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ULTRASTRUCTURAL CHANGES IN MAIZE LEAF CELLS INFECTED WITH *MAIZE DWARF MOSAIC VIRUS* AND *SUGARCANE MOSAIC VIRUS*

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Plant viruses create many changes in the morphology of the plant cell once the infection process has begun. This paper describes and compares the ultrastructural changes induced in maize cells by two isolates of *Maize dwarf mosaic virus* (MDMV), Spanish (MDMV-Sp) and Polish (MDMV-P), and one isolate of *Sugarcane mosaic virus* (SCMV) at 10 and 42 days post-inoculation: the concentration and arrangement of virus particles, inclusion bodies associated with infection, and other cytological alterations. The most important difference between maize cells infected with MDMV isolates and with SCMV-P1 was in the form of cytoplasmic cylindrical inclusions. In cells infected with MDMV only typical inclusions such as pinwheels and scrolls were observed, but laminar aggregates were also present in SCMV-infected cells. No virus particles were found in plant cell organelles. Specific virion arrangements occurred in cells infected with MDMV-Sp and SCMV. The most interesting new finding was of specific amorphous inclusions in the cytoplasm of MDMV-Sp-infected cells, which clearly differentiated the two MDMV isolates studied.

Key words: Maize, Potyviridae, cell ultrastructure, cytoplasmic inclusions, amorphous inclusion, plant viruses.

INTRODUCTION

Maize dwarf mosaic virus (MDMV) and *Sugarcane mosaic virus* (SCMV), belonging to the genus *Potyvirus* (Potyviridae), are serious maize pathogens causing leaf mosaic and stunting in maize plants (Gordon, 2004; Fuchs, 2004). The viruses were recently identified in Poland (Trzmiel and Jeżewska, 2008; Trzmiel, 2009). A characteristic feature of potyviruses is induction of specific inclusions in cells of infected plants.

Initially all mosaic virus isolates originating from maize were classified as strains of MDMV, and isolates originating from sugarcane as strains of SCMV. The former classification, based on differentiation of biological characters, was inadequate. Further investigations of serological and molecular properties led to the establishment of four groups of viruses, assigning the known virus strains to four species: (1) MDMV (MDMV-A, MDMV-D, MDMV-E, MDMV-F), (2) SCMV (MDMV-B, SCMV-A, SCMV-B, SCMV-D, SCMV-E, SCMV-SC, SCMV-BC, SCMV-Sabi), (3) *Johnsongrass mosaic virus*, JGMV (SCMV-JG, MDMV-O) and (4) *Sorghum mosaic virus*, SrMV (SCMV-H, SCMV-I, SCMV-M) forming a *Sugarcane mosaic virus* subgroup (Shukla et al., 1989). Lesemann et al. (1992) studied the structure of cytological inclusions and other cytological alterations induced by representative isolates of the four viruses belonging to the SCMV subgroup, and differentiated the viruses based on cytopathology.

There are many reports on ultrastructural changes observed in plant cells resulting from potyvirus infections in different hosts, but little information on the ultrastructural alterations in maize cells infected with maize mosaic viruses, particularly SCMV.

We studied the cytopathological effects of infection by isolates of MDMV and SCMV in maize leaf cells. We compared the effects of two MDMV isolates, Polish and Spanish, and examined the cytopathology of SCMV infection.

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Abbreviations: CI – cytoplasmic inclusion; CCI – cytoplasmic cylindrical inclusion; AI – amorphous inclusion; ER – endoplasmic reticulum; MDMV – *Maize dwarf mosaic virus*; SCMV – *Sugarcane mosaic virus*.

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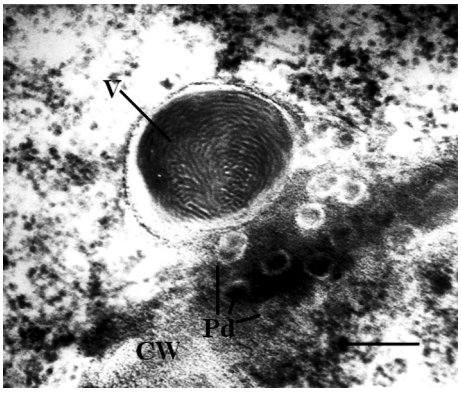

Fig. 1. Particles of MDMV-P, coil near cell wall at 10 dpi. CW – cell wall; Pd – plasmodesmata; V – virus particles (arrow). Bar = 200 nm.

