Journal of Plant Biology, March 1999, 42(1) : 63-70

Modified Plasmodesmata in *Sorghum* (*Sorghum bicolor* L. Moench) Leaf Tissues Infected by Maize Dwarf Mosaic Virus

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The early acute response (EAR), a type of hypersensitive response, is defined by small chlorotic spots at the base of the youngest leaf of sorghum (*Sorghum bicolor* L. Moench) cultivar HOK, and usually appears within five days after inoculation with maize dwarf mosaic virus strain A (MDMV-A). These chlorotic spots become necrotic one to two days later and the leaf tissues are rapidly killed. In leaf tissues showing EAR, plasmodesmal fields contained many modified plasmodesmata of various sizes and structures within thickened cell walls. The membranous vesicles and tubules, derived from the extended terminal structures of modified plasmodesmata, were blocked by callose-like deposits in the area between the cell wall and plasmalemma. Also observed were two opposite-directed channels united via a central cavity at the middle lamella of the cell wall, one end of which was connected to the plasmalemma, but the other end sealed off to form a bulbous extension. The localized structure, an extraprotoplasmic sac containing aggregates of elongated virus-like particles associated with the modified plasmodesmata, was located between the plasmalemma and the cell wall. The sac was bound by membranes, and appeared to be sealed and completely excluded from the protoplasm. Extraprotoplasmic sacs appeared to be derived from the terminal extension of modified plasmodesmata, and these modification seem to be related to restriction of the viral spread.

Keywords: cell-wall abnormalities, early acute response, extraprotoplasmic sac, Maize Dwarf Mosaic Virus (MDMV), modified plasmodesmata, viral spread

Young seedlings are generally more sensitive than aged plants when inoculated with the virulent strain of a virus. When such a strain is inoculated into certain monocot hosts, symptoms are expressed as an early acute response (EAR) at the base of the youngest leaf in the terminal whorl and as a late chronic response showing systemic mosaic in the old leaves (McKinney and Greely, 1965). The EAR is possibly associated with the restriction of viral spread, but not necessarily with the restriction of viral replication. Although the potential significance of this phenomenon was first recognized about 30 years ago, the mechanisms by which EAR operates have not been resolved.

Cell-wall abnormalities accompanying modification of plasmodesmata are frequently associated with an increase in the number of paramural bodies between the cell wall and the plasmalemma in virus-infected leaf tissues (Bassi et al., 1974; McMullen et al., 1978; McMullen and Gardner, 1980; Choi 1996). Such structures often occur near plasmodesmata, complex trans-wall membranous structures, which are intercellular connections between neighboring cells. Plasmodesmata are involved in intercellular transport, cell-to-cell communication, cell differentiation, and plant growth and development (Robards and Lucas, 1990; Lucas et al., 1993). In addition, they are known to function as a major pathway for the spread of plant viruses from cell to cell (Hull, 1989; Deom et al., 1992; Fujiwara et al., 1993).

Virus-like particles (VLPs) within the plasmodesmata of virus-infected plants have been observed with an electron microscope. In previous investigations, the modified plasmodesmata were not empty but often contained VLPs aligned in a single or a double row (Kim and Fulton, 1973; Allison and Shalla, 1974; Murant et al., 1975; Weintraub et al., 1976; Robards and Lucas, 1990). On the other hand, many plant viral genomes encode specific movement proteins (MP) which interact with components of the plasmodesmata and facilitate viral spread between cells (Atabekov and Taliansky, 1990; Lucas et al., 1990; Deom et al., 1990, 1991; Berna et al., 1991; Wolf et al., 1991). Since the size exclusion limit (SEL) of plasmodesmatal structures is not compatible with the passage of plant viruses, the pore structures must be expanded sufficiently to allow the viral spread. Like viral spread through the modified plasmodesmata, inhibition of viral cell-to-cell movement via plasmodesmata by callose-like deposits in areas surrounding local lesions has been described (Allison and Shalla, 1974). Recently, it has been suggested that the

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deposit of callose in the constricted neck region of plasmodesmata could affect the pathway available for solute movement (Oleson and Robards, 1990).

