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Cytological Modification of Sorghum Leaf Tissues Showing the Early Acute Response to Maize Dwarf Mosaic Virus

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Sorghum leaf tissues showing the early acute response to systemic infection with maize dwarf mosaic virus (MDMV) strain A, contained unusual virus-induced cytological modifications including cell wall thickenings and protrusions, intercellular vesicles termed as "paramural bodies", modified plasmodesmata, abnormal plastids, and cylindrical inclusion bodies. Abnormal cell wall, some of which associated with paramural bodies, was frequently contained modified plasmodesmata. Various abnormal plastids were located within infected cells of leaf tissues showing the early acute response. The most important changes in chloroplast seen in the tissues are the presence of small vesicles, deformation of membranes, reduction in granal stack height, disappearance of osmiophilic globules and degeneration of stuctures. The cytological modification was not occurred in nucleus but a group of degenerated mitochondria with abnormal membranes attached to cylindrical inclusion bodies were observed. It was hard more or less to prove the relationship clearly between virus and cellular organelles in virus replication.

Keywords: early acute response, maize dwarf mosaic virus (MDMV), abnormal cell wall, modified plasmodesmata, abnormal plastid

Disease symptoms on sorghum leaves induced by maize dwarf mosaic virus (MDMV), depend upon the strain of virus involved, sorghum genotype, and environmental factors (Jarjees and Uyemoto, 1983; Seifers, 1984; Antignus, 1987). Some sorghum cultivars produced symptoms by early acute and late chronic responses after mechanical inoculation with MDMV-A strain. The early acute response consisted of short chlorotic spots on the youngest leaves in the terminal whorl of inoculated sorghum plants and usually appeared within 5 days after inoculation, which became necrotic 1-2 days later. The infected plants then developed the late chronic response showing typical systemic mosaic symptoms (Choi *et al.*, 1986).

In cells of MDMV infected sorghum plants showing the early acute response, the modifications were frequently occurred in the cell wall and cellular organelles. Cell wall abnormalities including wall thickenings and protrusions appeared as physical barriers to cell-to-cell spread of virus not only in cells surrounding necrotic local lesions of hosts (Tu and Hiruki, 1971; Allison and Shalla, 1974), but also those in systemically infected leaf tissues (Kim and Fulton, 1973; McMullen *et al.*, 1977; McMullen and Gardner, 1980). In several virus-host interactions, these abnormalities with intercellular vesicles have been frequently associated with an extension of plasmodesmata between the cell wall and plasmalemma (Bassi *et al.*, 1974; McMullen *et al.*, 1978; McMullen and Gardner, 1980). Although the vesicular formations between the cell wall and plasmalemma, termed "paramural bodies" (Marchant and Robards, 1968), have been often found in healthy plant tissue, an increase in their size or number associated with viral infection was common. Plasmodesmata are morphologically intercellular connections between neighboring cells, and they are involved in intercellular transport, cell-to-cell communication, cell differentiation, and plant growth and development (Gunning and Robards, 1976; Robards and Lucas, 1990; Lucas *et al.*, 1993). The extension through the plasma membrane-lined channel within plasmodesma is a desmotubule, the modified extension of endoplasmic reticulum, which is known

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as a major pathway for cell-to-cell spread of plant viruses (Hull, 1989; Deom *et al.*, 1992).

The cytopathological effects by some viruses give rise to intranuclear inclusions of various kinds with or without masses of virus particles in nucleus, cause vesiculation in perinuclear space, or modify the size and shape of nucleus (De Zoeten *et al.*, 1972; Rushing *et al.*, 1987). The development of abnormal membranes in mitochondria has been described in several virus infections, but no relation has been established to virus replication (Francki, 1987). Several viruses induce small vesicles near the periphery of chloroplasts which differ from those of the tymovirus-induced type in that they do not appear to have necks connecting them to the cytoplasm. Even though the vesicles or other changes induced by barley stripe mosaic virus (BSMV) or turnip yellow mosaic virus (TYMV) appear to be closely related with the virus replication, those induced by many other viruses appear to be degenerative consequences of infection (Hatta and Matthews, 1975; Lin and Langenberg, 1985).