MATERIALS AND METHODS

VIRUSES

The three virus isolates used in this study were a Polish isolate of *Maize dwarf mosaic virus* (MDMV-P2) originating from fodder maize (*Zea mays* ssp. *indentata* Montg.) in Wielkopolska Province, a Polish isolate of *Sugarcane mosaic virus* (SCMV-P1) isolated from sweet maize (*Zea mays* ssp. *saccharata* Koern.) in Wielkopolska Province, and a Spanish isolate of *Maize dwarf mosaic virus* (MDMV-Sp) obtained from Dr. M.A. Achon of the Department of Plant Production and Forest Science at the University of Lleida (UdL), Spain.

METHODS

Breeding lines of maize (W-401, F2, sweet maize cv. Waza) were inoculated at the three-leaf stage with MDMV-P2, SCMV-P1 and MDMV-Sp, respectively. Control plants were inoculated with water. All experimental plants were grown in an insect-proof greenhouse during summer 2007. Tissues from systemically infected leaves showing mosaic symptoms were taken for examination. The leaf samples were collected and fixed 10 and 42 days after inoculation.

Pieces of leaves were fixed in 3% glutaraldehyde in phosphate buffer (pH 7.2) for 3 h at room temperature. The pieces were washed four times (1 h each) in buffer. The material was postfixed for 2 h in 2% osmium tetroxide in the same buffer at 4°C, and washed again in the buffer, dehydrated in an ethanol and acetone series, embedded in Epon 812 resin, and after polymerization cut with a glass knife on a Reichert OM-U2 ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate. The sections were examined with a Philips EM-201 electron microscope at 80 kV in the Institute of Plant Protection, National Research Institute in Poznań.

RESULTS

The effects of virus infection on maize leaf cell ultrastructure were examined at 10 and 42 days post-inoculation (dpi). The main objects of interest were the distribution and arrangement of virus particles, cytoplasmic inclusions, and changes in organelle ultrastructure.

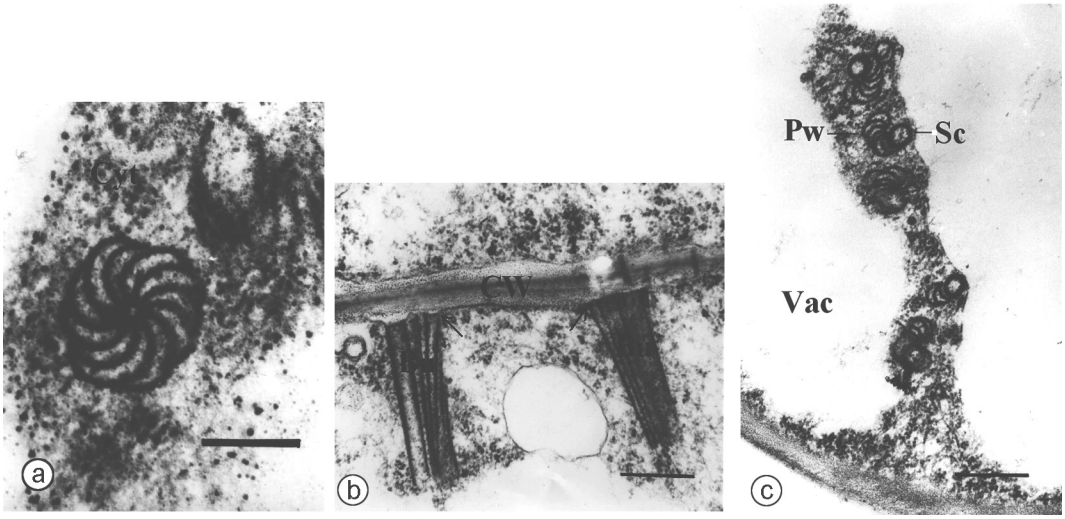

Fig. 2. Inclusions in maize mesophyll leaf cell infected with MDMV-P. (**a**) Typical pinwheels in cross section in cytoplasm at 42 dpi. Cyt – cytoplasm. Bar = 200 nm, (**b**) Pinwheels in longitudinal section seen as bundles associated with plasmalemma (arrows), perpendicular to cell wall at 10 dpi. Bu – bundles; CW – cell wall. Bar = 500 nm, (**c**) Protrusion of cytoplasm into vacuole at 42 dpi. PW – pinwheel; SC – scroll; Vac – vacuole. Bar = 500 nm.