An extraprotoplasmic sac, has been found to be frequently associated with the modified plasmodesmata (Allison and Shalla, 1974; McMullen et al., 1977; McMullen and Gardner, 1980). The sac appears to be a closed structure that originates from the modified plasmodesmata. It was found to be sometimes filled with aggregates of VLPs, embedded in the callose-like deposit between the plasmalemma and the cell wall, but often contained small spherical granules rather than virus particles (McMullen and Gardner, 1980). The structure is assumed to be a protective response by the plant to restrict the spread of virus (McMullen et al., 1977), but its nature is unknown. If the modification of plasmodesmata for viral restriction is induced by biochemical mechanisms similar to the viral spread through the modified plasmodesmata, the VLPs localized in the extraprotoplasmic sac should be similar to the form involved in the spread.

In this paper is described the occurrence and the spatial relationship of cell-wall abnormalities, including cell-wall thickenings, paramural bodies, modified plasmodesmata and extraprotoplasmic sacs in sorghum leaf tissues infected with maize dwarf mosaic virus (MDMV) A strain. The investigations reported here suggest how the modified plasmodesmata and extraprotoplasmic sacs restrict cell-to-cell viral spread in sorghum leaf tissues showing EAR.

MATERIALS AND METHODS

Virus and Plants

MDMV strain A was maintained in johnsongrass and sugarcane mosaic virus strain maize B (SCMV-MB) in N28 corn plants, and both served as viral sources (Choi, 1996). The inoculum sources were prepared by grinding the infected leaves in deionized water (1:5, w/v) using a mortar and pestle. Both surfaces of the third and emerging fourth leaves were dusted lightly with 600-mesh carborundum, and rubbed twice by the finger wipe method. MDMV-A was inoculated into sorghum (*Sorghum bicolor* L. Moench) cv HOK and SCMV-MB into cv Pioneer8680. Mock-inoculation was performed by inoculation with deionized water on carborundum-dusted leaves. Tissues of the fifth leaf of HOK showing EAR induced by MDMV-A were harvested within five days after inoculation and those of Pioneer8680 showing typical mosaic symptoms within two weeks after inoculation, and both were prepared for the cytopathological studies.

Electron Microscopy

Infected sorghum leaf tissues were cut under a drop of 2.5% (v/v) glutaraldehyde in 0.1 M potassium phosphate buffer (pH 7.2) and fixed in the same buffer solution for 60 min under vacuum at 4°C. Post-fixation was in 1 ml. of cold 1% (v/v) osmium tetroxide in 0.1 M potassium phosphate buffer (pH 7.2) for 60 min. The tissues were dehydrated in a cold acetone series starting at 25% with changes every 15-30 min up to 100% acetone, soaked for 2-4 h at room temperature in a mixture of Spurr low-viscosity plastic and Bo-Jax Mixture (23:77, v/v) with DMP-30 (Tridimethylamino methyl phenol) used as the accelerator (Choi, 1996), transferred to a fresh plastic mixture in "micron" molds and polymerized at 60°C for 24 h. Thin sections were made with a Porter Blum MT-2 ultramicrotome, stained with uranyl acetate followed by lead citrate, and observed with a Hitachi HU-12A electron microscope at 100 kV.

RESULTS

Early Acute Response (EAR)

Virulent strains of a virus can induce rapid functional and structural modifications of the most susceptible leaf tissues during the early stages of infection. EAR of sorghum cv HOK consisted of small chlorotic spots at the base of the youngest leaf (fifth) in the terminal whorl, and usually appeared within five days after inoculation with MDMV-A. These chlorotic spots became necrotic one to two days later, and rapidly killed the leaf tissues, but EAR developed neither on the fifth leaf tissues of cv Pioneer8680 showing systemic mosaic symptoms by SCMV-MB nor on those of mock-inoculated HOK sorghum plant. The EAR, therefore, is a type of hypersensitive response that eventually takes the form of necrosis. The infected plants then developed a late chronic response showing typical mosaic symptoms on the third and fourth leaves.

