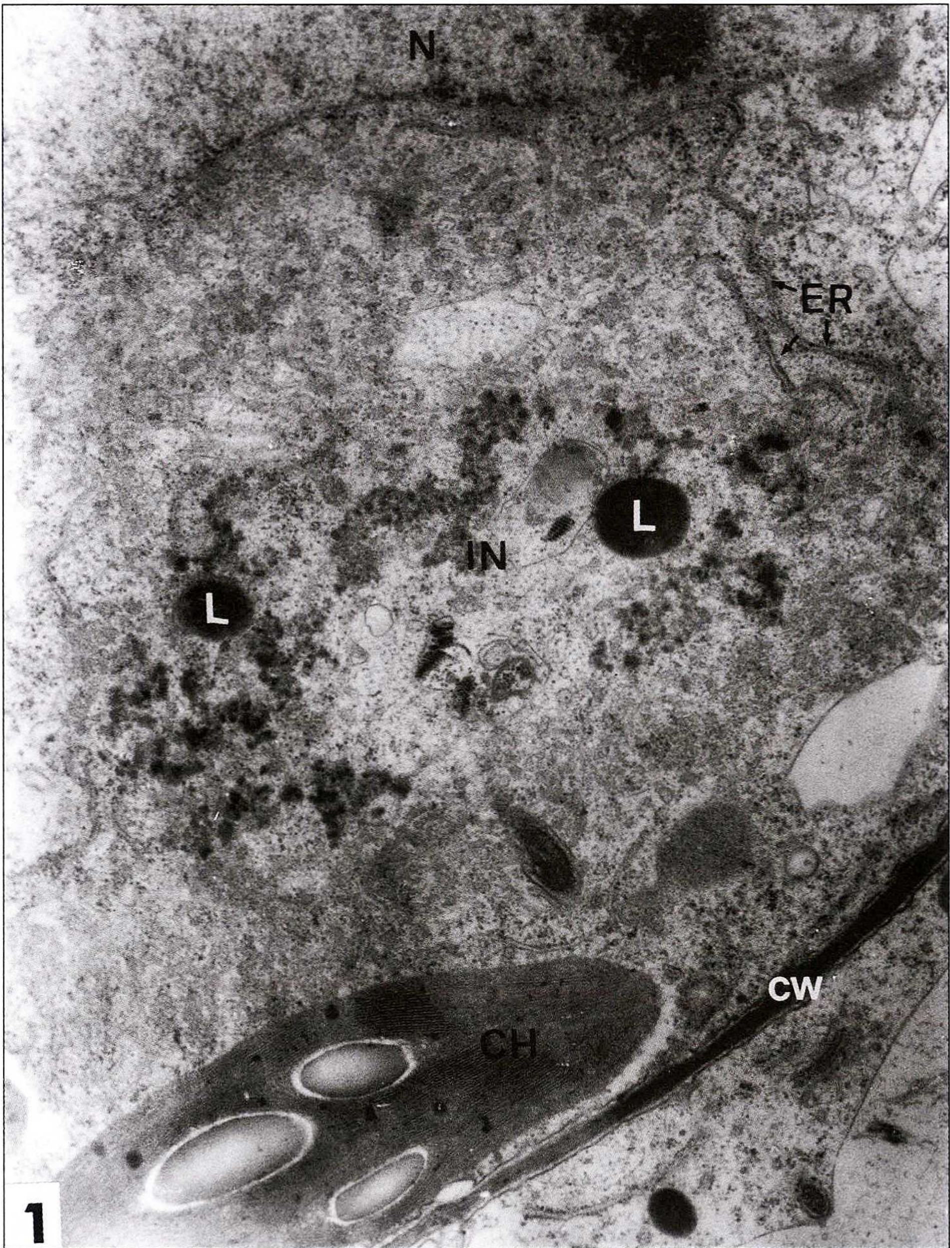

Fig. 1. A thin section of the fragment of large membranous inclusion (IN) between cell wall (CW) and nucleus (N.). CH – chloroplast, ER – endoplasmic reticulum, L – lipid body. Magn. 18.000×