None of the leaf cells of plants infected with any of the three virus isolates showed cytoplasmic inclusions in the plasmodesmata. Nor did they show virus particles in cell organelles.

MDMV-P2

Flexuous virus particles loosely distributed in the cytoplasm were seen at both 10 and 42 dpi. As early as 10 dpi and also at 42 dpi, inclusions of large coils of virus particles appeared in close contact with cell walls and plasmodesmata (Fig. 1).

Inclusions typical for potyviruses – pinwheels and scrolls – were found in the cytoplasm of mesophyll cells at both time points. In cross section the pinwheels were seen as 11-armed structures (Fig. 2a), and in longitudinal sections as bundles. At 10 dpi the bundles were perpendicular to the cell wall, mostly in contact with plasmodesmata (Fig. 2b); at 42 dpi they were dispersed in the cytoplasm, usually in contact with membranes of endoplasmic reticulum (ER). The bundles were sometimes at one end but more commonly at both ends of the plasmodesmata. In mesophyll cells, characteristic protrusions of the cytoplasm into the vacuole were found at 42 dpi. Pinwheels and scrolls occurred inside the protrusions. At 42 dpi the number of scrolls increased. The majority of scrolls were closely associated with pinwheel arms (Fig. 2c).

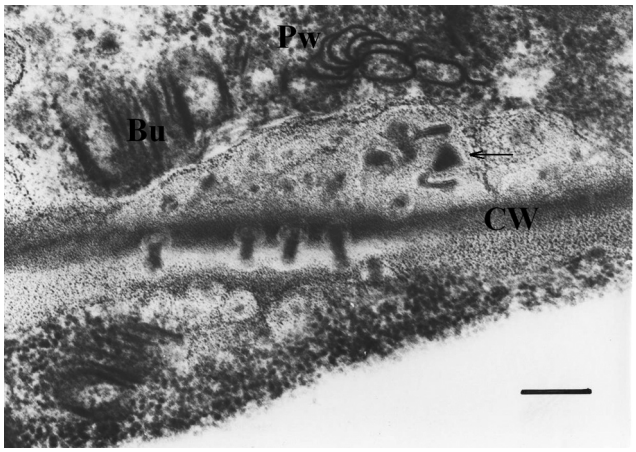

Fig. 3. Cytological alterations induced by MDMV-P in maize mesophyll leaf cells: irregular thickening of cell walls at 10 dpi, unidentified structures in cell wall (arrow). Bu – bundle; CW – cell wall; PW – pinwheel. Bar = 250 nm.

At 10 dpi the cell walls of many mesophyll cells already were thickened and contained unidentified osmiophilic structures (Fig. 3) which later, at 42 dpi, projected into the cytoplasm.

Chloroplasts, mitochondria and nuclei were not altered in ultrastructure.

