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ULTRASTRUCTURAL CHANGES IN LEAF CELLS INFECTED WITH *ARABIS MOSAIC NEPOVIRUS* II. IN *CHENOPODIUM QUINOA* PLANTS

Lidia Zielińska, Henryk Pospieszny

Institute of Plant Protection
Miczurina 20, 60-318 Poznań, Poland
e-mail: H.Pospieszny@ior.poznan.pl

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Abstract: The ultrastructural changes in leaf cells of *Chenopodium quinoa* caused by *Arabis mosaic virus* (ArMV) infection have been investigated in electronmicroscopic studies. The techniques of negative staining and embedding sections from leaves in epoxy resin were applied. ArMV virions have been found in the cytoplasm and either singularly scattered or forming different arrangements in the vacuoles as well. Tubules with virions have been observed only rarely. Two types of inclusions have been identified and their structure illustrated. Ultrastructural changes in leaf cells of *C. quinoa* caused by ArMV infection are partially similar and partially different from those occurring on *Phaseolus vulgaris* plants infected with ArMV. It might suggest that some ultrastructural changes are typical for the virus and some are characteristic for the host plant.

Key words: *Arabis mosaic nepovirus*, cytopathology

INTRODUCTION

The results from the studies on the identification of *Arabis mosaic virus* (ArMV) isolated from *Laburnum vulgare* have been presented in another paper (Pospieszny et al. 2001).

This paper is the result of studies inspired by earlier ultrastructural investigations on *Phaseolus vulgaris* plants infected with this virus (Zielińska and Pospieszny 2001). Gerola et al. (1965; 1966) describing the ultrastructural changes in leaf cells of *Chenopodium amaranticolor* and *Petunia hybrida* have proved the differences caused by the ArMV infection. They were related to the ArMV virus preferences to certain types of cells and the presence or absence of the virus in an inclusion's regions. In the first paper on this subject some ultrastructural changes in bean leaves have been illustrated with reference to those presented by Gerola et al. (1965; 1966). This fact led to the undertaking of other investigations on the ultrastructural

changes in *Chenopodium quinoa* leaves and to compare the results with other studies carried out on other plant species infected with ArMV (Gerola et al. 1965; 1966; Zielińska and Pospieszny 2001). Initial observations had already proved the presence of inclusion in *C. quinoa* leaves different from than in *P. vulgaris* cells.

MATERIALS AND METHODS

C. quinoa plants having systemic symptoms caused by ArMV infection were the subjects of the research. *C. quinoa* plants at a growth stage of 2 pairs of leaves were inoculated mechanically with an inoculum. Samples consisting of leaves with systemic symptoms and samples of roots were collected 10 days after inoculation. Specimens for the identification of virus particles were prepared using the negative staining of fresh juice. For ultrastructural studies leaves with the systemic symptoms were taken 14 days after inoculation. The technique of fixing leaves was described in an earlier paper (Zielińska and Pospieszny 2001).

RESULTS

Ten days after inoculations the presence of polyhedral virus particles (about 30 nm in diameter) was detected with a method of negative staining of extracts from *C. quinoa* leaves and roots. Singularly scattered viruses were found in the extracts from the first leaf (inoculated) and upper leaves including top leaves but excluding the fourth leaf. Only a few short tubules with viruses were observed in the extracts from inoculated leaves and top leaves.

The ultrastructural studies of leaf cells of *C. quinoa* proved the presence of 2 types of inclusions in cytoplasm of parenchyma cells. Figure 1 shows the membranous inclusion consisting of densely arranged vesicles. Those inclusions covering smaller or larger cytoplasmic regions were located near cell nucleus or between chloroplasts and at time invaginating towards vacuole. The inclusions often contained lipid bodies and folded concentric membranes. Endoplasmic reticulum and Golgi structures were placed around cytoplasmic membranous inclusions. Concentrations of virus particles were found on the edges of some inclusions.

The second inclusion type had an electronically dense matrix of homogenous, oval or polymorphic bodies, abundantly concentrated in the selected cytoplasm area (Fig. 2). Loosely scattered virus particles were around those bodies (Fig. 2 – arrows). Membranes did not surround those inclusions. Both inclusion types were always placed in different, but not adjoining, regions of cell cytoplasm.