Modification of Plasmodesmata

The most distinctive modification of plasmodesmata was induced at the interface between the cell wall and plasmalemma in the cells of the fifth leaf of

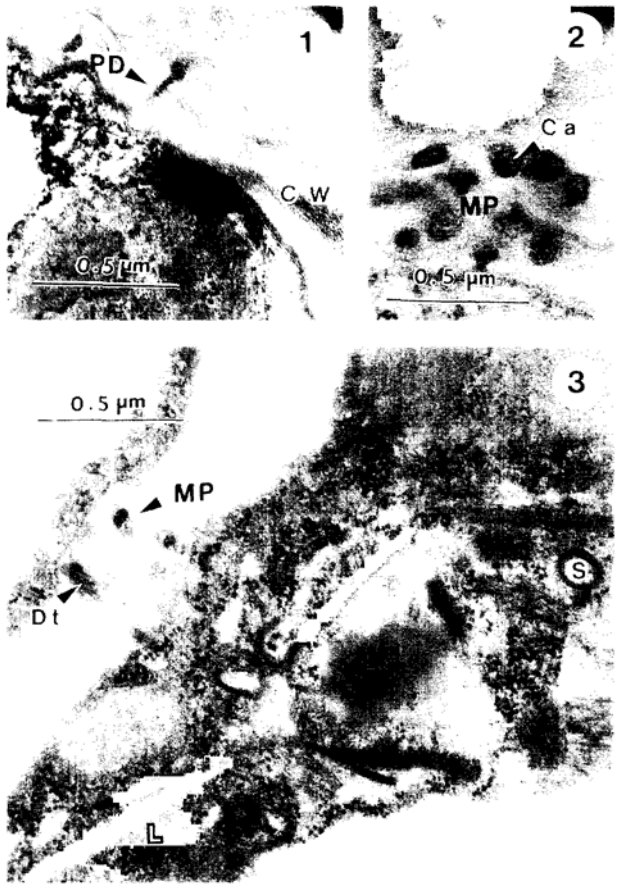


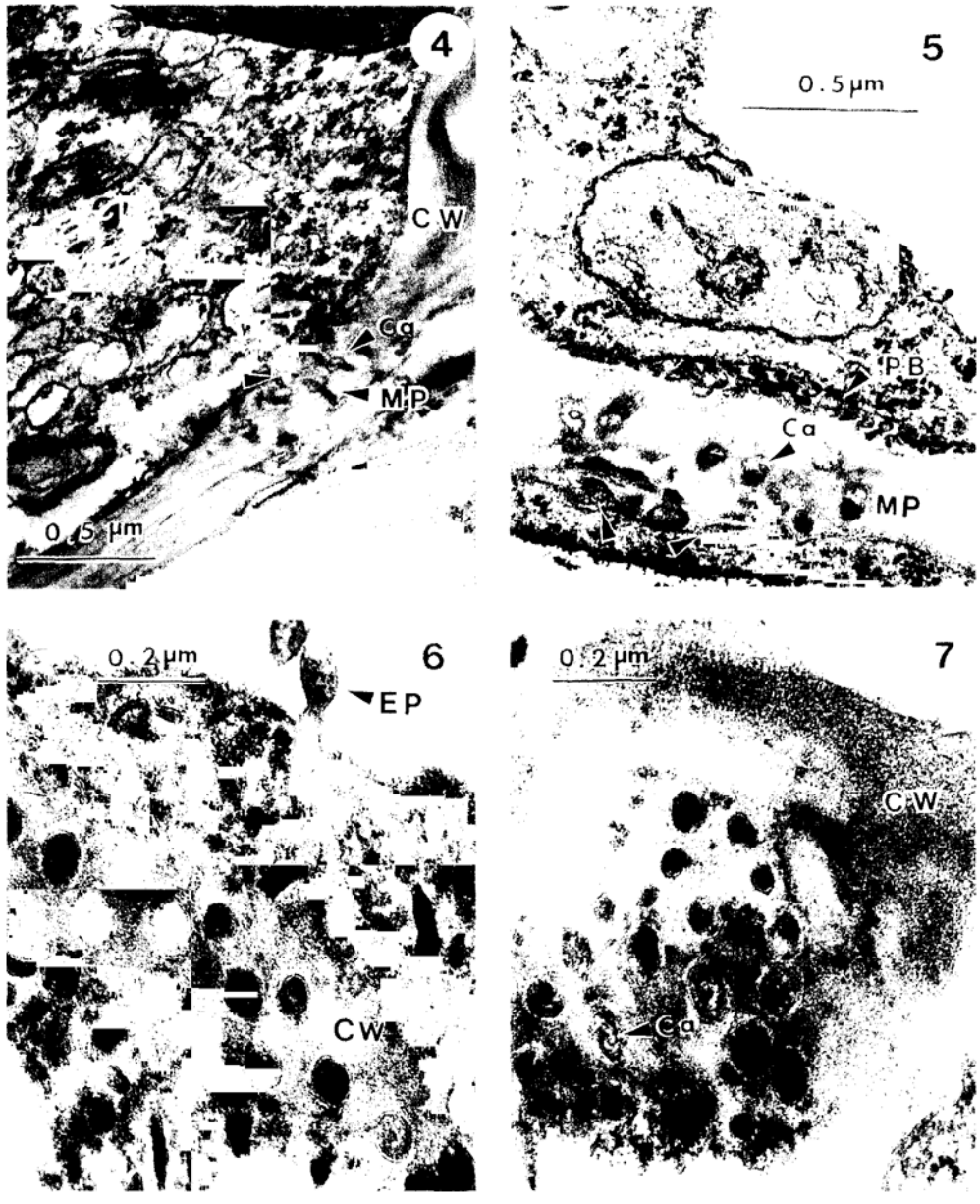
Figure 1. Normal feature of plasmodesmata in uninfected HOK sorghum plant. PD (plasmodesma), CW (cell wall). **Figures 2-3.** Electron micrographs of sorghum (*S. bicolor* L. Moench) leaf cells infected with SCMV-MB. **Figure 2.** A field containing various sizes of modified plasmodesmata (MP) in leaf tissues of Pioneer8680 sorghum plant showing systemic mosaic symptoms by SCMV-MB. Note the central cavities (Ca) within the plasmodesmata. **Figure 3.** Desmotubules (Dt) within size-modified plasmodesmata in leaf tissues of Pioneer8680 sorghum plant showing systemic mosaic symptoms. The cytoplasmic inclusions induced by SCMV-MB are evident; laminated aggregates (L), and scrolls (S).

infected HOK sorghum plants within five days after inoculation with MDMV-A. These changes were not observed in uninfected HOK sorghum plants (Fig. 1). In leaf tissues of Pioneer 8680 sorghum plants that developed systemic mosaic symptoms by SCMV-MB, development of cavities and desmotubules within the plasmodesmata were evident (Figs. 2 and 3), but accompanying cell-wall abnormalities were less prominent than in leaf tissues showing EAR by MDMV-A. Typical cytoplasmic inclusions, including laminated aggregates and scrolls, were induced by SCMV-MB (Fig. 3). An increase of modified plasmodesmata, and distinctive protrusions of plasmodesmata into the cytoplasm (Figs. 4, 9, and 10) were typical cell-wall abnormalities found only in the leaf tissues showing EAR. Cell-wall thickenings containing modified plasmodesmata, paramural bodies and/or large vesicles were often present together at the same site along the cell wall (Figs. 5, 12, and 15). The plasmodesmatal field contained many modified plasmodesmata of various sizes within thickened cell walls (Figs. 6 and 7). The filamentous materials filling the central cavities have been described, and their accumulation was related to the development of cavities (Ding et al., 1992), however the central cavities containing the filamentous materials were not found. The membranous vesicles and tubules were derived from the extended terminal structures of modified plasmodesmata, which were blocked off from the protoplasm by the callose-like deposits in the area between the cell wall and plasmalemma (Figs. 8 and 15). Based on studies using transgenic tobacco plants that expressed an MP, the callose deposit has been suggested to regulate the molecular SEL of plasmodesmata (Wolf et al., 1991).

