Plant Pathol. J. 17(4) : 201-204 (2001)

The Plant Pathology Journal
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Ultrastructural Aspects of the Mixed Infections with *Turnip mosaic virus* and *Ribgrass mosaic virus* in Oriental Cabbage

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(Received on May 13, 2001)

Ultrastructural observation was conducted for the cells of oriental cabbage, *Brassica campestris* ssp. *pekinensis* 'Chunhawang', inoculated simultaneously with *Turnip mosaic virus* (TuMV-Act2-4vq) and *Ribgrass mosaic virus* (RMV-Ca1dn2) which were known as major destructive viruses of oriental cabbage in Korea. In cells infected with RMV alone, the virus particles were located as bundle or scattering in cytosols and vacuoles, which were typical ultrastructures of tobamovirus. Vessels of xylem were compacted with RMV particles. The cells infected only with TuMV had the cluster of virus particles scarcely and the typical potyvirus inclusions of scrolls, pinwheels, tubes and laminated aggregates in cytosols. The TuMV particles were jammed lineally between tonoplasts. In double infection, the two unrelated viruses of TuMV-Act2-4vq and RMV-Ca1dn2 were located together in a cell, and typical properties of each virus were also observed. The potyvirus inclusions and the tobamovirus particles were mixed entirely in cytoplasm. The virus particles of RMV were presented strikingly near and in the center of potyvirus inclusions. In vascular cells, the tobamovirus particles were located abundantly than those in single infection. The potyvirus inclusions were embedded in the cluster of RMV particles in phloem parenchyma cells and the vascular elements were degenerated severely.

Keywords : mixed infection, oriental cabbage, RMV, TuMV.

Oriental cabbage is an important crop as a basic material of Kimchi that is one of the important traditional Korean foods. The crop improvement of oriental cabbage has been done in quality, yield and overcome of seasonality in Korea. However, the diseases of bacterial soft and viruses have been the big problems in the production of oriental cabbage.

Viruses occurring in oriental cabbage were *Cucumber mosaic virus* (CMV), *Turnip mosaic virus* (TuMV), and *Ribgrass mosaic virus* (RMV) in Korea. CMV and TuMV were reported (Lee, 1981). RMV occurred as the mixed infection rate of 63.4% with TuMV in oriental cabbage cultivated in summer and autumn seasons (Kim et al., 1993). The two viruses, RMV and TuMV, were quite different in ecological characteristics of transmission manners, physical properties, and morphology of virus particles. When the two viruses were inoculated mechanically at the same time to oriental cabbage, the symptoms of necrotic spots, midrib necrotic streaks and severe mosaic on leaves were revealed more severely than those of each single infection. There was limited information of intracellular morphology about the relationships of the two mixed viruses in replication, translocation and symptom expression on oriental cabbage.

Materials and Methods

Virus source and oriental cabbage used. The commercial cultivar of oriental cabbage, *Brassica campestris* ssp. *pekinensis* 'Chunhawang', was used for the artificial infection. Virus isolate used was Ca1dn2 for RMV isolated from oriental cabbage at field of National Horticultural Research Institute, Suwon, and Act2-4vq for TuMV from oriental cabbage at field of alpine area of Pyungchang, middle eastern area of Korea.

Making of mixedly infected plants. Same volumes of leaves of oriental cabbage 'Chunhawang' infected respectively with RMV and TuMV were homogenized with mortar and pestle in 4 vol. of 0.01M sodium phosphate buffer, pH 7.0. The two viruses were inoculated to healthy 'Chunhawang' grown to be 3 or 4-leaf stages with wooden towel dipped into inoculum mixture after dusting carborundum 600 mesh. After showing virus disease symptoms of necrotic spots on leaves and midrib necrotic streaks, the tissues were used as embedding materials for electron microscopy.