In *C. quinoa* cells there were recorded ArMV virions loosely scattered in the cytoplasm of mesophyll cells and within the phloem sieve elements (Fig. 3). In parenchyma cells there were often observed virus particles in linear arrangement near to cell walls, closely adjoining to plasmalemma from protoplast side. They were not surrounded by tubules (Fig. 4). Those virus arrangements were not detected within the protoplast. The crystalline aggregates of virus particles as well as semiconcentric virus particles were found in both cytoplasm (Fig. 5) and vacuoles (Fig. 6). The aggregates of empty capsids were not observed.

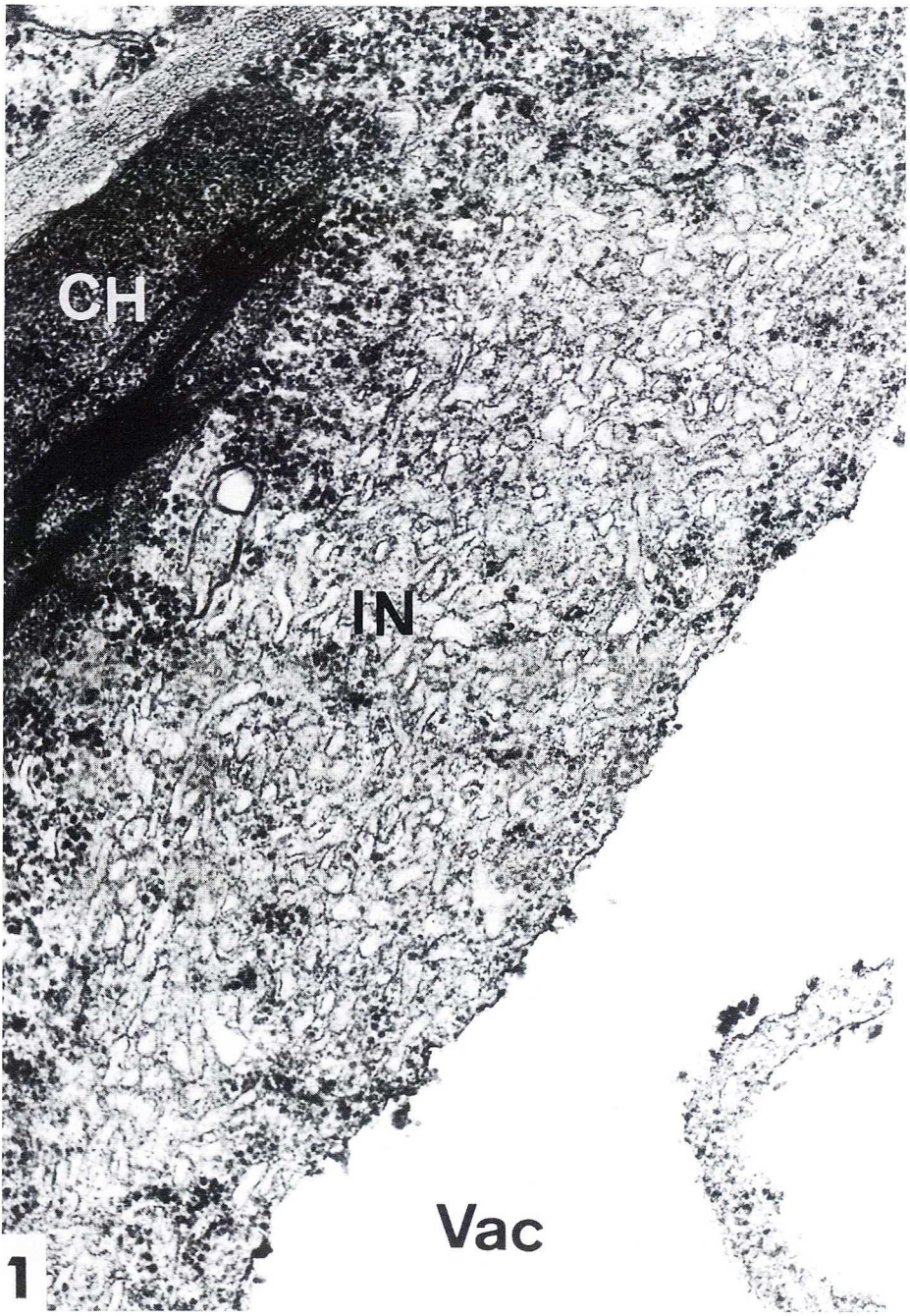

Fig. 1. Large membranous inclusion (IN) in the cytoplasm. CH – chloroplast, Vac – vacuole. Magn. 58 000 ×