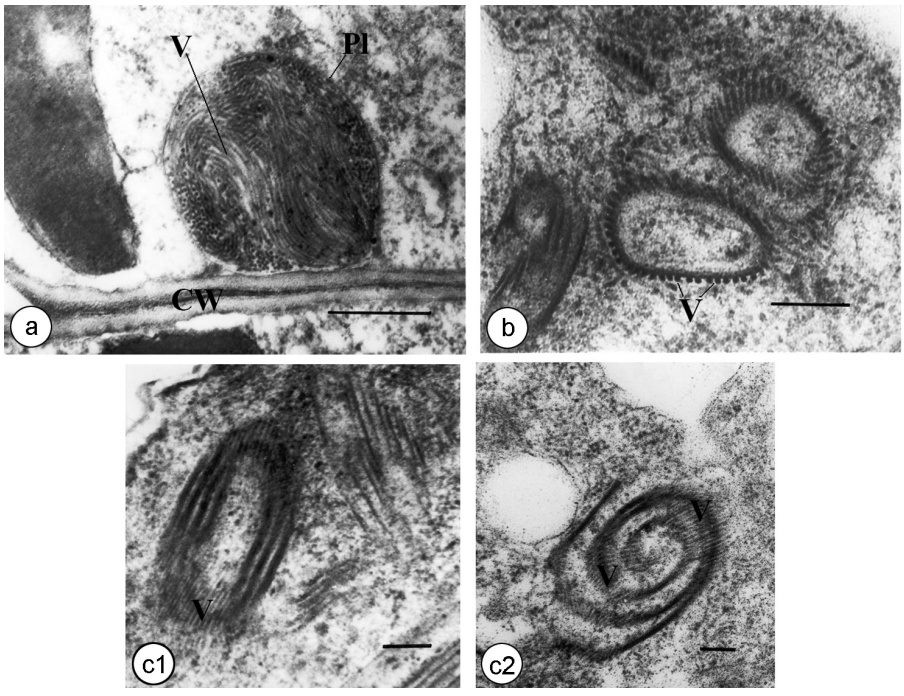

Fig. 4. Particles of MDMV-Sp. (**a**) Coil bound by single unit membrane near cell wall at 42 dpi. W – cell wall; Pl – plasmalemma; V – virus particles. Bar – 500 nm, (**b**) Virus particles in cross section attached to single membrane in the form of cylinder at 42 dpi. V – virus particles. Bar = 200 nm, (**c1,2**) Uniseriate sheets of virus particles appearing as a plate cut obliquely. V – virus particles. Bar = 200 nm.

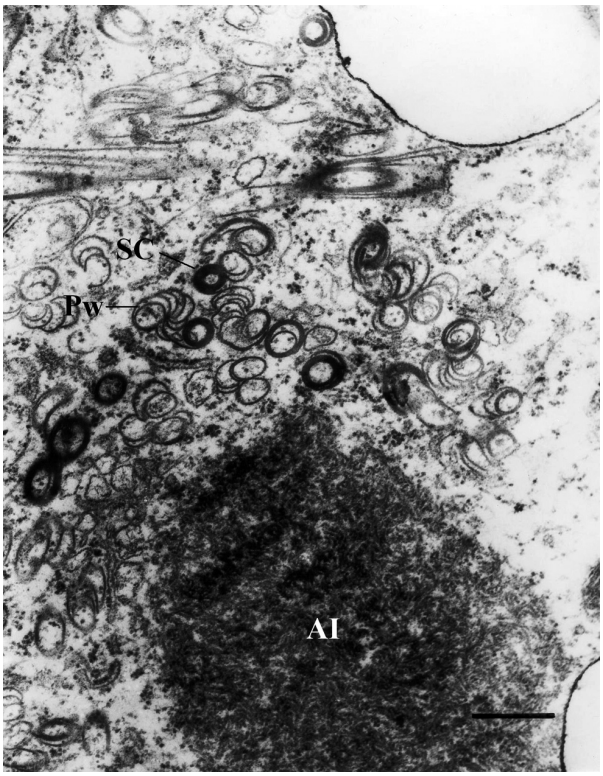

Fig. 5. Inclusions in maize leaf cells infected with MDMV-Sp: abundant pinwheels, scrolls and amorphous inclusion in cytoplasm at 42 dpi. AI – amorphous inclusion; PW – pinwheel; SC – scroll. Bar = 500 nm.

MDMV-Sp

Initially (10 dpi) only individual flexuous virus particles were seen in the cytoplasm of mesophyll cells and epidermis cells. The number of virions increased at 42 dpi and tended to aggregate in bundles. Coils of virus particles were observed in association with the cell wall. The coils occurred more frequently at 42 dpi, and some were packed in thin membranes, probably originating from the plasmalemma (Fig. 4a).

In the later stage of infection (42 dpi) the virus particles occurred at high concentrations and were arranged in frameworks not seen at 10 dpi. The frameworks were of two general types. In the first, virions were serially joined in single rows attached to a single membrane. The membranes were planar or else cylindrical with the virus particles located outside of them (Fig. 4b). In the second type, virus particles were serially joined in strongly osmiophilic plates. The plates were straight or more frequently helical. Sections of the plates revealed layers of virions cut longitudinally and/or obliquely (Fig. 4c1, c2).