The modified plasmodesmata observed in this study varied in length and diameter; they were membrane-bound and terminated in a bulbous extension, which was usually found between the plasmalemma and cell wall (Figs. 8, 9, 10, and 11). Two opposite-directed channels united via a central cavity at the middle lamella of the cell wall, one end of which was connected to the plasmalemma and the other end closed to form a bulbous extension (Fig. 11). Elongated plasmodesmata containing desmotubules (Robards and Lucas, 1990) extending into areas showing cell-wall abnormalities were often found (Fig. 12). Some of them appeared simple but extended in size, while others were complex plasmodesmata branched at either ends (Fig. 15), secondary plasmodesmata (Ding et al., 1992).

Extraprotoplasmic Sac

A localized extraprotoplasmic sac, containing filamentous aggregates of elongated VLPs, was often observed in cells from longitudinal sections of infected tissues (Fig. 13). These structures were located between the plasmalemma and the cell wall, bound by membranes, associated with modified plasmodesmata, and appeared to be sealed and completely excluded from the protoplasm. Structures similar to extraprotoplasmic sacs were frequently found without contents (Fig. 14), while others contained electron-

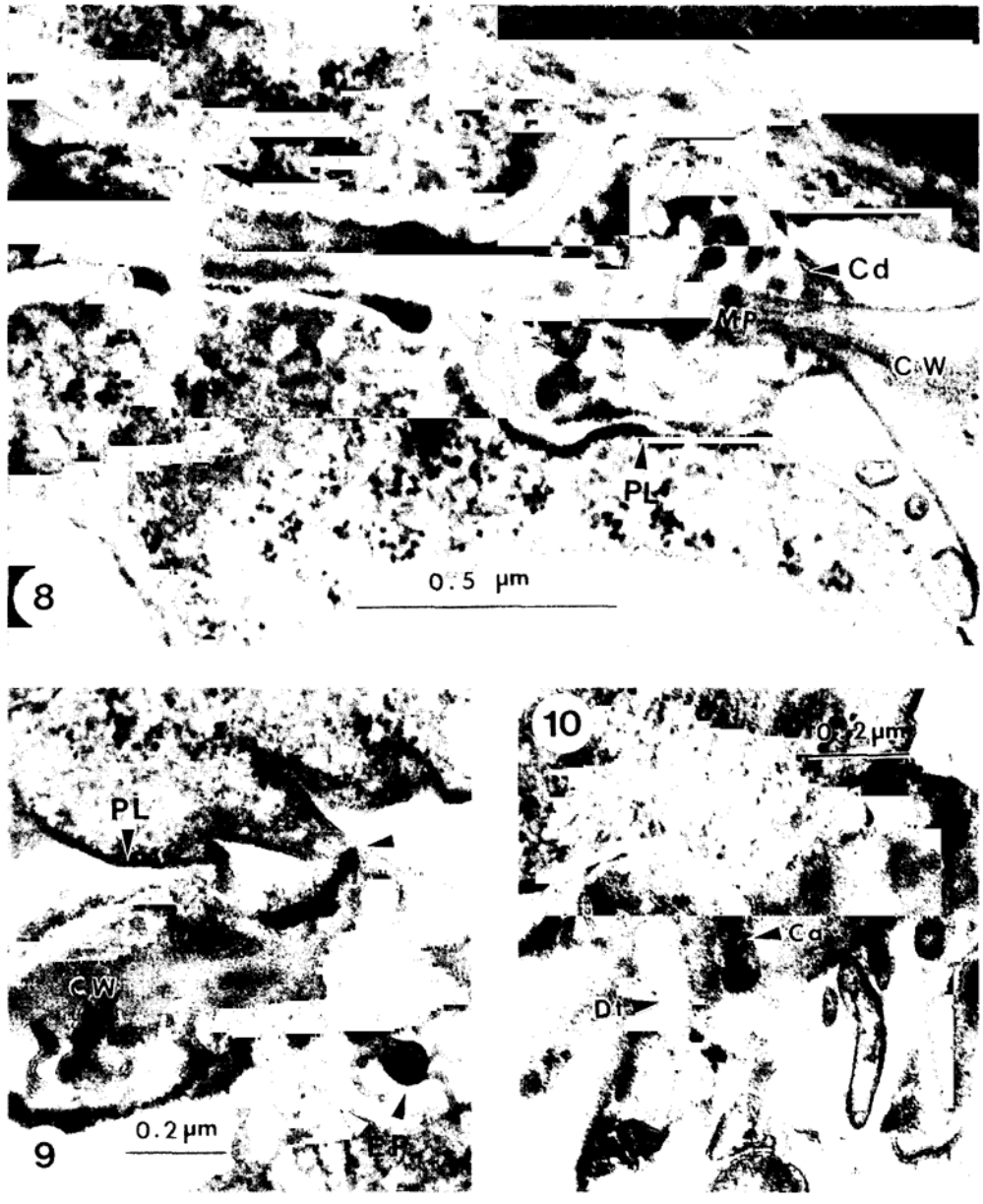


Figures 4-7. Electron micrographs of sorghum (*S. bicolor* L. Moench) leaf cells infected with MDMV-A. **Figure 4.** Electron micrograph showing cylindrical inclusions (CI) in the cytoplasm and cell wall abnormalities associated with modified plasmodesmata induced by early acute response in the sorghum cultivar HOK inoculated with MDMV-A. MP contains an extended protrusion (unlabeled arrowhead) and a central cavity (Ca). **Figure 5.** Accumulation of paramural bodies (PB) and flattened membranous vesicles (unlabeled arrowheads) localized with modified plasmodesmata are present together at the same site. **Figure 6.** A cross-sectional view of a field containing many plasmodesmata within thickened cell walls (CW). Note an extended protrusion of plasmodesma (EP). **Figure 7.** A cross-sectional view of a field containing various sizes of plasmodesmata surrounded by thickened cell walls. Some of the plasmodesmata contain central cavities.