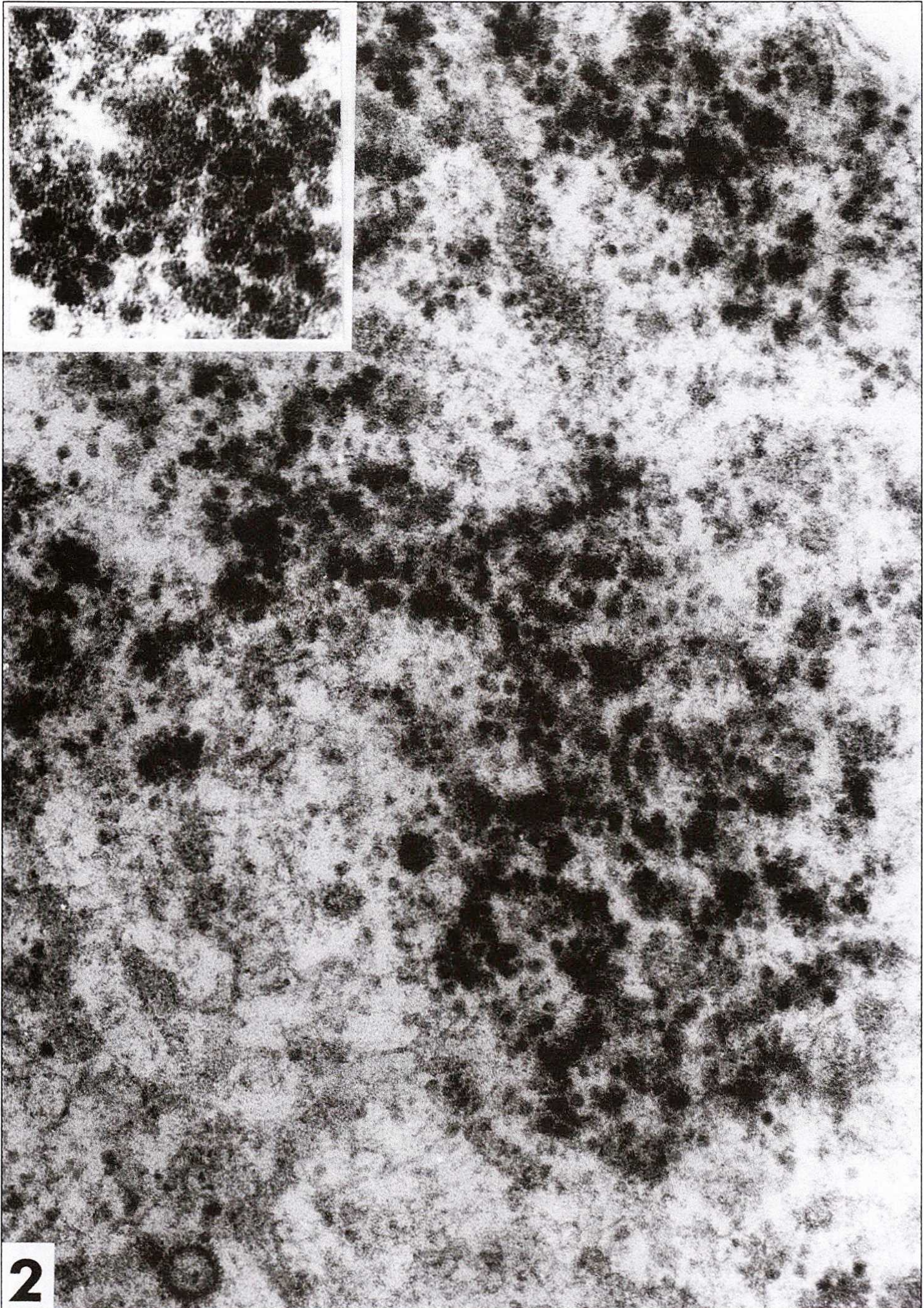

Fig. 2. A fragment of membranous inclusion containing amorphous material with viruses. Magn. 91.000x. Inset contains ArMV particles in magnification. Magn. 175.000×

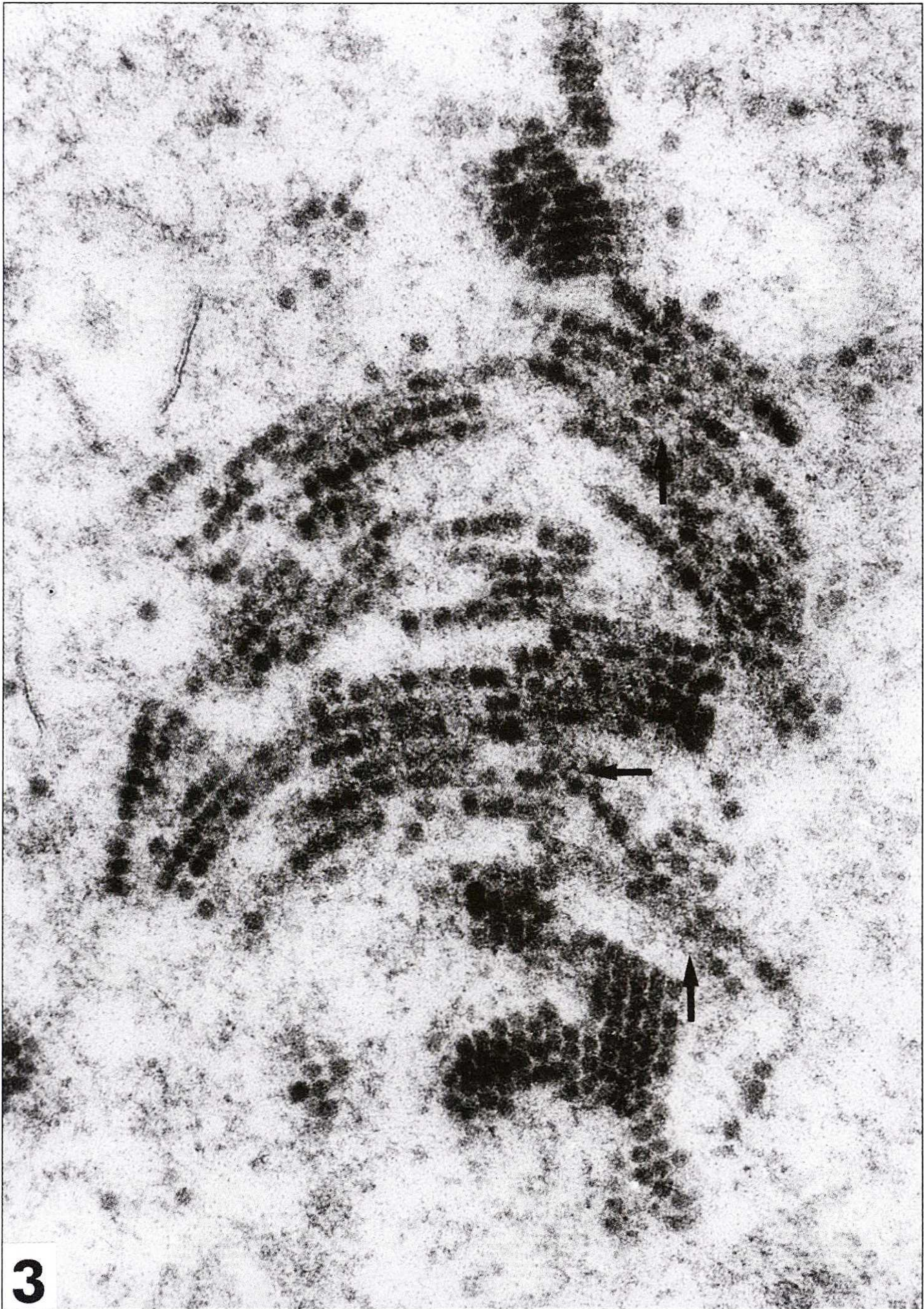

Fig. 3. A part of the inclusion with nucleoprotein-dark stain ArMV particles and empty capsids (arrows). Magn. 130.000×