Electron microscopy. Tissues for electron microscopy were embedded in epon resin by the normal procedures. The infected tissues were cut into about 1 × 3 mm and then fixed in 2.5% glutaraldehyde overnight. The fixed tissues were post-fixed with 2% osmium tetroxide after washing with 0.05 M Millonig's phos-

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phate buffer. The double fixed tissues were soaked overnight in 1% uranyl acetate after washing with distilled water. Dehydration of the tissues with an ethyl alcohol series was proceeded through 50% to 100% in 6 steps for 20 min, respectively. The epon resin-embedded infected tissues was hardened at 70°C and 100°C for 120 min and 60 min, orderly. The ultra-thin sections were stained with 2% uranyl acetate and 0.5% lead citrate for 2 min and 5 min, respectively.

Results

Single infection of RMV-Ca1dn2. In mesophyll cells of oriental cabbage 'Chunhawang', virus particles of RMV-Ca1dn2 presented as compacted masses, stacked-band structures or scattered in cytosols and vacuoles. The virus particles were also distributed as a bundle shape and scattered in phloem cells, furthermore, vessels of xylem were compacted with RMV particles (Fig. 1).

Single infection of TuMV-Act2-4vq. General ultrastructures of oriental cabbage 'Chunhawang' infected with TuMV Act2-4vq isolate, were shown as a mass of virus particles and typical inclusions of potyvirus. Virus particles were aggregated like threads on a bobbin in cytoplasm of mesophyll cells. TuMV particles were arrayed on the tonoplast and located near or inner part of inclusions. The laminated aggregates of straight long and short curve could be seen easily. The other inclusions of scrolls, tubes and pinwheels were mainly located in cytoplasm (Fig. 2).

Mixed infection of RMV and TuMV. The cells infected mixedly with TuMV-Act2-4vq and RMV-Ca1dn2 isolates, had each virus particles and potyvirus inclusions. In cytoplasm of oriental cabbage 'Chunhawang', the virus particles of RMV and TuMV were scattered mixedly and the specific feature was that RMV particles were located at inner part of scroll potyvirus inclusions (Fig. 3). In vascular

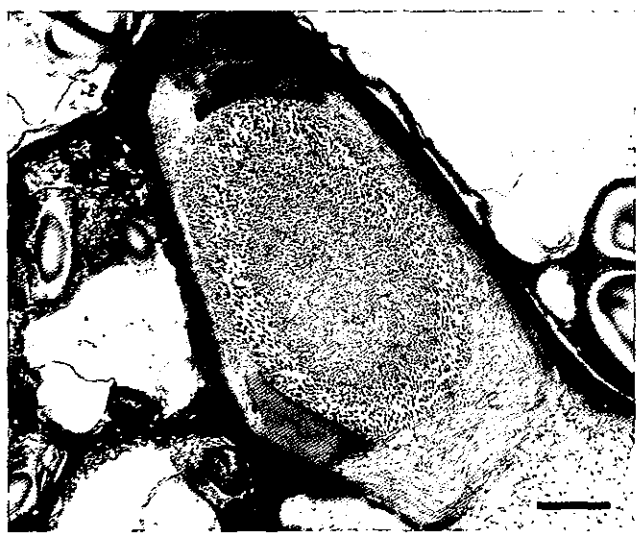

Fig. 1. Large cluster of virus particles was shown in xylem vessel of oriental cabbage 'Chunhawang' infected singly with *Ribgrass mosaic virus*. The virus particles were sectioned crossly and obliquely. Bar=250 nm.

Fig. 2. *Turnip mosaic virus* produced typically the inclusions of laminated aggregates (LA) and cylindrical tubes (CT) in cytosols. Bar=200 nm.