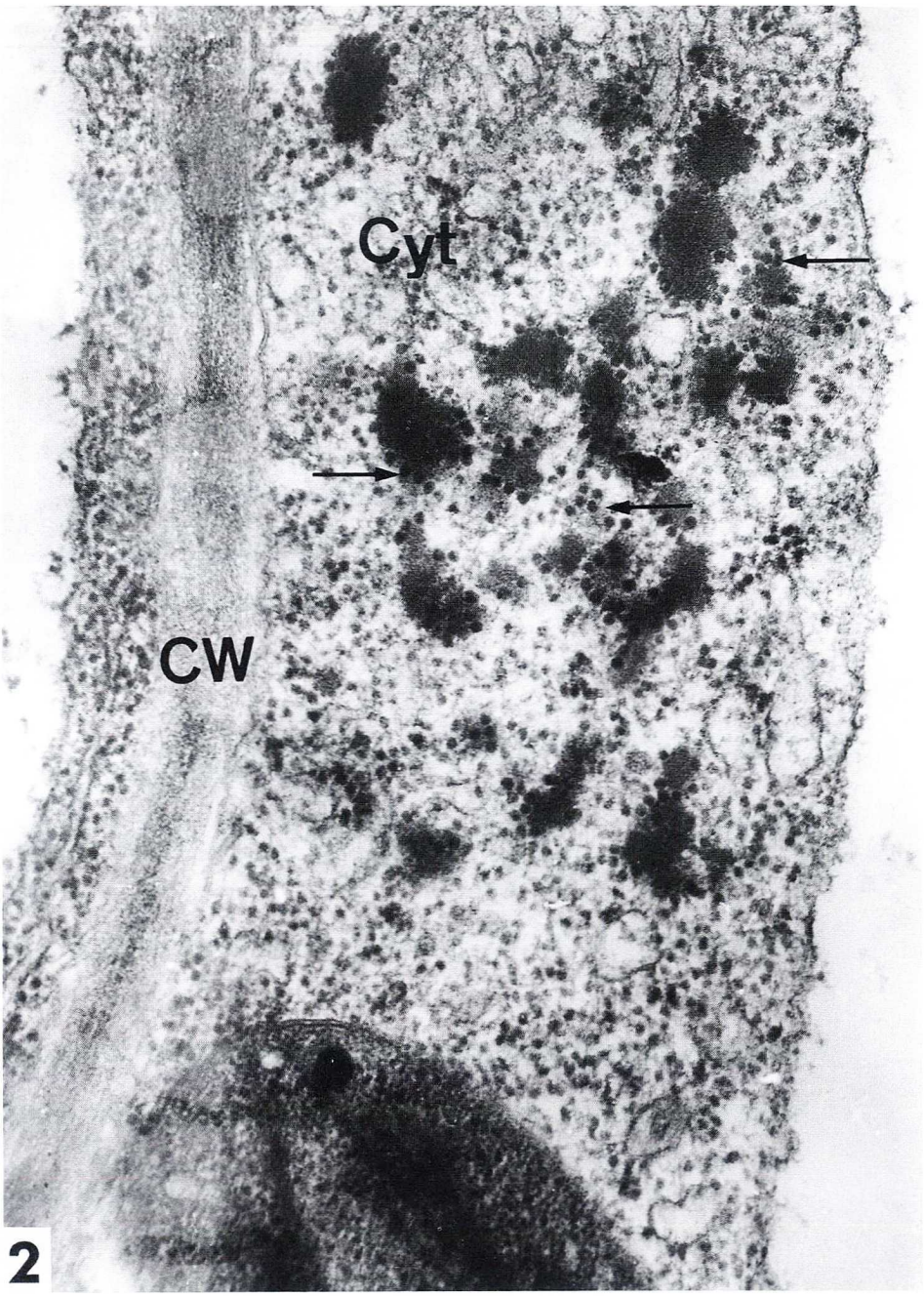

Fig. 2. The inclusion consisting of electronically dense bodies. Cyt – cytoplasm, CW – cell wall, arrows – viruses. Magn. 73 000×