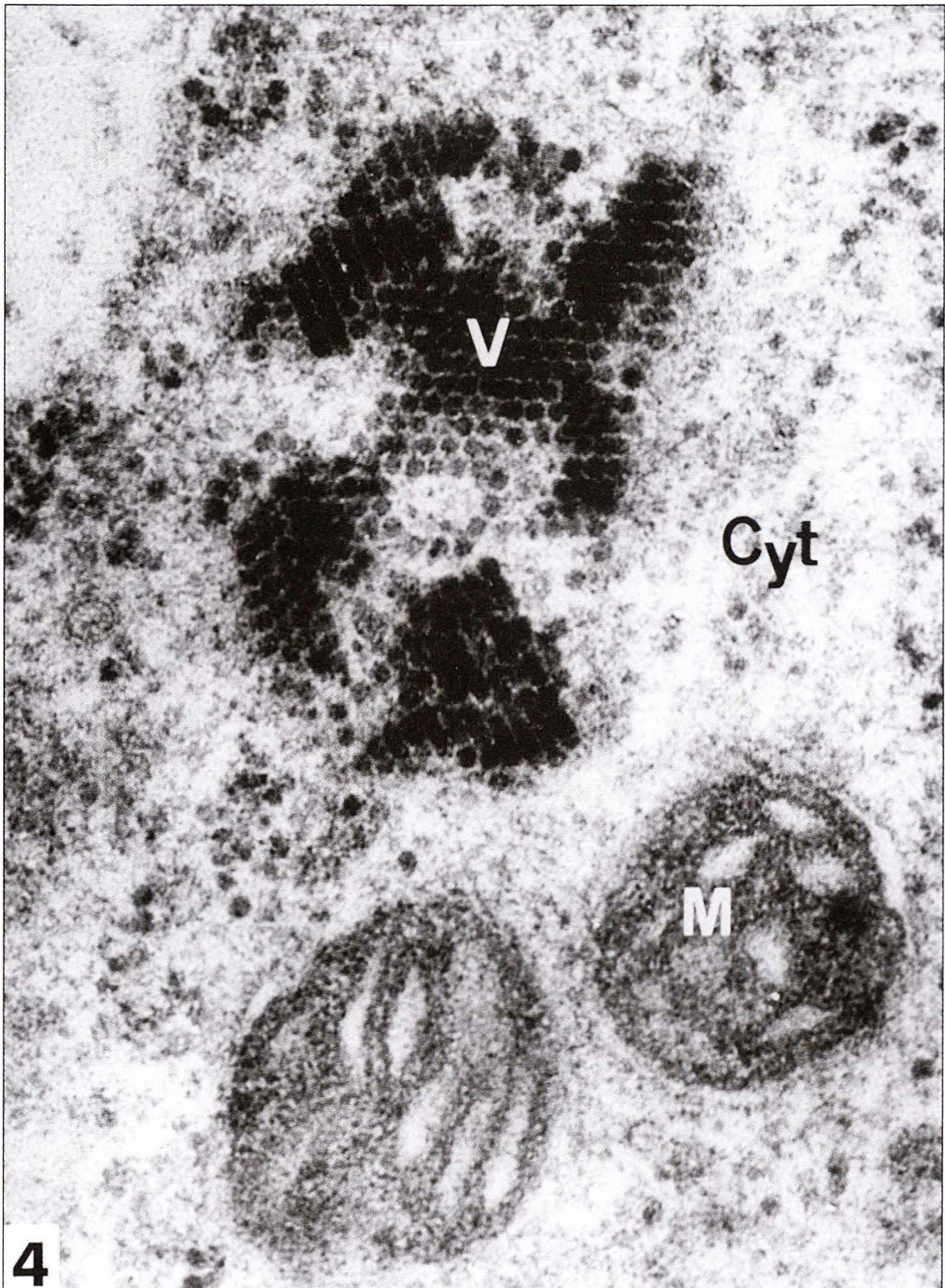

Fig. 4. A portion of crystalline aggregated virus particles in the cytoplasm. V – viruses, Cyt – cytoplasm, M – mitochondrium. Magn. 110.000×

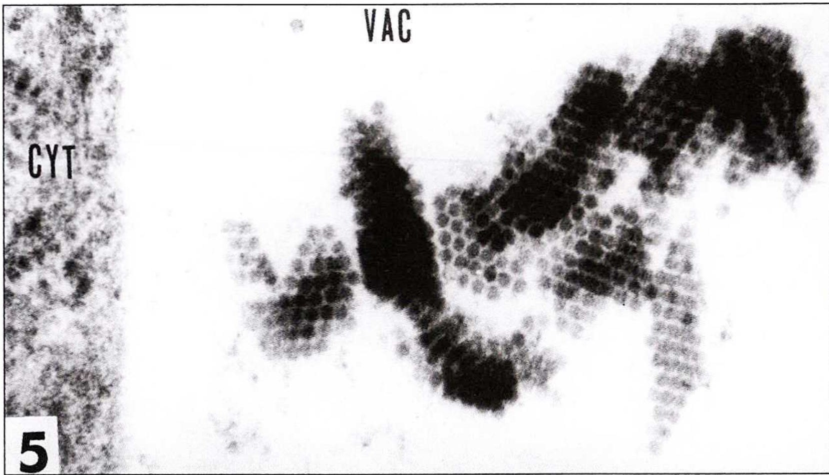

Fig. 5. A crystalline aggregate of virus particles in the cell vacuole. Vac – vacuole, Cyt – cytoplasm. Magn. 110.000×