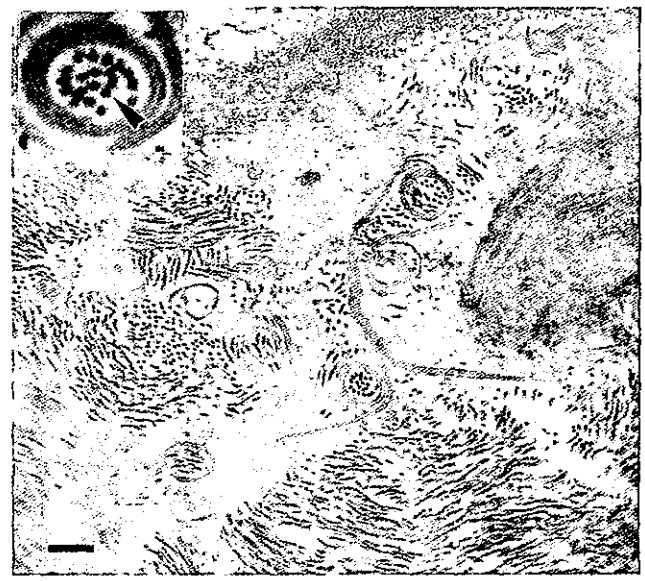

Fig. 3. In mixed infection of *Turnip mosaic virus* and *Ribgrass mosaic virus*, the inclusions of potyvirus and tobamovirus particles were presented mixedly in mesophyll cells of oriental cabbage. Furthermore, filling-up of the tobamovirus particles in the inner part of scroll potyvirus inclusions (arrow head) was striking. Bar=250 nm.

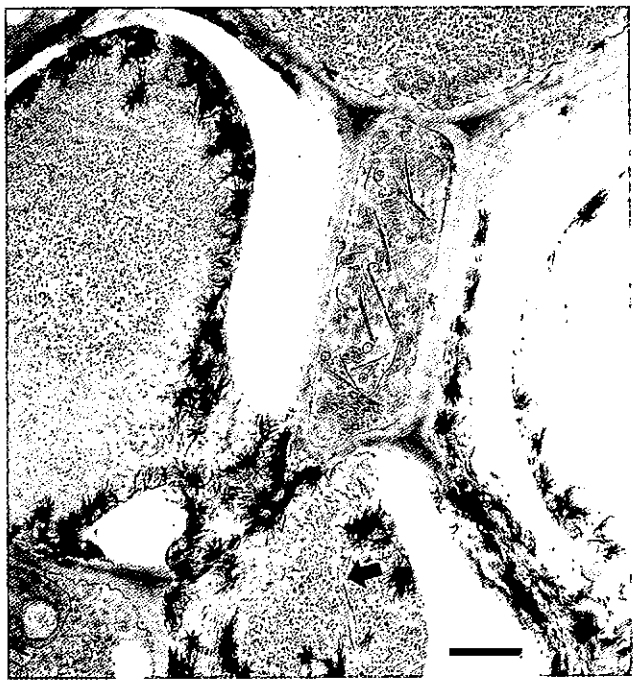

Fig. 4. Xylem vessels sectioned crossly were filled up the virus particles of *Ribgrass mosaic virus* and potyvirus inclusions (arrow) were embedded. Bar=1,000 nm.

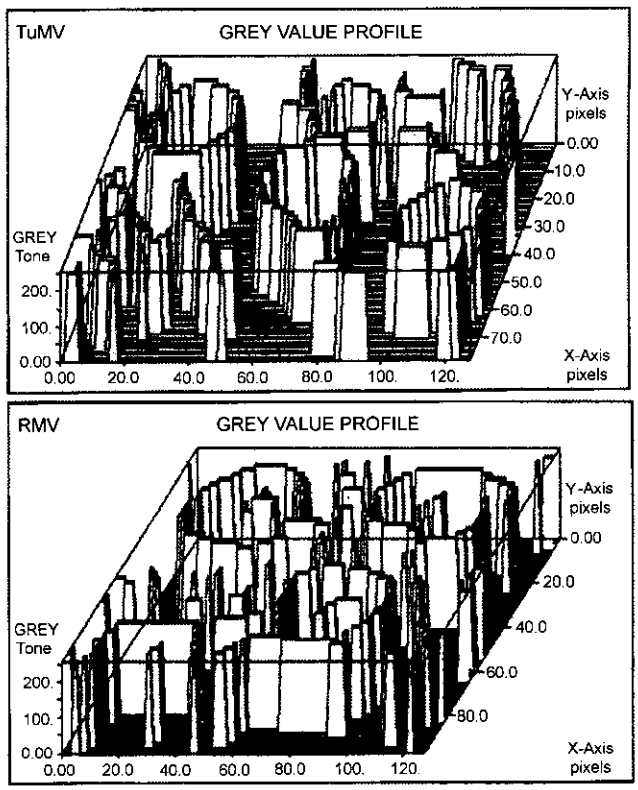

Fig. 5. Grayvalue profiles of virus particles in ultra-thin sections scanned by image analyzer, IBAS 2.0. The virus particles of *Turnip mosaic virus* (TuMV) were shown single-layered tubes, however, those of *Ribgrass mosaic virus* (RMV) had a central canal each virus particle showing two-layered tubes.