The purpose of this paper is to investigate the cytological modifications including cell wall abnormalities, paramural bodies, modifed plasmodesmata, degenerated mitochondria, and abnormal plastids in sorghum leaf tissues showing the early acute response by MDMV-A strain.

MATERIALS AND METHODS

MDMV strain A was maintained in johnsongrass grown in a steam-sterilized mixture of soil : sand : peat (2 : 1 : 1) under a temperature controlled greenhouse. The inoculum sources were prepared by grinding the infected leaves with a mortar and pestle in the deionized water (1 : 5, w/v). Both surfaces of the emerging third and fourth leaves of sorghum cultivar HOK at the fourth-leaf stage were rubbed by finger-wipe method with carborundum. Mock inoculation to plant as a control was made by rubbing buffer onto carborundum-dusted leaves. Infected leaf tissues showing the early acute response were harvested within 5 days after inoculation and prepared for electron microscopy as described previously (Choi and Gardner, 1994).

RESULTS

Cell Wall Abnormalities

The most distinctive ultrastructural modifications were induced at the interface between the cell wall and plasmalemma within 5 days after inoculation with MDMV-A in HOK sorghum leaves, however no modifications and symptoms were observed in the mock-inoculated control plant. Examination of leaf tissues showing the early acute response revealed cell wall abnormalities, wall thickenings (Figs. 1, 2, 3, 4, 5) and protrusions of the cell wall into the cytoplasm (Figs. 6, 7, 8). The cell wall thickenings due to the deposition of additional materials occurred, particularly in proximity to plasmodesmata (Figs. 1, 2, 3, 4, 5). These deposits have been characterized as callose to restrict virus spread from cell to cell, which usually appeared in leaf tissue showing necrotic local lesions by virus infections (Allison and Shalla, 1974). The protrusions often appeared to be finger-like projection (Figs. 7, 8).

There were various paramural bodies containing an extensive accumulation of electron-dense membranous vesicles and/or tubules between the plasmalemma and cell wall, which appeared to be derived from the contents of plasmodesmata (Figs. 1, 4). A central cavity at the middle of the cell wall (Fig. 2) is a structure for multiple branches of plasmodesmata to be united as described previously (Ding *et al.*, 1992). The modifications of plasmodesmata were induced by extension of their size and/or length (Figs. 2, 4, 5). Extended plasmodesmata were electron-dense, membrane-bound, terminated in bul-

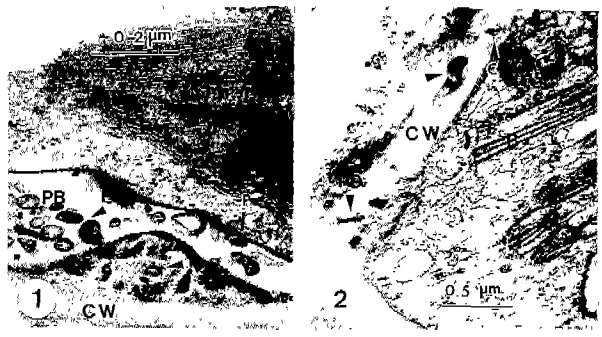

Fig. 1. Paramural bodies and flattened membranous vesicles (unlabeled arrowhead) associated with extended plasmodesmata, are present together and cause wall thickenings between the plasmalemma and cell wall of HOK sorghum leaf tissues showing the early acute response infected with MDMV-A. PB, paramural bodies; EP, extended plasmodesmata; CW, cell wall; P, plasmalemma.
Fig. 2. Modified plasmodesmata (PD) by extension of bulbous terminals or size (unlabeled arrowhead) in HOK sorghum leaf infected with MDMV-A. Note the bundle (B) inclusions and central cavity (Ca) of plasmodesmata.