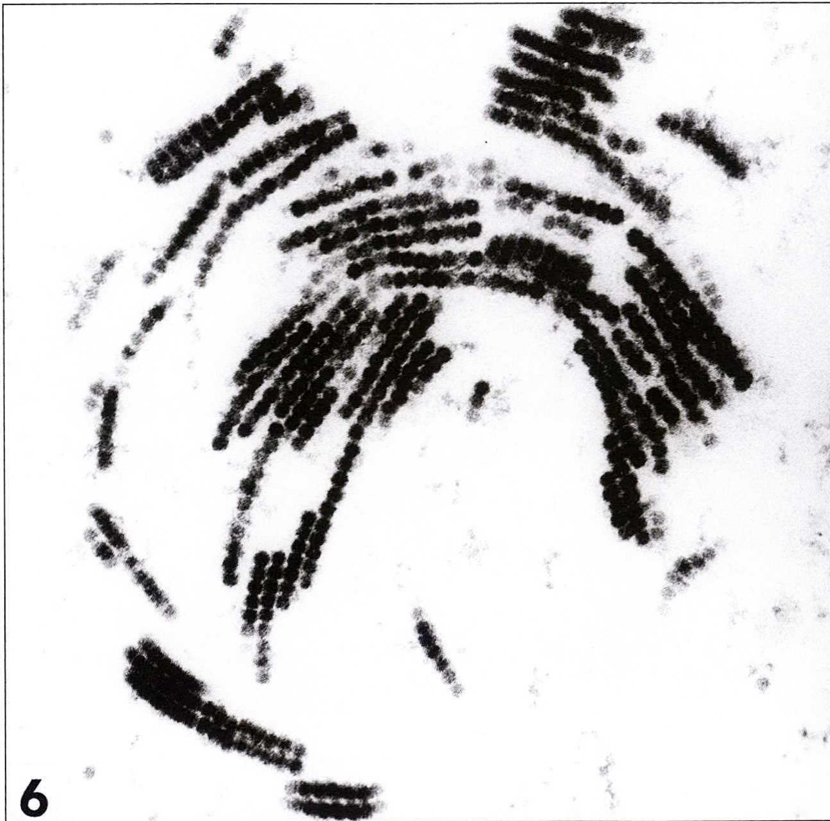

Fig. 6. Semiconcentric layers of aggregated virus particles in the vacuole. Magn. 105.000×

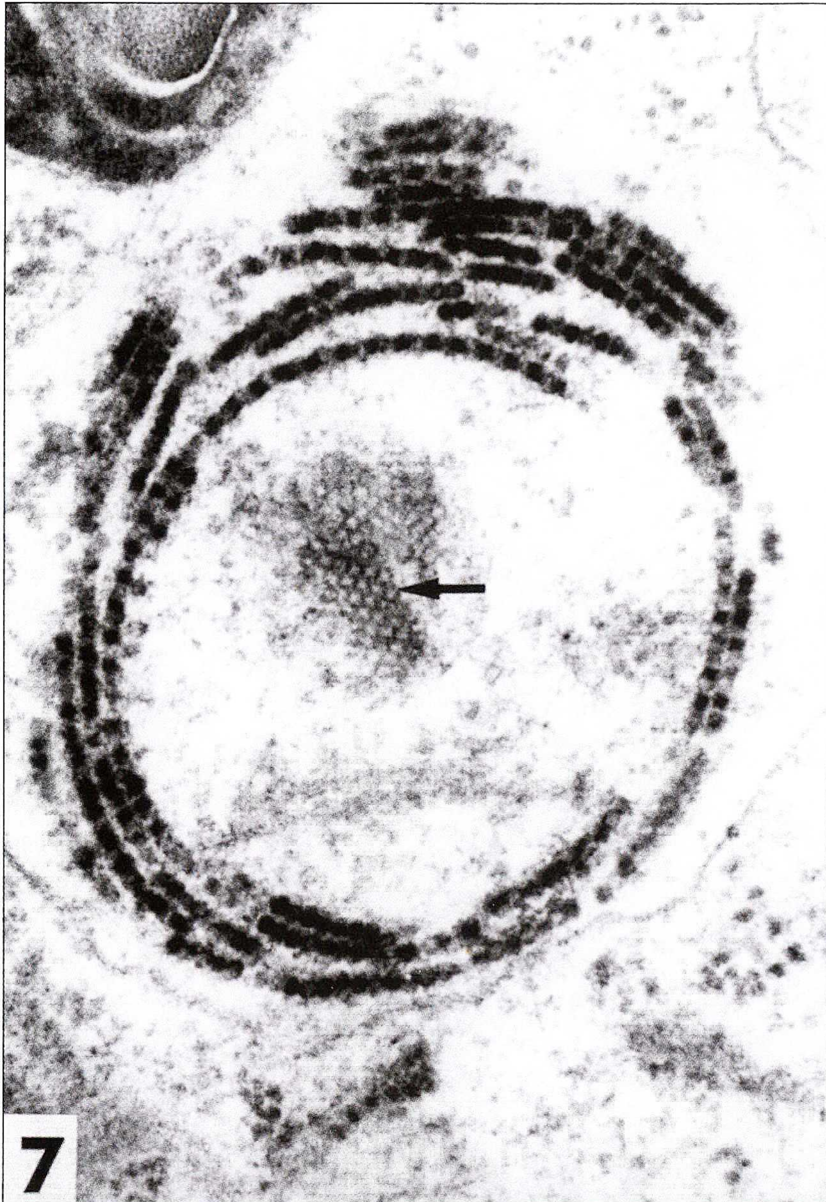

Fig. 7. Concentric layers of aggregated virus particles with group of empty capsids (arrow). Magn. 85.000×