bundles, RMV particles presented abundantly in sieve tubes and xylem vessels (Fig. 4), and the typical potyvirus inclusions of TuMV were also located in the inner part of the mass of RMV particles in the vascular elements (arrow). Generally, vascular tissues were degraded severely when the two viruses infected mixedly.

Greyvalue profile. Virus particles of TuMV and RMV were analyzed by an IBAS 2.0 image analyzer. The cross-sectioned virus particles of RMV had the typical central canal, but those of TuMV were not shown the central canal (Fig. 5). The analysis of cross-sectioned virus particles in the cells infected mixedly with TuMV and RMV gave the same morphologies with vertically sectioned virus particles of TuMV and RMV.

Discussion

Virus diseases were common in crops and generally occurred by one or more viruses. Oriental cabbages in field were infected mixedly with *Turnip mosaic virus* (TuMV) and *Ribgrass mosaic virus* (RMV) (Kim et al., 1994). TuMV is a member of the genus *Potyvirus*, and induces the specific cytoplasmic inclusions which are one of the indicators for the diagnosis of potyvirus infection in cells (Edwardson et al., 1978; 1984). The shapes of cytoplasmic inclusions (CI) are laminated aggregates, scrolls, pinwheels and so forth. Potyvirus inclusions were not known exactly about their cytopathic roles, however, they are involved in replication and translocation of virion's nucleic acid and protein components (Andrews and Shalla, 1974). RMV, a member of the genus *Tobamovirus* reported in oriental cabbage in Korea (Kim et al., 1993), located as banded aggregates and scattered in cytoplasms and vacuoles of all type cells, and it made a specific ultrastructure of spiral aggregate (Francki et al., 1985). However, the typical spiral aggregate could not be seen in cells of oriental cabbage infected only with Ca1dn2 isolate of RMV.

The both unrelated viruses of TuMV and RMV produced severe external symptoms and could be multiplied in a same cell doing simultaneous replication. The synergistic effects by the double infection of unrelated viruses were reported in cowpea stunt disease caused by mixed infection of *Blackeye cowpea mosaic virus* (BlCMV) and CMV in USA (Anderson et al., 1994; Pio-Ribeiro et al., 1978). The two unrelated viruses made specific arrangement of octagon; one isometric CMV particle and 8 filamentous BlCMV particles. In combination of TuMV-Act2-4vq and RMV-Ca1dn2, no specific combination occurred by the mixed infection of the two different viruses.

The occurrence of synergism in mixed infection of viral disease was also explained by translocation of virus particles in plant. In double infection of *Bean golden mosaic virus* (BGMV) and *Tobacco mosaic virus* (TMV) in bean, BGMV isolated in phloem tissues could invade the non-phloem tissues such as mesophyll and epidermal tissues by TMV translocation (Carr and Kim, 1983). TuMV located generally in phloem, mesophyll and epidermal cells but scarcely in vessels of xylem in single infection. However, TuMV particles in oriental cabbage infected with the two unrelated viruses presented mixedly with RMV particles in potyvirus inclusions in this experiment. It might involve the inducing of severe symptom as midrib necrotic streak and whole necrosis. In cells of oriental cabbage infected doubly with TuMV and RMV, the virus particles of RMV and TuMV might be involved in the replication of the both viruses (Matthews 1991). Tissue localization by the interaction between host and pathogen being host specificity was lost by the mixed infection (Gill and Ching, 1981; Carr and Kim, 1983). The intra-mechanisms of virus replication, localization, cytopathic effects and metabolic changes by mixed infections are still unknown with the degrading of resistance to virus diseases.

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