Ultrastructural Aspects of Citrus Infected with *Citrus yellow mosaic virus*

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ABSTRACT. Citrus yellow mosaic is caused by a badnavirus, *Citrus yellow mosaic virus* (CYMV). The causal virion is a bacilliform particle measuring approximately 130 x 30 nm. CYMV-infected sweet orange leaf and bark tissues were prepared for transmission electron microscopy and viewed. Aggregates of virions were previously found free in the cytoplasm of cells. Some unusual structures which may be inclusion bodies or viroplasms were found. Comparisons are made with the ultrastructure reported for *Cauliflower mosaic virus* (CaMV), the type member of the *Caulimoviridae*, and *Cacao swollen shoot virus* (CSSV), a badnavirus that is molecularly similar to CYMV.

Citrus yellow mosaic virus (CYMV) is a proposed member of the newly established family *Caulimoviridae* and the genus *Badnavirus* (11). This is based on its serological relationship with other badnaviruses and PCR amplification using degenerate primers designed from the conserved badnavirus sequences (2). The virus was first described in India in 1975 (5) and was studied by Ahlawat et al. (1, 2). It is widely distributed and found throughout India in sweet orange and pummelo (1) causing symptoms of a yellow mosaic of the leaves and a yellow flecking along the veins. Affected trees produce less fruit and the fruit from infected trees produces less juice and ascorbic acid. In severe cases the infected trees are often abandoned due to their loss in productivity. Ahlawat et al. (2) reported a partial characterization of CYMV in 1996. The virus was graft and dodder transmitted to 14 citrus species and cultivars.

Badnaviruses are characterized by non-enveloped bacilliform particles of 30 × 130-150 nm that contain a circular double-stranded DNA genome of 7.1-7.6 kb. The genomes of six badnaviruses including CYMV have been cloned and sequenced. Huang and Hartung (7) analyzed the nucleotide and deduced amino acid sequences of CYMV and found it to be most closely related to *Cacao swollen shoot virus* (CSSV).

Little is known about the cytopathology of badnaviruses. Electron microscopic studies of plants infected with *Rice tungro bacilliform virus* (RTBV), the type member of the RTBV-like group of the *Caulimoviridae*, have shown accumulation of virus in the cytoplasm of companion cells (12) and of a some xylem parenchyma cells (4) but no association has been found with inclusion bodies or viroplasms (4, 6). This is different from *Cauliflower mosaic virus* (CaMV), the type member and most studied of the *Caulimoviridae*, which is found in almost all cell types and within characteristic cytoplasmic viroplasms (3, 10, 13). CSSV, a badnavirus, occurs as aggregates in the cytoplasm but also has not been associated with structures such as inclusion bodies, viroplasms or membranes (8). Ahlawat et al. (2) showed the presence of CYMV as aggregations of virus particles in cells of infected sweet orange but did not find any inclusions or viroplasms. A study was initiated to determine more information on the ultrastructure of CYMV in infected citrus sweet orange tissues.

Virus Isolate. The virus isolate used in these studies is isolate B175 from the Beltsville Exotic Virus collection, USDA, ARS, Fruit Lab, Beltsville, MD. This isolate was graft transmitted from materials sent to the collection by K. Manjunath from

a naturally infected sweet orange tree in Andhra Pradesh, India.

Tissue fixation and preparation. Leaf and bark (phloem) tissues were excised from CYMV and healthy sweet orange plants. The tissue was cut into 2 × 2 mm pieces and immediately fixed in 3% glutaraldehyde in 0.066 M phosphate buffer, pH 6.8 for 12 h at 4°C. The tissue was then washed in the same phosphate buffer and post-fixed in 2% osmium tetroxide in 0.066 M phosphate buffer, pH 6.8 for 12 h at 4°C. After washing in buffer, the samples were dehydrated in an acetone series and embedded in Spurr's resin. Thick (0.75 µm) and ultrathin (60 nm) sections were prepared for light and electron microscopy.

Since it was previously reported that CYMV particles occurred throughout the cytoplasm of the cells, ultrathin tissue sections of infected leaf tissues were viewed first. In CYMV infected leaves, some free aggregates of bacilliform virions were found (Fig. 1), however in some of the same cells, large inclusions or viroplasms were also found (Fig. 2A, 2B). The inclusions appeared as a dark staining mass of materials which appeared to contained the bacilliform virions as described for CYMV. No inclusions or viroplasms were seen in the nuclei of the cells or in the chloroplasts. The nuclei and nucleoli appeared to be normal (Fig. 2A). No virions were found associated with these structures.