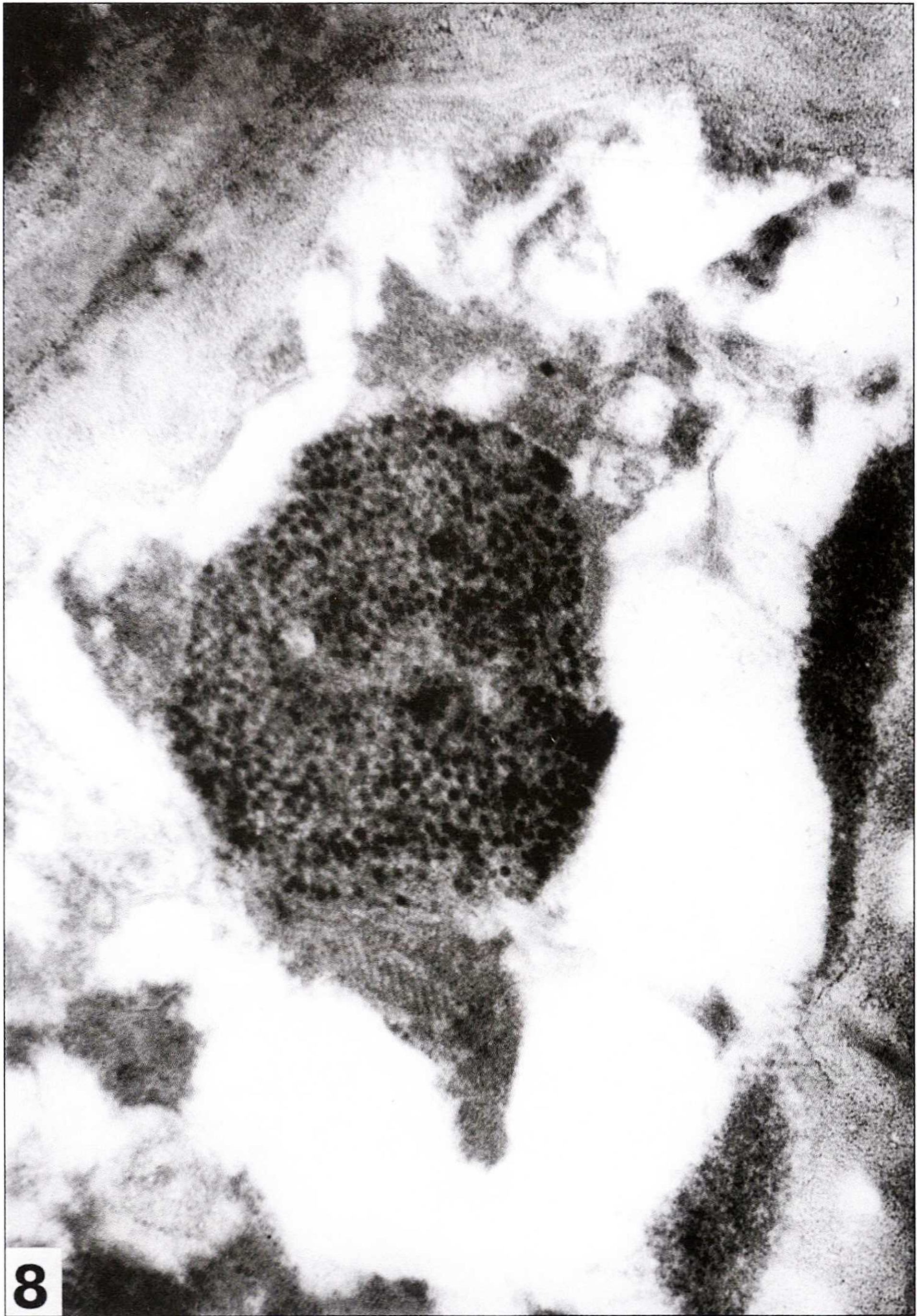

Fig. 8. A fragment of sieve element with viruses in mass of necrotic substance. Magn. 79.000×