MDMV-Sp, like MDMV-P2, generated typical cytoplasmic cylindrical inclusions (CCI), pinwheels and scrolls (Fig. 5). In the late stage of infection the pinwheels were more abundant.

A characteristic feature of MDMV-Sp only in the second stage of infection was the formation of amorphous, thin-fibrous inclusions which were highly electron-dense, located in the cytoplasm of mesophyll cells and not surrounded by a membrane. Numerous inclusions, the size of chloroplasts, took up much room in the cytoplasm (Figs. 5, 6a). Mitochondria were more frequent than in healthy cells, often disposed in groups or around the nucleus. (Fig. 6b). In the late stage of infection there were important alterations in the morphology of mitochondria: they were irregular in shape, forked, elongated or fragmented in separate units (Fig. 6a).

In mesophyll cells, vesicular structures appeared already at 10 dpi. Their number increased in the course of infection and by 42 dpi they were abundant in mesophyll and epidermis cells. Usually they were filled with fibrous and tiny-granular material. The structures occurred in association with endoplasmic reticulum (Fig. 6c).

In mesophyll and epidermis cells at 42 dpi, strong deformations of the cell walls were seen, consisting in the deposition of unidentified small-granular material with inserts of amorphous, fibrous, electron-dense structures (Fig. 6d).

The chloroplasts and nuclei of the cells were not altered.

SCMV-P1

Virus particles were seen in mesophyll and phloem cells at 10 as well as 42 dpi. At 10 dpi, single virions were loosely distributed in the cytoplasm and as coils near the cell wall.

At 42 dpi, virus particles occurred in very high concentrations in the form of bundles (Fig. 7a), which were specific arrangements with single arrays of virus particles separated by longitudinally disposed particles in cross section (Fig. 7b). The particles were irregularly associated with lamellar aggregates, one-layer rows perpendicular to the tonoplast or to the lamellar aggregate inclusion. Coils of virions were seen near the cell wall or even inside the cell wall, neighboring plasmodesmata.

In the cytoplasm of the virus-infected cells, protein inclusions such as pinwheels, scrolls and lamellar aggregates were routinely observed. Pinwheels and scrolls were found more frequently in the early stage of infection (Fig. 8a,b). Lamellar aggregate inclusions were more numerous in the late stage of infection. They were seen in cells of all kinds of plant tissues, including xylem tracheary elements (Fig. 8c). These inclusions were differently shaped as planar plates or flexuous structures. All three forms of cylindrical cytoplasmic inclusions were tightly associated, with many virus particles and vesicle structures, occasionally containing thin fibrils around them.

