

リンゴクロロティックリーフスポットウイルス,リンゴステムグルーピングウイルス,ならびにカンキツタターリーフウイルス感染Chenopodium quinoa葉組織の比較電子顕微鏡観察

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Comparative Electron Microscopy of *Chenopodium quinoa* Leaves Infected with Apple Chlorotic Leaf Spot, Apple Stem Grooving, or Citrus Tatter Leaf Virus

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Key words: cytopathology, *Chenopodium quinoa*, apple chlorotic leaf spot virus, apple stem grooving virus, citrus tatter leaf virus.

Flexuous filamentous particles of apple chlorotic leaf spot virus (ACLSV)¹⁾, apple stem grooving virus (ASGV)²⁾, and citrus tatter leaf virus (CTLV)^{3,4)} range 600~750 nm in length, but resemble closteroviruses⁵⁾ in morphology. Closteroviruses cause phloem necrosis and their particles are found to be confined to the phloem cells associated with vesicles containing nucleic acid-like fibrils⁶⁾. However, little information is available in cytopathology of ACLSV-, ASGV-, or CTLV-infected tissues. Small projections containing masses of elongated particles have so far been observed in cytoplasm of chlorotic areas in the sections of ACLSV-infected *Chenopodium quinoa*⁷⁾. CTLV particles were reported to be found only in phloem cells of the infected *C. quinoa* leaves⁴⁾. To elucidate the relationship between the three viruses and closteroviruses, electron microscopy on *C. quinoa* tissues infected with ACLSV, ASGV, or CTLV was conducted.

Viruses and plants. ACLSV P-205, ASGV P-209, originally isolated from apple infected with top-working disease⁸⁾, and CTLV Li-23⁴⁾ from lily were used. *C. quinoa* plants mechanically inoculated with either of the viruses and the healthy controls were incubated in a greenhouse at 20~25 C. Systemic leaves of the infected plants were sampled at 14~21 days after inoculation for cytopathological study.

Electron microscopy. Pieces of leaves from the infected plants and the healthy controls were prefixed in 5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol and embedded in Spurr's resin. Ultrathin sections were cut by a Reichardt ultramicrotome and stained with uranyl acetate and lead citrate. A Hitachi H-300 electron microscope was used for observation.

Ultrastructural studies of ACLSV-, ASGV-, or CTLV-infected plants revealed the occurrence of filamentous particles of 650~750 nm in length which appeared in mesophyll as well as phloem parenchyma cells, but not in epidermis or sieve elements. The particles were ob-

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served singly or as aggregates in the cytoplasm (Figs. 1~10). No severely degenerated cells in the phloem were observed. Deformation of cell organella was observed, however, no specific vesicles associated with virus particles were found in the infected cells. No cytopathological changes were observed in the healthy controls.

Virus particles appeared much more concentrated in the ACLSV-infected cells compared with the other two samples. ACLSV particles accumulated in cytoplasm or between tonoplast and cytoplasm, resulted in round virus aggregates of 0.5~1.5 μm in diam. (Figs. 5, 6). They also crystallized in cytoplasm observed as parallelograms in the sections (Figs. 8, 9). ACLSV particles were found in the nuclei as well (Fig. 10).

Relationships among the three viruses and to closteroviruses. Infection by a definite member of closteroviruses accompany severe phloem necrosis in the infected tissues. Their virus particles and the associated vesicles were observed confined to the phloem cells⁶⁾. However, severe phloem necrosis was not detected in ACLSV-, ASGV, or CTLV-infected plants in this study. Those virus particles, which were not accompanied by the vesicles, were observed not only in phloem cells but also in mesophyll cells despite of the previous observation for CTLV⁹⁾. These facts, as well as the characteristics such as transmissibility by sap, through seeds, but not by aphids^{1,2,4)}, strongly indicate that the three viruses should be excluded from closteroviruses.

ASGV has been transferred to a new group capillovirus^{9,10)} along with a serologically related virus, potato virus T¹¹⁾. Close relationship between capilloviruses and CTLV was also suggested by Bar-Joseph and Murrant¹²⁾. No obvious differences in cytopathology between ASGV- and CTLV-infected cells were detected in this study.

Considerable differences in various characteristics between ACLSV and ASGV were reported^{1,2,13)}. ACLSV particles appeared more concentrated in cells compared with the other viruses and often observed as large aggregates in this study. Although further investigations on different isolates are needed to confirm the results, the observed differences in cytopathology between ACLSV- and ASGV-infected plants support the idea that the two viruses should be classified into different groups¹³⁾. Further studies on similar viruses around closteroviruses may reveal some interesting new knowledge for these filamentous viruses.

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和 文 摘 要

大木 理・吉川信幸・井上成信・井上忠男：リンゴクロロティックリーフスポットウイルス，リンゴシステムグルーピングウイルス，ならびにカンキツタターリーフウイルス感染 *Chenopodium quinoa* 葉組織の比較電子顕微鏡観察

Closterovirus に比べて粒子長はやや短い粒子形状がよく似ているリンゴクロロティックリーフスポットウイルス (ACLSV)，リンゴシステムグルーピングウイルス，カンキツタターリーフウイルスの3種について，それぞれを単独に感染させた *C. quinoa* 葉組織切片中でのウイルス粒子の分布様式，内部病徴を電顕観察した。これら3種のウイルスはいずれも篩部細胞だけでなく葉肉組織の柔細胞の細胞質中に散在して，あるいは集塊として観察され，顕著な篩部壊死や closterovirus に特徴的な小胞構造は認められなかった。したがって，これら3種のウイルスは closterovirus とは区別して分類するのが適当と考えられた。なお，ACLSV は感染細胞中の粒子密度が格段に高く，平行四辺形状の結晶として観察される点など，他の2種とはやや異なる所見を示した。

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Explanation of plates**Plate I.**

Figs. 1, 2. Filamentous particles of apple stem grooving virus in cytoplasm of mesophyll cells in the infected *Chenopodium quinoa*.