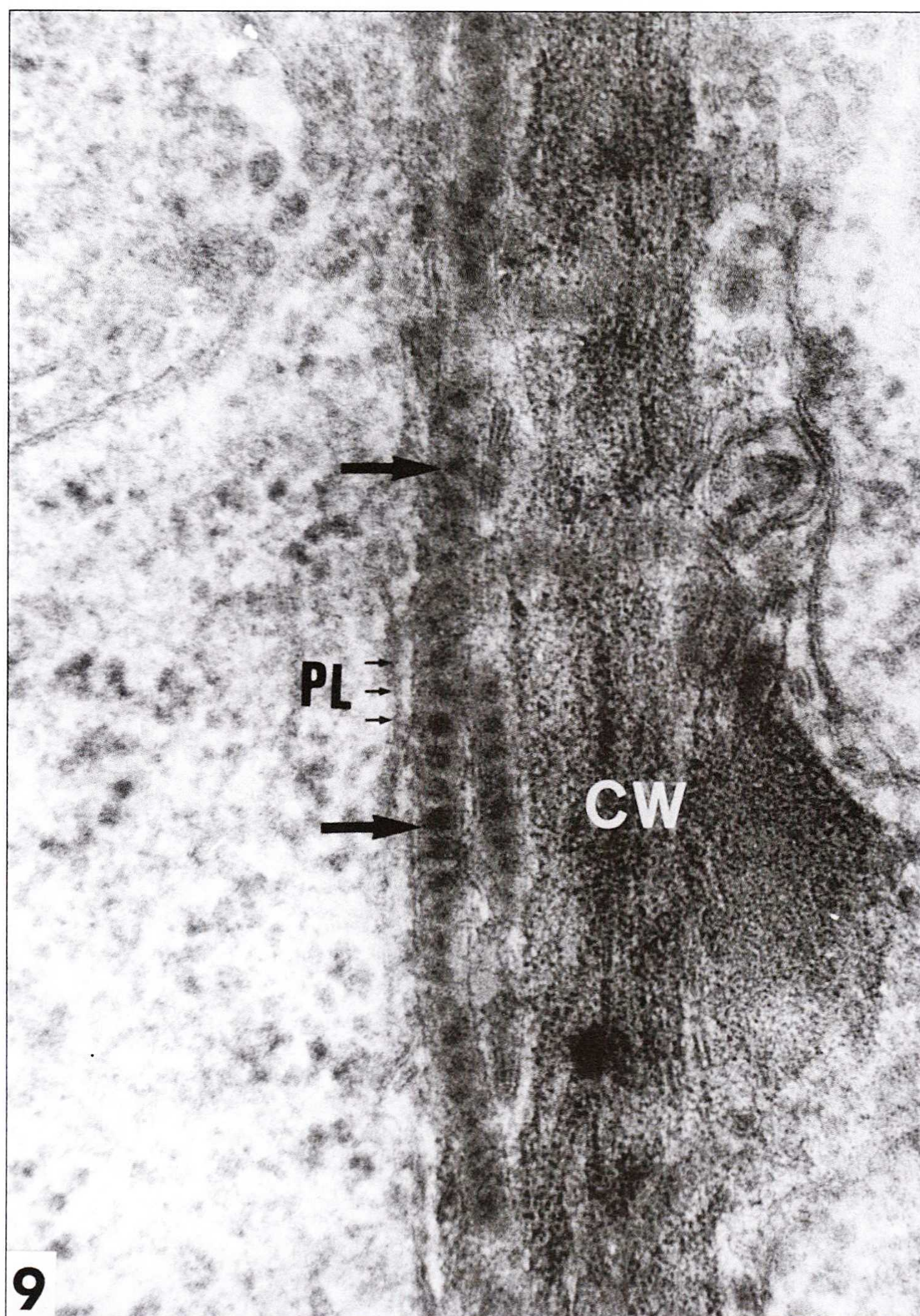

Fig. 9. Tubules containing virus particles (arrows) between the plasmalemma and cell wall. Pl. – fragment of plasmalemma, CW – cell wall. Magn. 156.000×

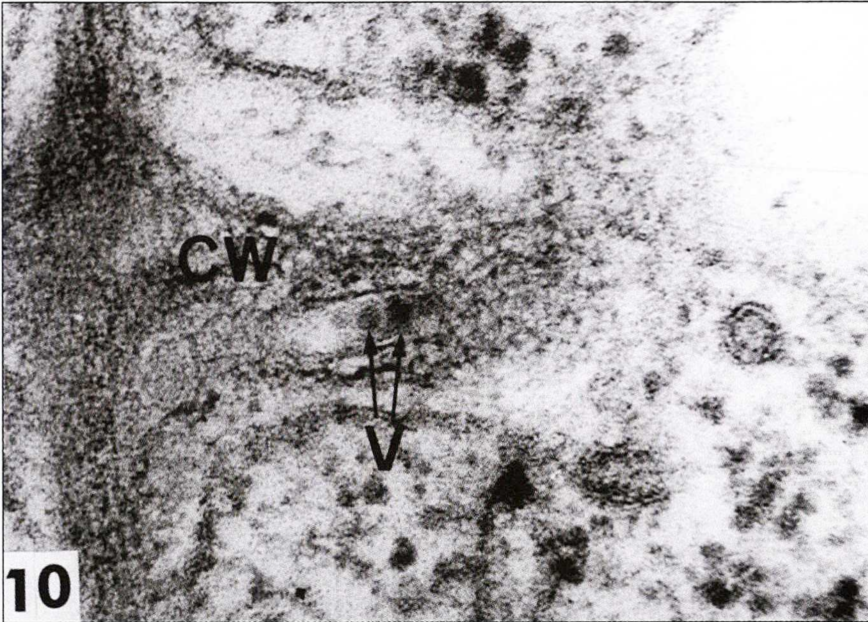

Fig. 10. A protrusion of cell wall (CW) in the direction of protoplast with viruses (V) in the plasmodesmata canal inside. Magn. 185.000×