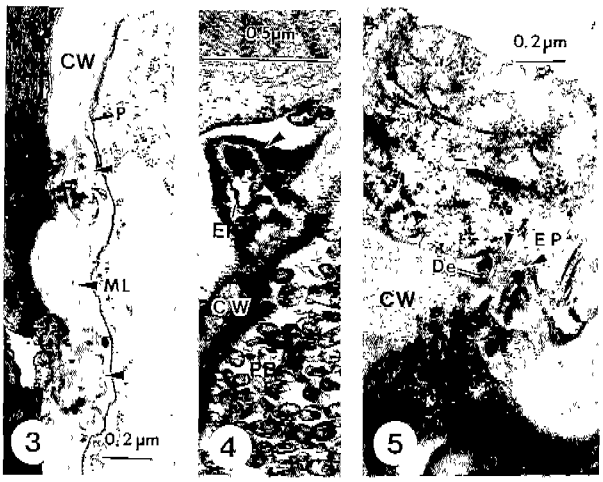

Fig. 3. Cell wall abnormalities accompanying paramural bodies at both sides of the cell wall (unlabeled arrowheads). ML, Middle lamella.

Fig. 4. A number of paramural bodies and extended membranous plasmodesmata associated with cell wall thickenings (unlabeled arrowheads), are present together between the plasmalemma and cell wall. EP, extended plasmodesmata.

Fig. 5. Electron micrograph showing cell wall thickenings (unlabeled arrow) by extended plasmodesmata terminated in bulbous protrusions and desmotubules (De) appear to traverse the canal within plasmodesmata.

bous protrusions, and contained no virus particles (Figs. 1, 4, 5). Some plasmodesmata containing desmotubules (Robards, 1971) were often found (Fig. 5).

Cell organelles

A variety of ultrastructural abnormalities have been found in chloroplasts of MDMV-infected leaf tissues showing the early acute response, some of which are disrupted or pleomorphic structures. Some cylindrical inclusions frequently attached to the outer membrane of chloroplast often clumped together (Fig. 9). Cells in infected leaf tissues frequently contained more than one type of plastid modifications, most of which were characterized by the electron density of stroma and the presence of osmiophilic globules. One of these defective chloroplasts was the most common type observed which contained a less degenerated grana and an electron-dense stroma with a number of osmiophilic globules but seems regular in shape (Fig. 9). The second type was found to have peripheral vesicles and few osmiophilic globules. This type frequently possessed small vesicles arranged in a row peripherally adjacent to

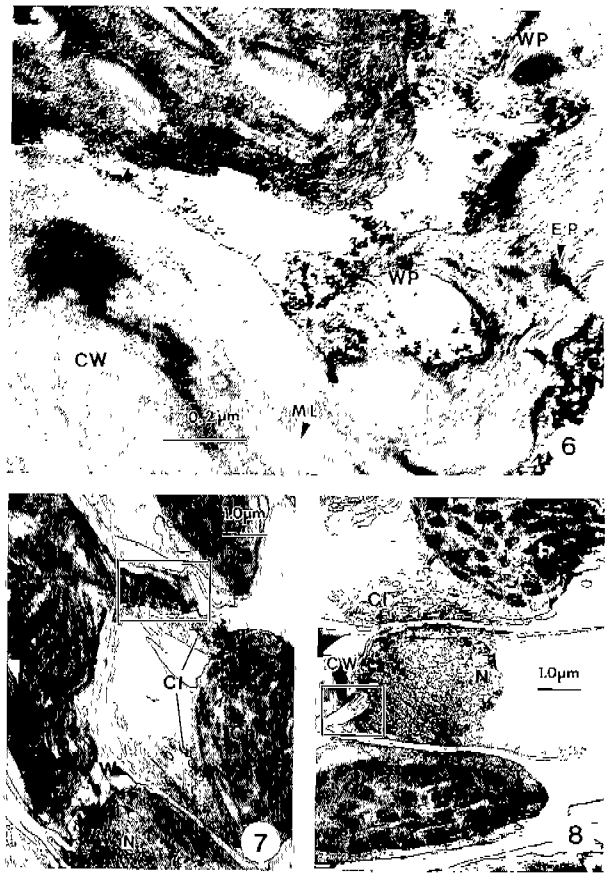

Fig. 6. Ultrastructure of cell wall protrusions (WP) involving extended plasmodesmata. ML, Middle lamella.

