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In Situ Localization of Plant Viral Genes and Gene Products in Infected Cells

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In situ hybridization and immuno cytochemical staining have been the powerful tools for localization of specific nucleic acids and proteins in tissue sections for the studies of cell biology, development and genetics. In this study, bamboo mosaic virus (BaMV) was used as a model virus to demonstrate ultrastructural locations and movement of BaMV RNA and the proteins it encoded in infected cells. BaMV has a single-stranded, plus-sense RNA genome with five conserved open reading frames (ORFs). Young leaves of green bamboo (Bambusa oldhamii) showing systemic mosaic symptom were harvested for electron microscopy. Leaf tissue pieces were fixed with glutaraldehyde and embedded in Lowicryl HM20. Immuno- and *in situ* hybridization electron microscopy was performed. BaMV capsid protein and masses of virions were detected in the cytoplasm and vacuoles of infected cells by antiserum to BaMV capsid protein followed by gold-labelled goat anti-rabbit IgG complexes. At the early stage of infection, BaMV capsid protein was also detected within the nuclei, especially in the nucleoplasm of the rolled young leaf samples (Fig. 1). However, the electron-dense crystalline bodies (EDCBs) were not labelled with antiserum to BaMV capsid protein. On the other hand, EDCBs were detected by using anti-ORF2 serum in the cytoplasm as well as in the nuclei (Fig. 2) whereas the BaMV virions were not labelled. In addition, digoxigenin (Dig)-labelled riboprobe, corresponding to the 173 nts at the 3' end of BaMV RNA, was used to hybridize the viral nucleic acids in tissue sections. The hybrids were subsequently reacted with sheep anti-Dig antibody followed by immunogold. BaMV RNA was specifically detected within chloroplasts, motochondria and nuclei of infected cells at all stages of infection. BaMV virions and 'BaMV-specific EDCBs were also labelled (Fig. 3).

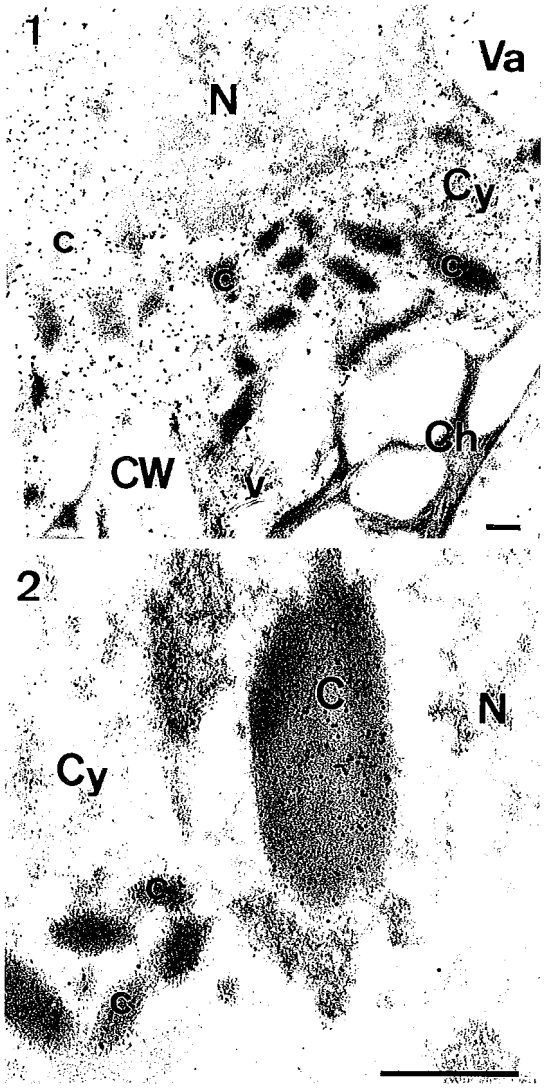

Fig. 1-2. Immunological detection of BaMV capsid protein and 28 kDa protein in glutaraldehyde-fixed, Lowicryl HM20-embedded and BaMV-infected green bamboo leaves. Thin sections were first stained with dilute anti-BaMV-CP serum (1) or anti-ORF2 serum (2), followed by gold-labelled goat anti-rabbit IgG complexes.

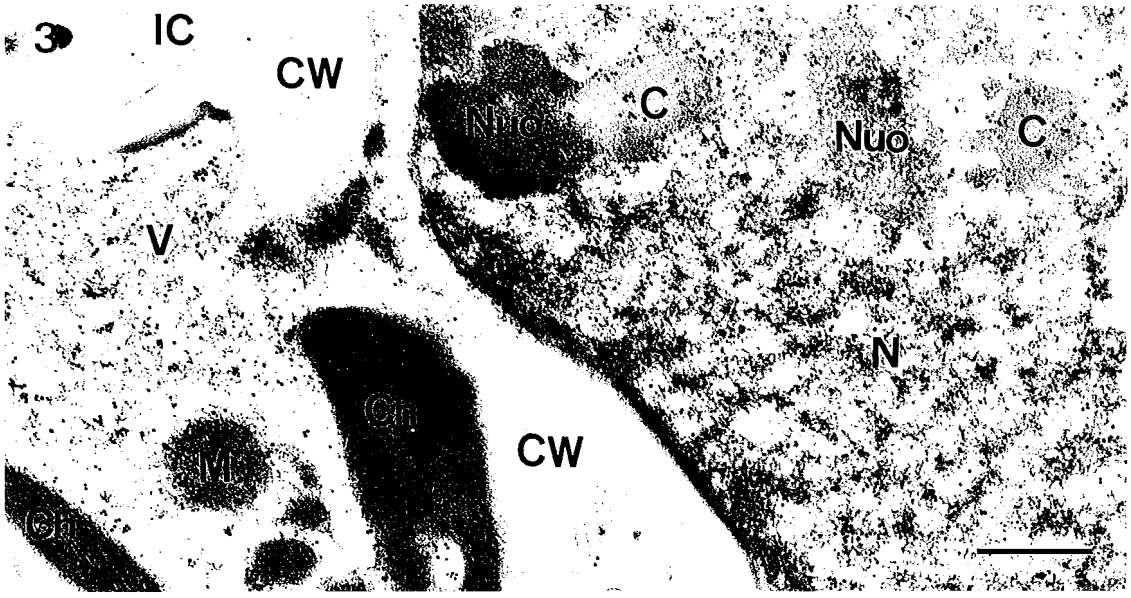

Fig. 3. *In situ* hybridization of BaMV RNA in BaMV-infected green bamboo leaves. Thin sections were hybridized with Dig-labelled riboprobe and detected with immunogolds.
C, electron-dense crystalline bodies; Ch, chloroplast; CW, cell wall; Cy, cytoplasm; IC, invtercellular space; M, mitochondria; N, nucleus; Nuo, nuleolus; Va, vacuole; V, virion. Bars = 500 nm.

The *in situ* localization of BaMV RNA and BaMV-encoded proteins provide important information about BaMV pathogenesis and gene expression.

REFERENCES

Lin NS, FZ Lin, TY Huang, YH Hsu. 1992. Phytopathology **82:** 731-735.

Lin NS, BY Lin, NW Lo, CC Hu, TY Chow, YH Hsu. 1994. J. Gen. Virol. **75:** 2513-2518.

Lin NS, CC Chen. 1991. Phytopathology **81:** 1551-1555.

Lin NS, CC Chen, YH Hsu. 1993. J. Histochem. Cytochem. **41:** 1513-1519.