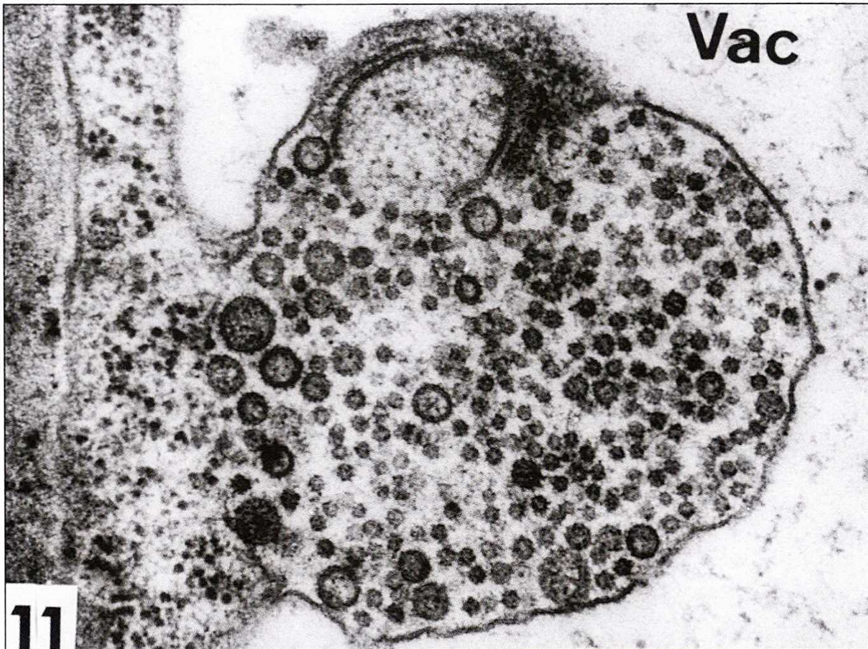

Fig. 11. An invagination of plasmalemmasome to vacuole. Vac – vacuole. Magn. 62.000×

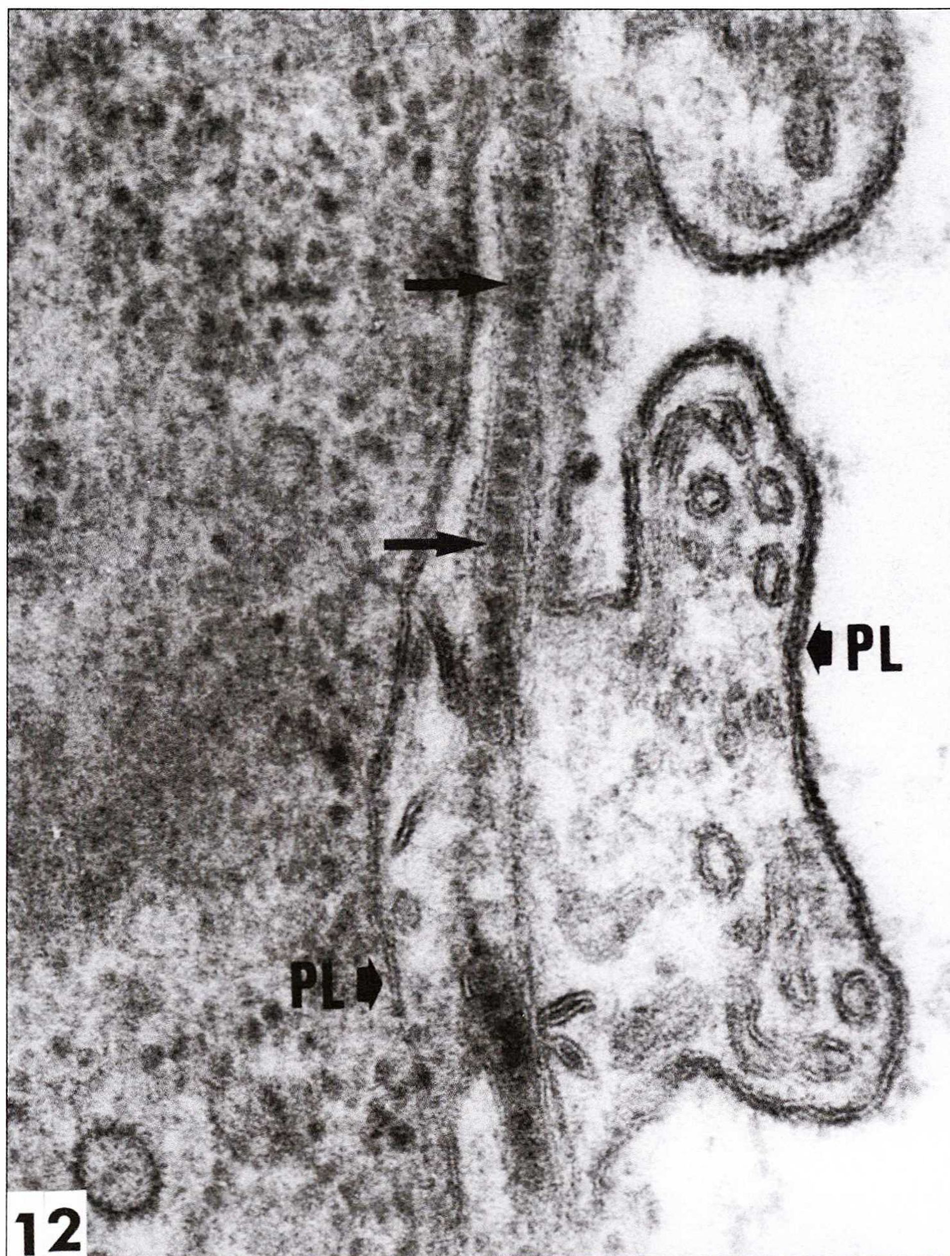

Fig. 12. A tubule with viruses (arrows) surrounded by plicated plasmalemma. Pl. – plasmalemma. Magn. 158.000×

The inclusions also include small crystalline aggregates of virus particles and semiconcentric and concentric layers of aggregated virus particles. It happens that they are also located in small vacuoles inside an inclusion. Often inclusions also contain a lipid body (Fig.1).