Fig. 7. Cell wall protrusions by wall abnormality (box) and finger-like projection into nucleus (+). CI, cylindrical inclusions; Ch, chloroplast; N, nucleus.

Fig. 8. Invagination of nucleus by finger-like protrusion of the cell wall

the chloroplast limiting membranes (Figs. 10, 11). These vesicles were bound by a single membrane with closed necks in continuity with the outer plastid membrane. Occasionally this type chloroplast was composed of twisted membranes forming tubular or vesicular networks (Fig. 14). The third type was irregular in shape, contained electron-transparent stroma caused in part by the scarcity or absence of ribosomelike particles normally found in the stroma. This type also had partially disrupted chloroplast limiting membranes, extensive intergranal lamellae and disrupted granal thylakoids (Figs. 11, 12). Some short granal stacks are formed by 2 through 6 thylakoids. The totally disrupted one characterized by missing the limiting membrane of chloroplast and the lamellar network (Fig. 13).

Compared to a normal chloroplast, all types con-

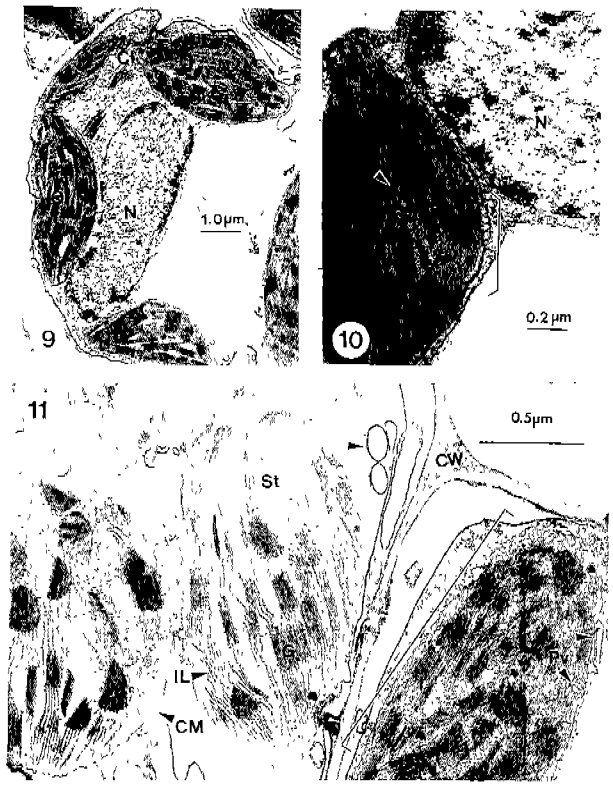

Fig. 9. Chloroplasts (Ch) in a cell of sorghum leaf showing the early acute response by MDMV-A appear to be normal in structure but contain several osmiophilic globules (O) . The cylindrical inclusions (CI) are attached to the limiting membranes of chloroplasts.

Fig. 10. Dense-stroma chloroplast with a number of peripheral vesicles but few osmiophilic globules are present in sorghum leaf tissues showing the early acute response by infection with MDMV-A. Bracket indicates the appearance of vesiculated area in a row.

Fig. 11. Electron micrograph of cells containing abnormal plastids·The cell on the left contains chloroplasts with partially disrupted limiting membranes, electron-transparent stroma, few grana, and extensive intergranal lamellae (IL). The cell on the right contains peripheral vesicles (Pv) in a row or a double row (bracket area). Some vesicles coalesce together to form large vesicles (unlabeled arrowheads). CM, chloroplast limiting membrane.