dense materials (Figs. 14 and 15). The development of extraprotoplasmic sacs appeared to generate from either the terminal extension of modified plasmodesmata (Figs. 13 and 14) or formation by plasmalemma (Fig. 15). However, the origin and function of the modification are still unclear.

DISCUSSION

The EAR may depend entirely or partly on the induced cytological modifications, which results from interactions between a specific sorghum cultivar (HOK) and MDMV strain A. It is assumed that EAR



Figures 8-10. Electron micrographs of sorghum (*S. bicolor* L. Moench) leaf cells infected with MDMV-A. **Figure 8.** A sunken areas of plasmalemma (PL) caused by cell-wall thickenings contains modified plasmodesmata within a callose-like deposit (Cd). **Figure 9.** Note the bulbous termination of extended plasmodesmata (EP). The open area (unlabeled arrowhead) is associated with a modified plasmodesmata, which is connected to the ruptured membranes of plasmalemma. **Figure 10.** Cell-wall abnormalities associated with extended plasmodesmata. Note the desmotubules within modified plasmodesmata.

results from the expression of host genes that restrict viral spread at the intercellular level but not virus replication. Hence, its occurrence is presumably correlated to cell-wall abnormalities. Cell-wall thickenings appeared as a physical barrier to viral cell-to-cell spread not only in the cells surrounding the necrotic local lesions of hosts (Allison and Shalla, 1974), but also in systemically-infected leaf tissues (Kim and Fulton, 1973; McMullen et al., 1977; McMullen and Gardner, 1980). Size-modified plasmodesmata in leaf tissues showing systemic mosaic symptoms by SCMV-MB were also observed, but distinctive cell-wall thickenings, accumulation of membranous vesicles and tubules, bulbous extension of plasmodesmatal ends, and localization of extraprotoplasmic sacs were predominantly in leaf tissues showing EAR by MDMV-A.