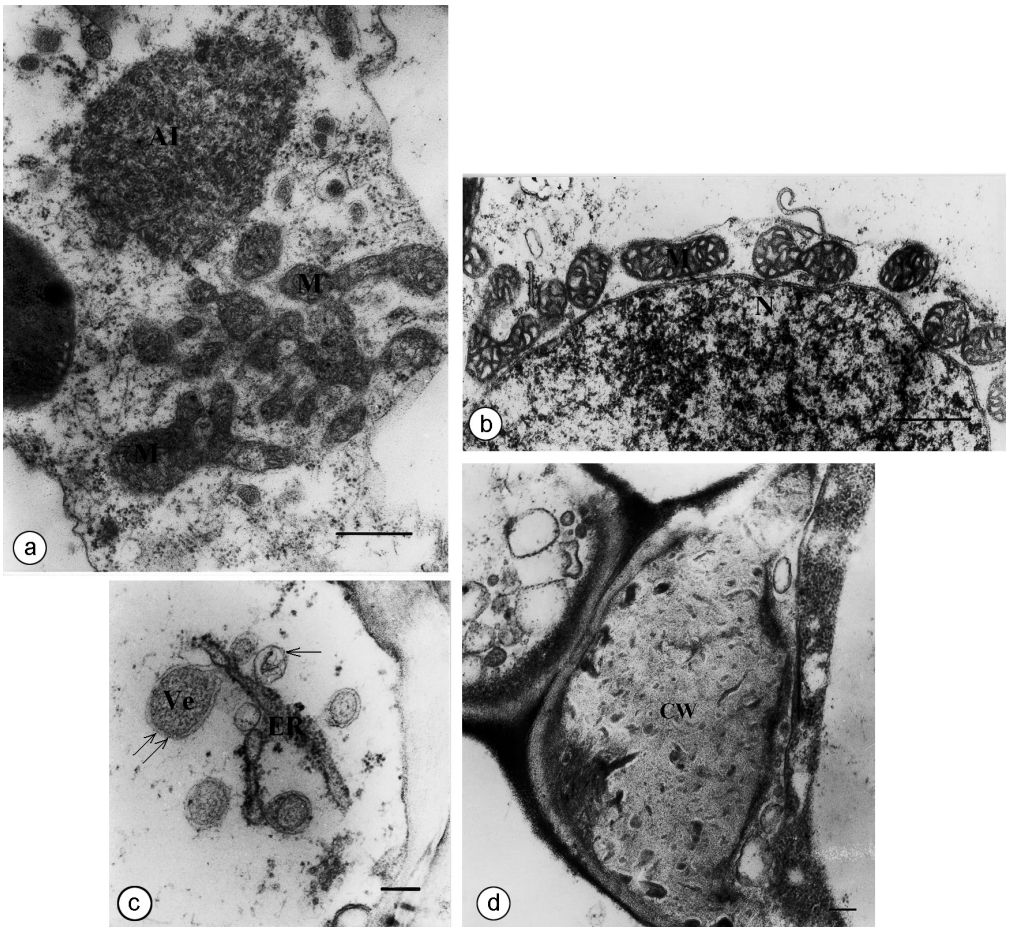

Fig. 6. Cytological alterations induced by MDMV-Sp in maize leaf cells. (**a**) Deformed mitochondria and amorphous inclusion in cytoplasm at 42 dpi. AI – amorphous inclusion; M – mitochondria. Bar = 100 nm, (**b**) Group of mitochondria around nucleus at 42 dpi. N – nucleus. Bar = 100 nm, (**c**) Vesicular structures with fibrous (arrow) and granular material (double arrows) in association with endoplasmic reticulum at 42 dpi. Ve – vesicle. Bar = 200 nm, (**d**) Deformation of the cell wall containing deposits of unidentified material at 42 dpi. CW – cell wall. Bar = 530 nm.

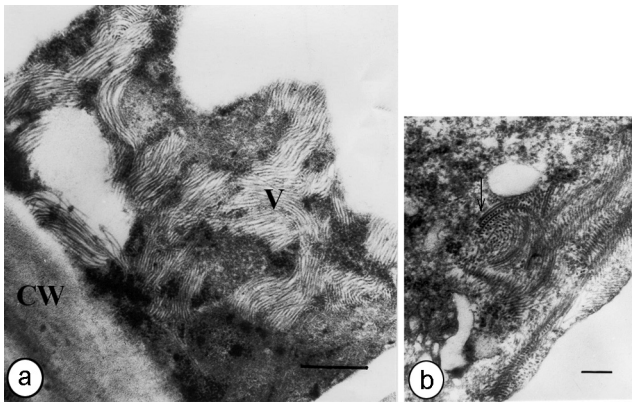

Fig. 7. Particles of SCMV-P. (**a**) Bundles of virus particles in seminecrotic mesophyll cell at 42 dpi. CW – cell wall; V – virus particles. Bar = 500 nm, (**b**) Arrangement of virus particles in cross and longitudinal sections (arrow) in mesophyll cell at 42 dpi. Bar = 200 nm.

Necrotic and semi-necrotic cells were often found in the late stage of infection (Fig. 7a).

As early as at 10 dpi the cell walls were thickened, with inserts of amorphous structures, and often projected into the protoplast (Fig. 9a). Later these alterations were very numerous, particularly in the regions of mesophyll cell necrotization or on the border between epidermis and mesophyll cells.

Mitochondria usually occurred in aggregates but were not deformed. The nuclei also appeared intact. The chloroplasts of bundle sheath cells were distinctly deformed (Fig. 9b).

