Evidence that Bean Golden Mosaic Virus Invades Non-phloem Tissue in Double Infections with Tobacco Mosaic Virus

By R. J. CARR and K. S. KIM*

Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701, U.S.A.

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SUMMARY

Bean leaves that had been doubly infected systemically with the legume strain of tobacco mosaic virus (CP-TMV) and bean golden mosaic virus (BGMV) were studied ultrastructurally. Virus particles and the cytopathological changes associated with each virus in single infections occurred within the same cell when plants were doubly infected, indicating that individual systemically infected host cells can multiply viruses of different nucleic acid composition. Although the cytopathic effects induced by BGMV are limited to phloem-associated cells in singly infected plants, the same effects were found in most leaf cell types in mixedly infected plants.

Mixed infections by plant viruses are common but have been relatively little studied (Matthews, 1981). Thus, information concerning the ultrastructure of mixed virus infections is sparse, although recent reports by Gill & Chong (1981) and Carr & Kim (1983), indicate that ultrastructural features in mixedly infected cells may be useful in discriminating related viruses or strains of the same virus.

This paper describes cytopathological effects occurring in leaf cells infected with the legume strain of tobacco mosaic virus (CP-TMV), an RNA virus with rod-shaped particles, and bean golden mosaic virus (BGMV), an isometric, DNA-containing geminivirus. The results suggest that BGMV infects cell types in doubly infected tissue that are not infected in singly infected leaves.

Bean plants (Phaseolus vulgaris L. cv. Cherokee Wax) were cultivated in a growth chamber in subdued light for a 16 h day and at a constant 32 °C, and 5- to 7-day-old bean plants were inoculated mechanically using sap from infected beans. Mixed inocula contained equal amounts of sap from plants infected with each virus. Inoculating either virus as part of a mixture did not appear to affect the efficiency of inducing mixed infections. Tissue samples were taken from infected trifoliate leaves 15 to 20 days after inoculation and prepared for electron microscopy as described previously (Carr & Kim, 1983).

Infection of bean with CP-TMV either singly or in mixed infection with BGMV resulted in the production of massive arrays of virus particles that were aligned along cell walls (Fig. 1 a) or protruded into the vacuoles of all leaf parenchyma cells. Particles of CP-TMV were also present in the chloroplasts and nuclei of infected cells (Fig. 1 a, d). The occurrence of CP-TMV particles in the nuclei of cells infected singly with CP-TMV, as well as in mixed infection with BGMV (see below), distinguishes this strain from the common strain of TMV (Honda & Matsui, 1969). Particles of the common strain of TMV occurred in the nuclei of tobacco leaf cells only if they were also infected with cucumber mosaic virus (Honda & Matsui, 1969).

The most consistently observed ultrastructural effects of BGMV infection were nuclear hypertrophy, segregation of the nucleolar fibrillar and granular regions, formation of intranuclear fibrillar rings (Fig. 1 and 2), and the appearance of small (18 to 20 nm) isometric virus-like particles (Fig. 1 b and 2a) in the nuclei of phloem parenchyma cells. These effects were the same as those previously described in P. vulgaris cv. Top Crop by Kim et al. (1978) except that there was an accumulation of what appeared to be perichromatin and interchromatin granules in the nuclei of infected P. vulgaris cv. Cherokee Wax leaf cells (Fig. 1 b, c). These granules were similar in appearance to granules described by Daskal (1981), and to
Fig. 1. Cytopathic effects of mixed infection with CP-TMV and BGMV. (a) A portion of a leaf mesophyll cell showing an intranuclear fibrillar ring (arrow) induced by BGMV, as well as clusters of CP-TMV particles in the cytoplasm and the chloroplasts (arrowheads). (b) Section through a mesophyll cell nucleus (N) showing fibrillar rings (arrowheads), virus-like particles (circled area; see also Fig. 2a) and a cluster of perichromatin granules (arrow) associated with BGMV infection, as well as CP-TMV particles (squared area). (c) Enlargement of perichromatin granules (arrowheads) as indicated by an arrow in (b). (d) Enlargement of the squared area in (b) showing CP-TMV particles (arrowheads). Bar markers represent 1 μm in (a, b) and 100 nm in (c, d).
perichromatin granules found in *Datura stramonium* cells infected by another geminivirus, the Florida isolate of Euphorbia mosaic virus (Kim & Martin, 1982).

Cells infected with both CP-TMV and BGMV contained particles and/or inclusions of both viruses (Fig. 1 and 2). Generally, young leaves contained a larger proportion of mixedly infected cells than did older leaves. Virus-like particles and inclusions associated with BGMV were always confined to the nucleus, whereas particles of CP-TMV were observed in the cytoplasm, nuclei and chloroplasts (Fig. 1a, b, d).

Previously, BGMV (Kim et al., 1978) and several other geminiviruses (Esau & Hoefert, 1973; Goodman, 1981) have been found to infect only phloem tissue cells. However, intranuclear effects induced by BGMV, such as fibrillar bodies, and virus-like particles were found in most cell types in plants infected with both BGMV and CP-TMV (Fig. 1 and 2). In some cells, characteristic inclusions and/or particles of both viruses were located within the same nucleus (Fig. 1b, d and 2a). The occurrence of these structures within the same cell shows that one cell can harbour and probably assemble viruses having genomes composed of different types of nucleic acid. Kamei et al. (1969) located particles of turnip mosaic virus (an RNA virus) and cauliflower mosaic virus (a DNA virus) within the same cell in inoculated *Brassica perviridis* leaves. The present study confirms the ability of single cells to harbour RNA and DNA viruses in systemic as well as local infections.

One of the major problems in studying phloem-limited viruses is the difficulty of purifying them (Takanami & Kubo, 1979), and low virus titre contributes to this difficulty. Because most cell types contain BGMV in mixedly infected plants, double infection with CP-TMV may be a way of increasing the concentration of BGMV in a propagation host. If a similar loss of host tissue specificity occurs with other phloem-limited viruses in mixed infections, it may aid in the study of these viruses.

Loss of virus host-tissue specificity as a result of mixed infection has been previously reported for two isolates of barley yellow dwarf virus (BYDV) (Gill & Chong, 1981); virus that was restricted to phloem in single infections was found in xylem tissue when plants were mixedly infected with the two isolates. However, we found BGMV-induced ultrastructural effects to be much more widespread in leaf cells than were BYDV particles in mixed infections. Perhaps the large degree of unrelatedness between CP-TMV and BGMV led to the total loss of the tissue specificity of BGMV observed here, whereas the relatively limited loss of tissue specificity in mixed infection by BYDV isolates was due to the similarity of isolates of a single virus.
Recently, it has been shown that tomatoes resistant to TMV became susceptible if the plants had been pre-infected with the unrelated potato virus X (PVX) as helper virus (Taliansky et al., 1982). It was concluded that PVX was facilitating the transport of TMV between tomato cells, and it is possible that in our study CP-TMV acted similarly as a helper virus to allow BGMV to move from phloem to other cell types such as mesophyll and epidermal cells.

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REFERENCES


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