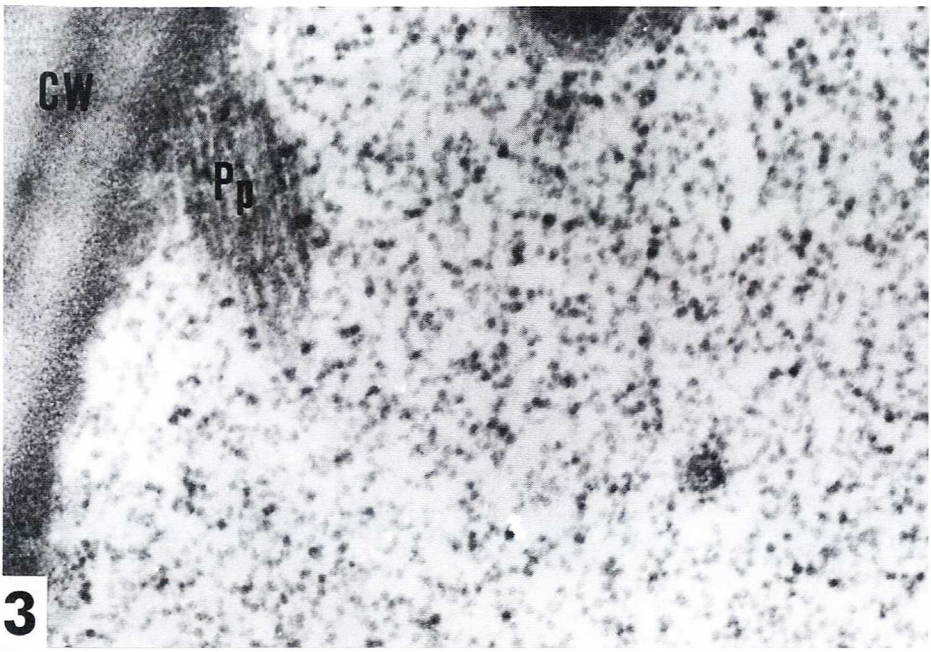

Fig. 3. Part of sieve element near cell wall (CW) with a large amount of virions and tubular P – protein body (Pp). Magn. 67 000 ×

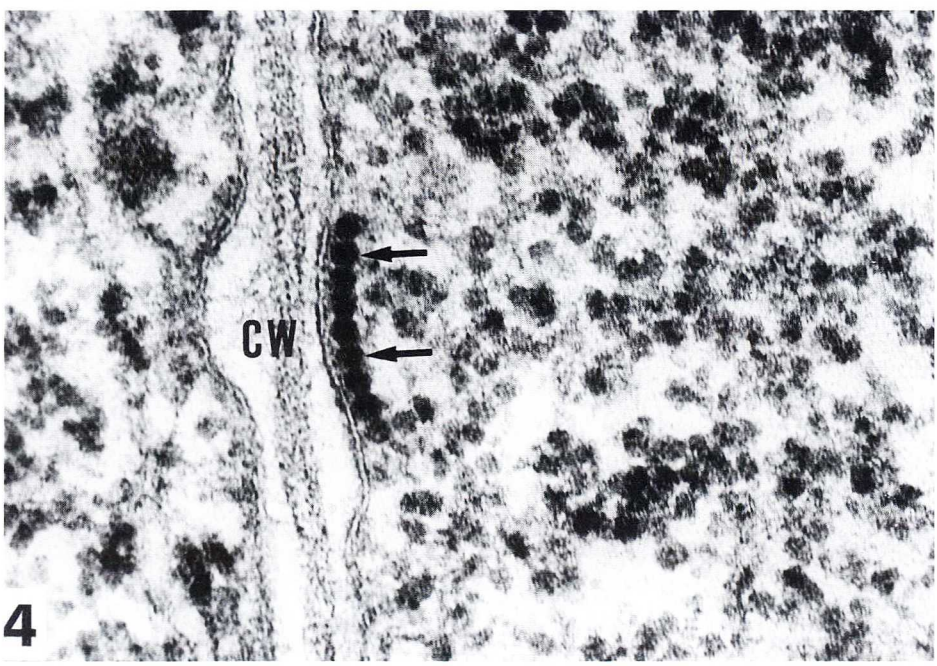

Fig. 4. Linear arrangement of virus particles near cell wall (arrows). CW – cell wall. Magn. 156 000 ×