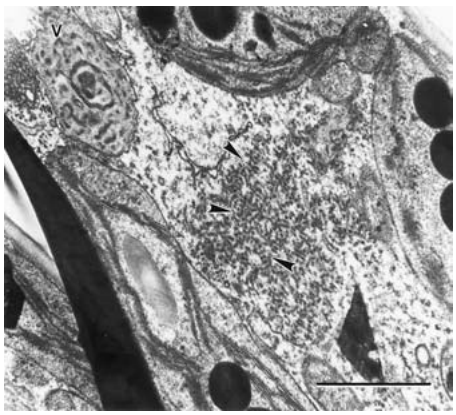

Fig. 1. Free *Citrus yellow mosaic virus* virions in infected citrus leaf tissue. Note large unusual body in upper left corner of micrograph. Scale marker = 1.0 µm.

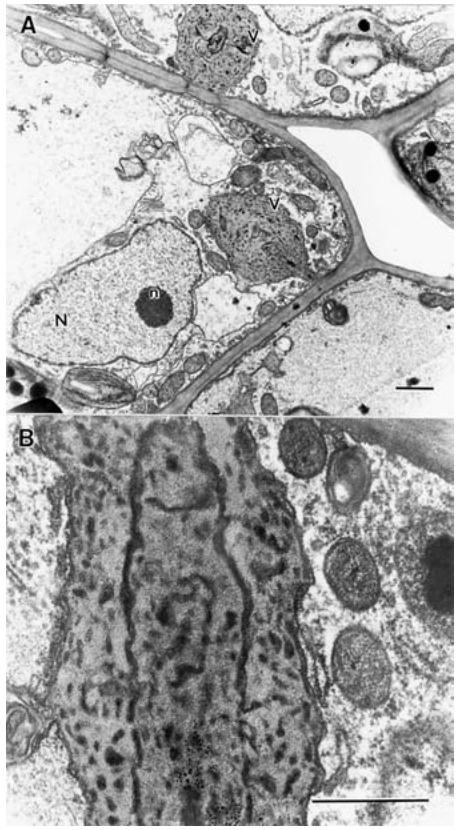

Fig. 2. Viroplasms in *Citrus yellow mosaic virus*-infected citrus leaf cells. 2A – Two adjacent cells containing viroplasms (inclusions) (V). The nucleus (N) and nucleolus (n) are clearly seen in the lower cell along with the viroplasm (V). Another viroplasm is seen in the upper cell. Scale marker = 1.0 µm. 2B – High magnification of a viroplasm. Scale marker = 1.0 µm.

Caulimoviruses and badnaviruses are similar, however they differ greatly in their genome size, particle shape, vectors and histopathology. CaMV is one of the most widely studied dsDNA viruses. CaMV induces characteristic viroplasms in the cytoplasm of infected plant cells (3, 10, 13). These viroplasms are sites of viral DNA synthesis and for the assembly of

virions. The viral coat protein may be confined to them as is much of the virus (14). Viroplasms are usually spherical and not membrane bound and have a fine granular matrix with some electron clear areas (3, 13). Virions are usually scattered throughout the viroplasm or are in irregular clusters. One strain of CaMV was found to induce viroplasms in chloroplasts but these structures did not contain virus (13).

Huang and Hartung (7) found that CYMV is molecularly similar to CSSV. CSSV has been found to occur as virus aggregates in leaves and petioles and in a few xylem parenchyma cells but was not associated with inclusion bodies or viroplasms like CaMV (8). RBTV, in another genus of the *Caulimoviridae*, occurs as single particles or aggregates of aligned particles in the cytoplasm of infected cells (4). No inclusion bodies have been associated with RBTV when plants were co-infected with *Rice tungro spherical virus*. There was no association with chloroplasts, nuclei or cell membranes.

Ahlawat et al. (2) found in ultrathin sections of CYMV, a large number of bacilliform particles in the cytoplasm of infected Mosambi sweet orange leaf tissues but not in the nuclei of infected cells like that found with Citrus leprosis virus (9). The virions were seen in loose packets in the cytoplasm. In our work reported here, we show that CYMV occurs as free virus aggregates and produces viroplasms or inclusions in the cytoplasm of infected cells. The inclusions or viroplasms are similar to described for CaMV, however there appears to be only matrix and no clear areas in the inclusions. It appears that virions are present in the viroplasms. These bodies also are similar to those found with other badnaviruses, but different from CSSV that is found only as aggregates of free virus in phloem tissues. A more extensive study is needed using labeled antibody and PCR techniques to determine the location of CYMV throughout infected plants and in the viroplasms.

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