Figs. 3, 4. Particles of citrus tatter leaf virus in cytoplasm of mesophyll cells in the infected *C. quinoa*.

Plate II.

Figs. 5~9. Particles of apple chlorotic leaf spot virus (ACLSV) in cytoplasm of mesophyll cells in the infected *C. quinoa*.

5, 6. Round virus aggregates.

7. Crystallized virus particles.

8, 9. Virus aggregates as parallelograms.

Fig. 10. ACLSV particles appeared in nucleoplasm of a mesophyll cell in the infected *C. quinoa*.

Bars represent 500 nm.

Abbreviations: cp, chloroplast; cw, cell wall; mt, mitochondrion; nu, nucleus; va, vacuole; vp, virus particles.

Plate I

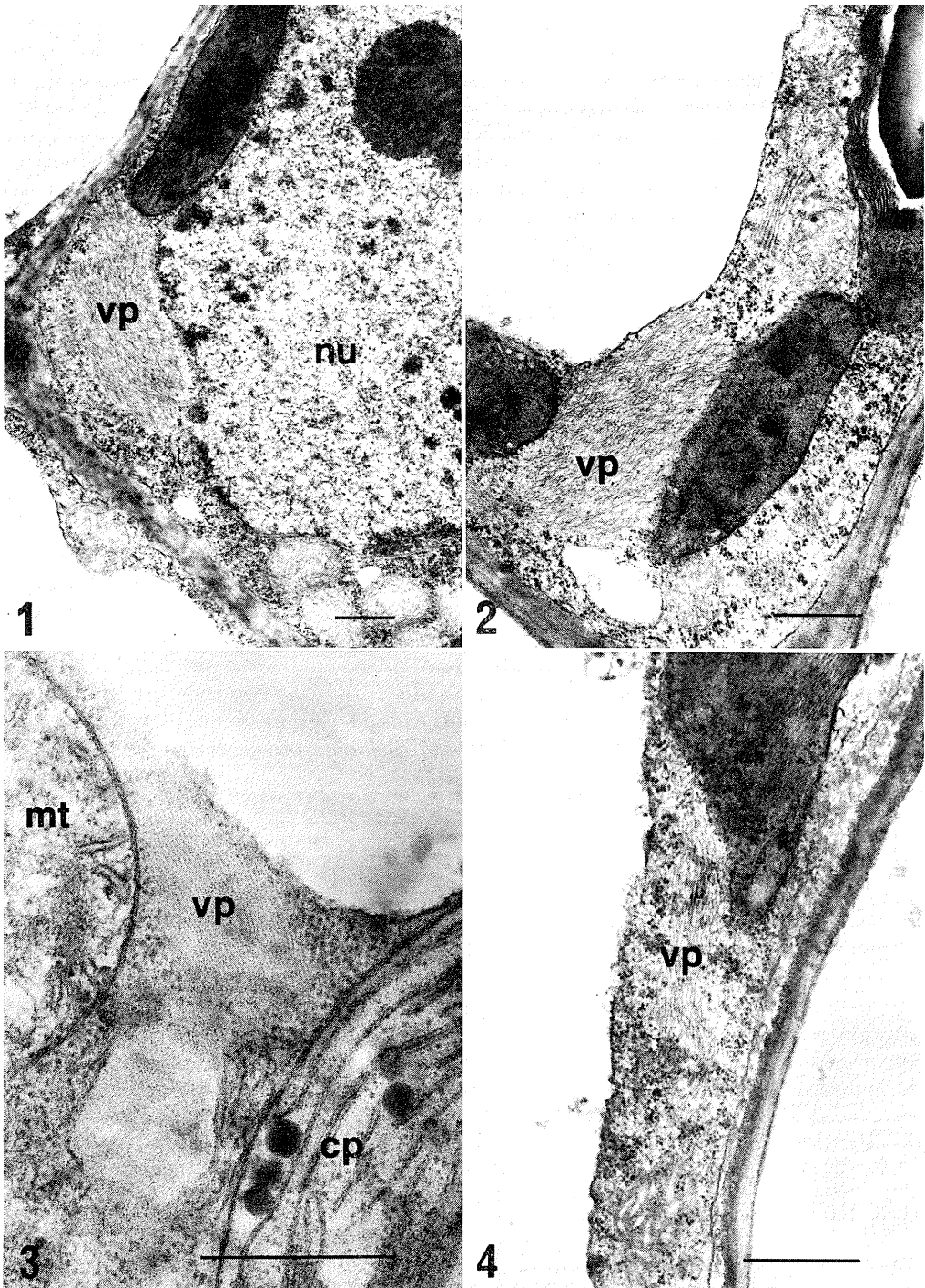


Plate II