The part of cytoplasm that was not included by inclusion and central vacuoles revealed the presence of ArMV located irregularly, as the crystalline aggregates of virus particles (Figs. 4, 5) in either semiconcentric (Fig. 6) or concentric layers of aggregated virus particles (Fig. 7). ArMV particles are spread out loosely and disorderly or are found as small aggregates near cell wall of mature sieve elements. Some sieve elements were already inactive. They either contained large accumulation of callose on sieve plates or firm aggregates of virus particles in a mass of necrotic substance (Fig. 8). Virus particles were not found neither in cell nucleus nor in chloroplast or in mitochondrium. The organells weren't changed as compared with a control.

Tubules containing virus particles were also identified in cytoplasm of parenchyma cells. They were similar to those detected in extract from crushed leaves except the length. Tubules present in tissue included at least 50 virus particles or many more. Also a frequency of observed tubules in tissue was small. It might be concluded that the tubules from plant extract were ripped apart while specimens were prepared. Most tubules in tissue was located near a cell wall. They were observed in area between the cell wall and plasmalemma, sometimes they appeared in two parallel rows (Fig. 9). Tubules that were positioned in the direction of plasmodesmas through which they often penetrate, also touched the cell wall.

Numerous examples of protrusions of the cell wall in the direction of protoplast and with plasmodesm and virus inside were found (Fig. 10). Disorder of plasmalemma observed near modified cell walls caused forming numerous plasmalemmasomes as a result of invagitation and vesiculation (Fig. 11). Invagitation of plasmalemma inside cell also caused pulling tubules together with virus. Sometimes tubules with viruses surrounded by plicated plasmalemma was observed in a central part of the cell (Fig. 12).

Single necrotic or seminecrotic cells together with aggregates of virus particles were found in the epidermis sphere and among parenchyma cells of bean leaves.

IV. DISCUSSION

Gerola et al. studies (1964; 1965; 1966) on ultrastructural changes in leaves of test plants *Chenopodium amaranticolor* and *Petunia hybrida* infected with ArMV showed high destruction of chloroplasts, occurrence of virus particles as the crystalline aggregates and as concentric layers. Characteristically concentric layers of aggregated virus particles, detected also in our study, were not discovered in other representatives nepoviruses group (Francki et al. 1985). We agree with Edwardson and Christie (1991) that this way of arrangment of viruses called spheroidal inclusions, distinguish ArMV among other viruses. According to Gerola et al. (1966) ArMV virus aggreagates were only present in phloem elements of *Petunia hybrida*, whereas in *Chenepodium amaranticolor* only in parenchyma

leaf cells, where photosynthesis takes place. Our studies revealed the ArMV particles occurrence in both mesophyl cells and phloem elements of *Phaseolus vulgaris* leaves as well.

Gerola et al. (1965; 1966) haven't mentioned tubules with virus at all. It should be concluded that they haven't observed their existence. While Walkey and Webb (1970) described tubules inclusions, in other words tubules with virus from nepoviruses, they also notified that there were no tubules in plants infected with ArMV and *Tomato black ring nepovirus* (TBRV) virus. The only information about ArMV virus particles lined up in single rows encircled by cytoplasmatic tubules was derived from Savino and co-author paper (1979) – according to Edwardson and Christie (1991).

Our studies confirm Savino and co-author research (1979). Additionally we assume that low concentration of tubules or absence of them in plants infected with ArMV virus might depend on the virus strain.

Walkey and Webb (1970) informed that their studies on *Cherry leaf roll virus* (CLRV) showed the largest concentrations of tubules with virus in indifferentiated meristematic cells and there were no tubules found in leaves.

Also our observations demonstrated that the young indifferentiated phloem cells contained the most numerous virus particles aggregates and tubules with virus ArMV.

Francki et al. (1985) and Brunt (1995) reveal that plant cells infected by all the nepoviruses contain cytoplasmic vesiculated bodies in cytoplasm. Probably both virus replication and virus cumulation takes place in the cytoplasmic vesiculated bodies. Our studies confirm their results. Exept for nucleoprotein ArMV particles, dark stained an empty capsids were also observed in inclusions in leaf cells of *Phaseolus vulgaris*. Francki et al. (1985) reported the similar phenomenon of aggregates of empty protein shells was observed in the nucleic of *Artichoke yellow ringspot virus* (AYRV) – infected cells and Tobacco ringspot virus (TRSV) – infected cells.

Jones et al. (1973) noticed on leaves of *Nicotiana clevelandii* infected with *Cherry leaf roll virus* (CLRV) cell wall protrusions in direction of cytoplasm, similar to those observed in our studies on leaf cells of *Phaseolus vulgaris*. However, Francki et al. (1985) suggest that those changes in cell walls are probable caused by an aging process affected by virus infection than by specific nepoviruses activity.

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ULTRASTRUKTURALNE ZMIANY W KOMÓRKACH LIŚCI PORAŻONYCH WIRUSEM MOZAIKI GĘSIÓWKI I. W ROŚLINACH FASOLI (*PHASEOLUS VULGARIS* L.)

STRESZCZENIE

Badania elektronomikroskopowe obejmowały obserwacje cząstek wirusa mozaiki gęsiówki (ArMV) oraz tubul z wirionami w ekstraktach z rozgniecionych liści fasoli, a także obserwacje zmian w ultrastrukturze komórek liści fasoli z objawami infekcji systemicznej.

Stwierdzono występowanie charakterystycznych inkluzji membranowych w obrębie cytoplazmy i opisano ich strukturę.

Wiriony ArMV znajdowano luźno, nieregularnie rozrzucone w cytoplazmie, lub w postaci krystalicznych agregatów, albo w formie półkoncentrycznie, bądź koncentrycznie układających się skupisk.

W bliskim sąsiedztwie ścian komórkowych obserwowano nieliczne, lecz nieraz bardzo długie, tubule z wirusami. W badanym materiale licznie występowały protruzje ściany komórkowej do wnętrza protoplastu, często z wirusami oraz liczne plazmalemmasomy.