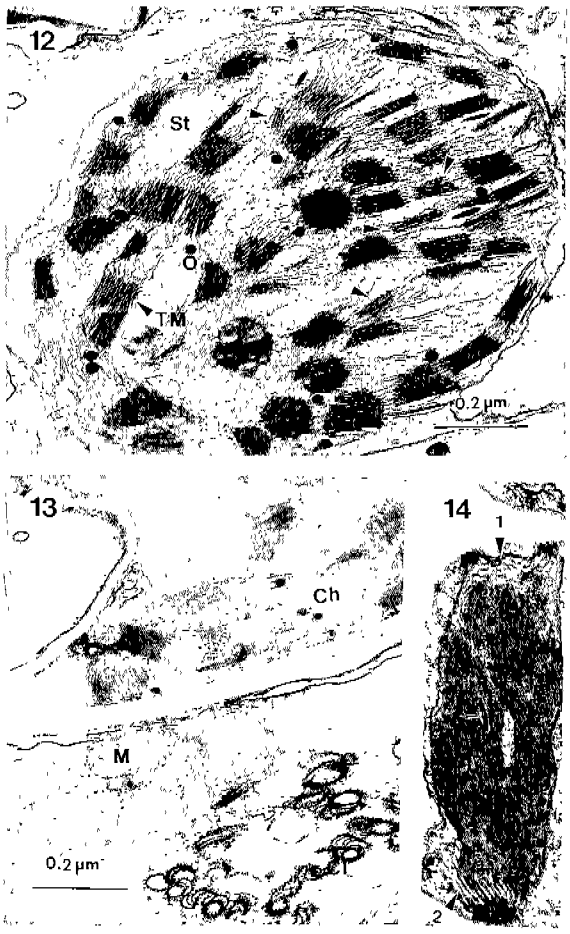

Fig. 12. An abnormal chloroplast, containing disarranged intergranal lamellae, disorganized grana and electron-transparent stroma, is surrounded by partially disrupted chloroplast limiting membranes. Some short granal stacks (unlabeled arrowheads) are formed from a few thylakoid membranes (TM).

Fig. 13. The totally disrupted chloroplast characterized by the disappearance of chloroplast limiting membranes. Some cylindrical inclusion bodies attached to the membranes of disrupted mitochondria (M) are evident.

Fig. 14. Electron-dense stroma of a chloroplast contained twisted vesicular (1) and helical array of tubular networks (2). S, starch.

tained the deformed granal stacks fused by thylakoid membranes each other and disarranged internal membranes, since thylakoid membranes undergo progressive disruption and marked disarrangements. A number of osmiophilic globules are present in less disrupted chloroplasts, however the number of osmiophilic globules decreased gradually following progressive disruption of thylakoid structures (Figs. 9, 10, 11).

Nuclei were often invaginated, but insignificantly affected in integrity, by the cell wall protrusions (Figs. 7, 8). In a group of degenerated mitochondria with abnormal membranes were often found. There were no clear evidences of virus particles or inclusions within mitochondria or nuclei except the inclusions associated with near mitochondria in infected cells (Fig. 13).

DISCUSSION

Sorghum leaf tissues showing the early acute response by MDMV infection cause various cytological modifications, most of which appear to be structural abnormalities of cell walls and organelles. These abnormalities persist at least for a time just before necrosis development which eventually kill the leaf tissues when inoculations are made to young seedlings. Presumably they are induced by interactions between cells of a monocot host and a virus at the early stage, which express the resistance at the cellular level to minimize the viral spread from cell to cell. Ironically some cells in the green portion of chronically infected leaf tissues showing mosaic symptoms, although containing viruses, do not undergo degenerative event in response to infection. Even though cells in this region support virus replication, as evidenced by the presence of virions in the cytoplasm, the cells involving cellular organells appear relatively unmodified (Gardner, 1967).