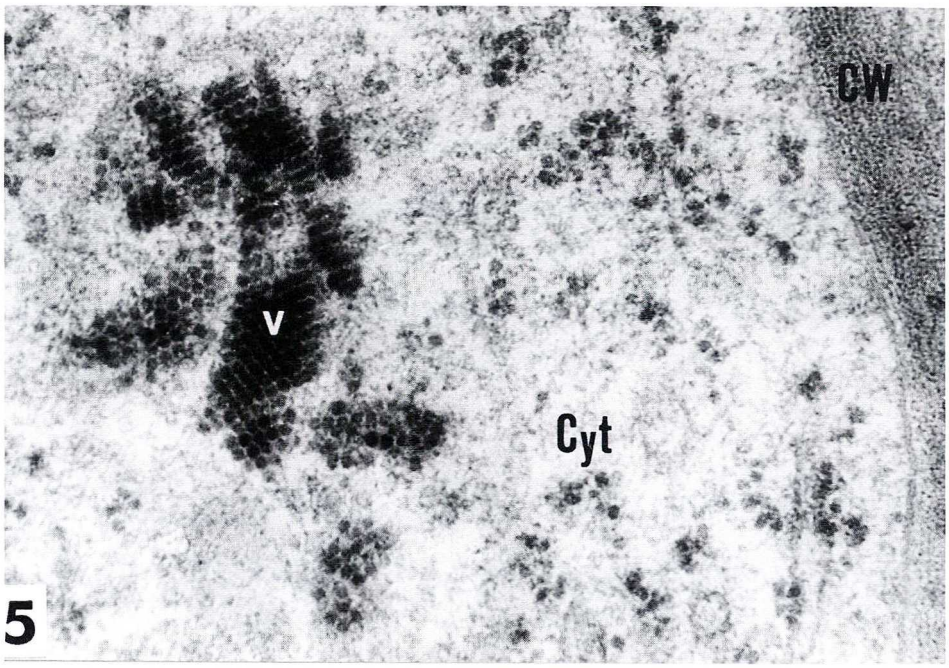

Fig. 5. Crystalline aggregate of virus particles in the cytoplasm. V – viruses, Cyt – cytoplasm, CW – cell wall. Magn. 64 000 ×

Few short tubules with virus particles were detected in cytoplasm of parenchyma cells. They were placed within cells and near cell walls. However, there was no protrusion of cell walls toward the protoplast. Plasmodesmas in cell walls often contained virus particles but seldom were encircled by tubules (Fig. 7).

In leaf cells of *C. quinoa* virus particles were not present and also there were no symptoms of severe deformation. Nevertheless, the chloroplast from samples of chlorotic leaves had numerous distended areas between gran disks.

DISCUSSION

The ultrastructural changes in leaf cells of *C. quinoa* caused by ArMV infection were similar but not identical to those observed in *P. vulgaris* leaves (Zielińska and Pospieszny 2001). At some point they differed significantly.

ArMV virus particles were detected in *C. quinoa* and *P. vulgaris* in both parenchyma mesophylle cells and phloem cells. The virus particles were similarly arranged in aggregates in cytoplasm and vacuoles. However, the frequency of occurrence of crystalline aggregates with tubules containing virus particles was much lower in *C. quinoa* cells than in *P. vulgaris*. Also plasmalemmasomes occurred much less frequently in *C. quinoa* and there were no empty virus capsids and cell wall protrusions as compared with *P. vulgaris*.

Šarić and Wrischer (1975) studying *Grapevine fanleaf virus* in *Nicotiana clevelandii* cells have recorded the presents of very large virus aggregates in central vacuoles.

Fig. 6. Semiconcentric layers and crystalline aggregate of virus particles in the vacuole. Magn. 107 000 ×