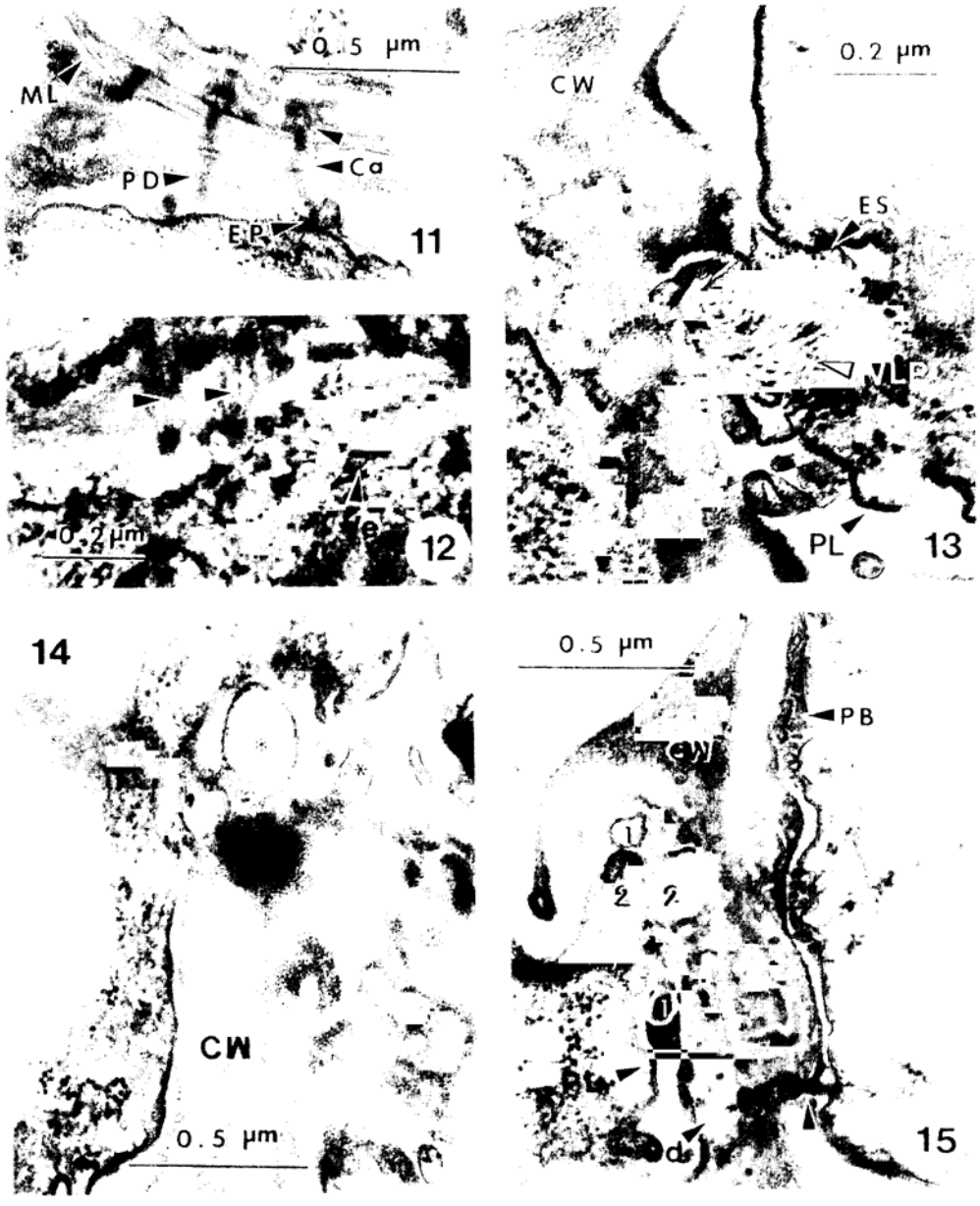


Figure 11-15. Electron micrographs of sorghum (*S. bicolor* L. Moench) leaf cells infected with MDMV-A. **Figure 11.** A longitudinal section of extended plasmodesmata with bulbous protrusions (EP), connected by two central cavities separated by middle lamella (ML) in HOK sorghum leaf tissues showing early acute response. **Figure 12.** Cell-wall abnormalities accompanying vesicles (Ve) at one side of the cell wall. The desmotubules within modified plasmodesmata are evident. **Figure 13.** A longitudinal section through extraprotoplasmic sac (ES) and its association with extended plasmodesmata in HOK sorghum leaf tissues infected with MDMV-A. Note the localized aggregates of virus-like particles (VLP) within sac and that limiting membranes of the sac are not continuous with the plasmalemma. **Figure 14.** The formation of membrane-bound sacs (*) located between the plasmalemma and cell wall (CW). **Figure 15.** Extraprotoplasmic sacs, empty extraprotoplasmic sacs (1) and sacs with electron-dense contents (2) located between the plasmalemma and cell wall. Also notes the vesicle formation within the callose-like deposits at both sides of the cell wall, associated with extended protrusions of plasmodesmata, some of which are branched (unlabeled arrowhead).

It has been suggested that cell-wall abnormalities in barley leaf tissues showing EAR reduce the systemic spread of barley stripe mosaic virus (McMullen et al., 1977). Likewise, the EAR of sorghum leaf tissues may be a transient response to viral infections that result in the death of leaf tissues. It is postulated that MDMV

triggers the host cell to produce abnormalities such as plasmodesmata blocked by callose-like deposits during the early infection process, and this modification may restrict the viral spread.

The extended bulbous terminals in the modified structure of plasmodesmata were located outside the thickened cell walls, and they were often continuous with membranous vesicles. It has been suggested that the termination of extended plasmodesmata by a callose-like deposit may be at the origin of the extraprotoplasmic sacs (McMullen et al., 1977; McMullen and Gardner, 1980). Because different types of unusual structures were present together at the same site, this spatial relationship may reflect the formation of those structures.