An accumulation of paramural bodies and modified plasmodesmata could be involved in the synthesis of new cell wall material and resulted in the cell wall thickenings or protrusions, because they were present together at the same site (Bassi *et al.*, 1974). Paramural bodies were speculated as exocytic transporting metabolites, as an endocytic assistance of transporting materials into cell, or as a transient response of the protoplast to unfavorable condition (Kim and Fulton, 1971), however their origin and function are still unclear.

A number of unusual structures, the modified plasmodesmata, were often contained rows of virus-like particles formed a single row or a double row (Kim and Fulton, 1973; Murant *et al.*, 1975; Weintraub *et al.*, 1976). They were similar to myelinic bodies which were associated with tubules containing virus particles (Kim *et al.*, 1974). In a cross sectional view of such virus-induced structures they were also observed but empty in this study. It has been suggested that the formation may pass through plasmodesmata and be transfered to the paramural bodies (Kim and Fulton, 1973). This hypothesis strongly supports the view that the cell wall thickenings consist of new wall material with similar structures to bulbous terminals of extended plasmodesmata and they are continuous with paramural bodies. It is obvious from the present evidences that MDMV may trigger the host cell to produce additional cell wall material for the formation of wall thickenings. Such cell wall abnormalities in systemic infection might be a transient expression to virus infection but were not effectively to inhibit the systemic spread of virus particles.

Unlike animal cells, plant cells are surrounded by cell wall, therefore, plant viruses must be more active than animal viruses in their spread from cell to cell. To carry out this, plant viruses encode movement proteins (MPs) that are essential for the infection of a host plant, but not required for the replication or encapsidation of virus (Hull, 1991). Recent molecular studies have suggested the viral form for spread as MP-associated filaments (Citovsky *et al.*, 1990, 1992; Citovsky and Zambryski, 1991). Because of the ultrastructural similarities between tobacco mosaic virus (TMV) MP-associated filaments and intact TMV particles, the viral form for spread from cell to cell is currently under speculations and arguments.

It seems just as likely that different kinds of plastids observed in a single cell could be intermediate steps in plastid disorganization. The progressive disruption of chloroplast apparently was in part responsible for the degeneration of grana and the lack of an electron-dense stroma, the formation of small peripheral vesicles, and other abnormalities in chloroplasts by MDMV infection. It appears that in leaf tissues showing the early acute response these degenerative processes continue until plastid structure is completely disorganized. It can be supported by some cells, although containing masses of virus, contain plastids with few ultrastructural abnormalities (McMullen *et al.*, 1978). Mention the peripheral vesicle formation, an interpretation was given either as an invagination of both limiting plastid membranes (Hatta and Matthews, 1974, 1975) or as an exclusion of the inner plastid membrane to form vesicular membranes in BSMV-infected cells (McMullen *et al.*, 1978). More likely the latter hypothesis is supported by the peripheral vesicle formation in MDMV-infected tissue. The definite evidences of virus/vesiculated-plastid relationship to support association with virus synthesis has been lacking in this study.

Since the normal chloroplasts are known to contain few osmiophilic globules, an increase in size and/or number of osmiophilic globules is closely related with the breakdown of thylakoids in chloroplast affected by the physical or abiotic treatments (He *et al.*, 1994; Ahn *et al.*, 1995). However, the more disrupted the integrity of thylakoids structure, the less accumulated osmiophilic globules are found in the chloroplasts of MDMV-infected cells. If the

former hypothesis is right, it is possible to interpret that osmiophilic globules are temporally increased after inoculation with MDMV. However, these were not last due to the necrosis development which is a type of hypersensitive reaction of sorghum leaf tissues following the early acute response.

The most important changes in chloroplast found in leaf tissues showing the early acute response are the presence of small vesicles, the deformation of membranes, the reduction in granal stack height, the disappearance of osmiophilic globules, and the degeneration of stuctures. The cytological modification could not be detectable in the nucleus of which size or shape seemed normal, otherwise their modification could be induced with minor by MDMV. A group of degenerated mitochondria with abnormal membranes attached to cylindrical inclusion bodies were observed, though, it was difficult more or less to prove the relationship clearly between virus and cellular organelles in virus replication.

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