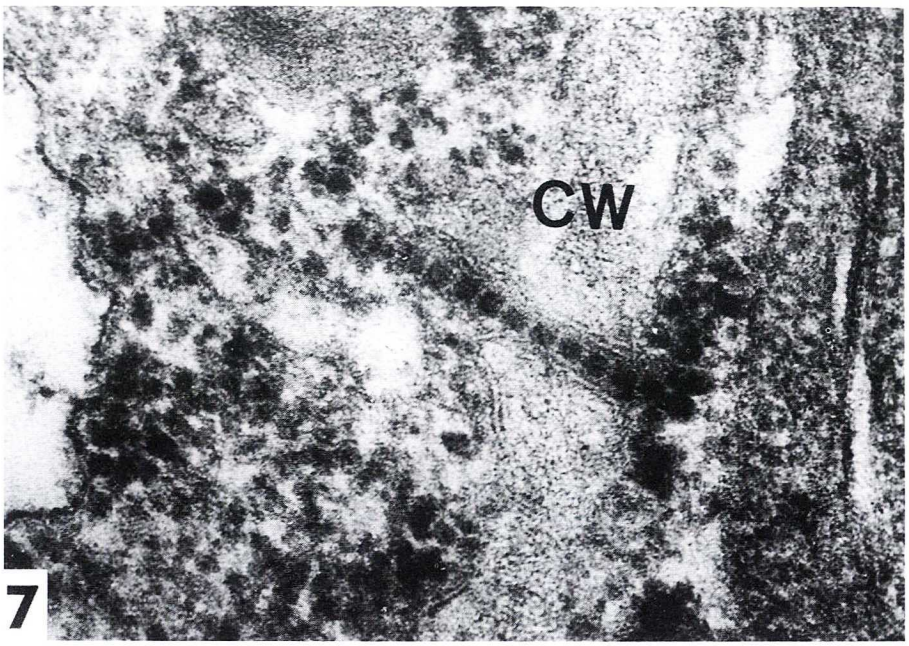

Fig. 7. Virus particles associated with a plasmodesma. CW – cell wall. Magn. 156 000×

Particles of this virus retained the same row arrangement as they had in the cytoplasm. Our studies confirm those results. ArMV viruses in *P. vulgaris* cells (Zielińska and Pospieszny 2001) as well as in *C. quinoa* cells did not change the layout after translocation from cytoplasm to vacuole. Above-mentioned authors (Šarić, and Wrischer 1975) have believed that moving virus particles to vacuole is probably the way viruses are removed from the cytoplasm. This statement seems to be very applicable because there were no virus aggregates in the adjoining cytoplasm. The proof for this conclusion is the statement made by Francki and others (1985) pointing out the frequent occurrence of different nepoviruses in vacuoles in metabolic active cells with undestroyed tonoplast.

Two inclusion types were found in parenchyma leaf cells of *C. quinoa*. The first type is the membranous inclusion, typical for nepoviruses, described by Francki et al. (1985) and Brunt (1995). The second inclusion type included only numerous, oval, homogenous, and electron dense bodies. Gerola at al. (1965) have observed similar pictures of round, circular electron dense bodies in close proximity to concentric arranged ArMV virus particles in leaf cells of *C. amaranticolor*. This type of inclusion has no relation to membrane structures recorded either in *P. vulgaris* leaves (Zielińska and Pospieszny 2001) or in *Petunia hybrida* (Gerola et al. 1966) infected by ArMV. One can conclude that this type of inclusion occurring at a certain time after infection with ArMV can be related only to *Chenopodium* species.

An interesting observation is ArMV coexistence with inclusions. In our studies the virus particles were found in both types of inclusions in *C. quinoa* and in the inclusions in *P. vulgaris* cells. (Zielińska and Pospieszny 2001). Conversely, the re-

sults from other investigations revealed the presence of large virus aggregates in inclusions in *C. amaranticolor* leaves (Gerola et al. 1965) and the absence of virus particles in inclusions in *P. hybrida* leaves (Gerola et al. 1966).

Additionally, the characteristic, linear arrangements of virions next to plasmalemma have been observed in *C. quinoa* leaf cells. It can be assumed that this is either the first stage of forming semiconcentric or concentric virus aggregates – typical only for ArMV or the beginning of tubule initiations with viruses. Jones et al. (1973) declared on the basis on sets of ultrathin sections that tubule membrane containing viruses were a plasmalemma prolongation.

ArMV viruses did not cause serious chloroplast deformations in studied leaves of *C. quinoa* as compared with changes produced by this virus in *P. hybrida* leaves (Gerola et al. 1966).

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POLISH SUMMARY

ULTRASTRUKTURALNE ZMIANY W KOMÓRKACH LIŚCI PORAŻONYCH WIRUSEM MOZAIKI GĘSIÓWKI
II. W ROŚLINACH KOMOSY RYŻOWEJ (*CHENOPODIUM QUINOA*)

W badaniach elektronomikroskopowych wykonanych metodami barwienia negatywowego i zatapiania wycinków liści w żywicach epoksydowych, opisano zmiany ultrastrukturalne w komórkach liści *Chenopodium quinoa* spowodowanych przez *Arabis mosaic virus* (ArMV). Wiriony ArMV znajdowano w cytoplazmie oraz we wakuolach pojedynczo rozrzucone lub w różnych układach. Tubule z wirionami znajdowano bardzo rzadko. Stwierdzono występowanie 2 typów inkluzji w cytoplazmie i opisano ich strukturę. Zmiany ultrastrukturalne w ko-

mórkach liści *C. quinoa* wywołane infekcją ArMV częściowo są podobne a częściowo różne od zmian stwierdzonych u *Phaseolus vulgaris* towarzyszących infekcji ArMV. Może to sugerować, że w tym przypadku część zmian ultrastrukturalnych jest charakterystyczna dla wirusa, a część jest właściwa gatunkowi rośliny.