Since the MP but no coat protein (CP) is required for cell-to-cell movement of TMV, the modification of plasmodesmata is proposed to be an MP-activated process (Citovsky et al., 1990, 1992; Citovsky and Zambryski, 1991; Ding et al., 1992). The TMV movement model is the most studied system but potyviral spread or cell-to-cell restriction may operate by different mechanisms (Hull, 1989; Atabekov and Taliansky, 1990; Maule, 1991; Dolja et al., 1994, 1995). The role of potyviral CP in virus movement has been suggested in studies of a CP mutant of tobacco etch virus it has been shown that the CP is required for both cell-to-cell and vascular movement (Dolja et al., 1994, 1995). Therefore, the potyvirus may increase the plasmodesmatal permeability in a different manner from that of TMV MP. In addition, the modified plasmodesmata in this study have a desmotubule radius similar to the average potyviral diameter. This structural modification of plasmodesmata, an increase in the physical diameter of the pores, may allow intact viral particles to pass through.

The filamentous aggregates of the VLPs localized in extraprotoplasmic sacs were frequently found to be connected by a plasmodesmatal canal in previous results (Allison and Shalla, 1974; McMullen and Gardner, 1980). It is assumed that the VLPs may be viral particles which were trapped in the extraprotoplasmic sac after passage through the plasmodesmata. Direct evidence for this hypothesis, however, will require immunogold-labelling with capsid-protein specific antiserum. The observations suggest that the viral spread from cell to cell through the plasmodesmata may be inhibited by cell-wall abnormalities, which would account for the restriction of virus in leaf tissues showing EAR. The modified structures described in this report represent a part of a defense mechanism of the host plant to minimize viral spread, whose function depends on the sensitivity of leaf tissues to viral infection.

ACKNOWLEDGEMENTS

I would like to thank Dr. E.-S. Kim and Dr. K.-B. Lee for careful review of the manuscript. This work was financially supported by a grant from the 1998 Central Research Fund of Pai Chai University.

Received December 7, 1998; accepted March 11, 1999.

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