DISCUSSION

VIRUS PARTICLES

The results of our findings concerning MDMV-P2 indicated low concentrations of the particles in

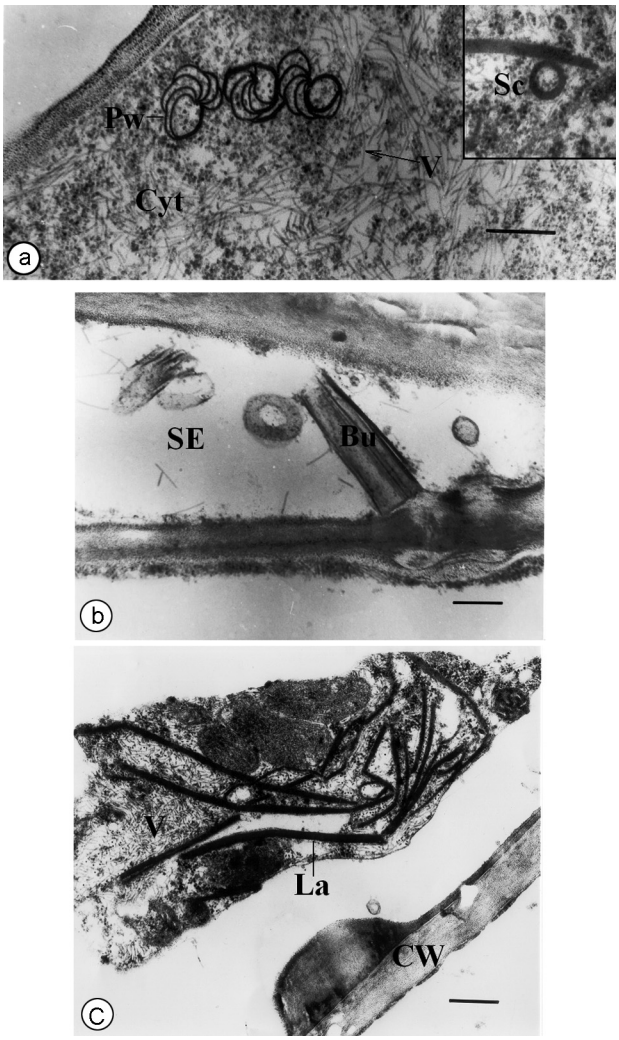

Fig. 8. Inclusions in maize leaf cells infected with SCMV-P. (**a**) Pinwheels, scrolls and virions (arrow) in cytoplasm of mesophyll cell. Inset: scroll and fragment of lamellar aggregate at 10 dpi. Cyt – cytoplasm; PW – pinwheel; SC – scroll; V – virus particles. Bar = 500 nm, (**b**) Bundles and few virus particles in sieve element at 10 dpi. Bu – bundle; SE – sieve element. Bar = 500 nm, (**c**) Lamellar aggregates and numerous virus particles in young xylem tracheary element at 42 dpi. CW – cell wall; La – lamellar aggregate; V – virus particles. Bar = 500 nm.

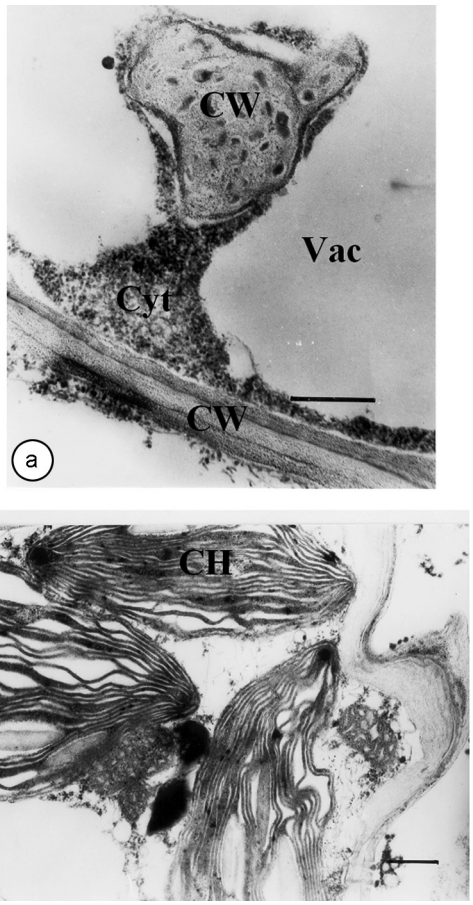

Fig. 9. Cytological alterations induced by SCMV-P in maize mesophyll leaf cells. (**a**) Cell wall thickening and protrusions of cell wall into cytoplasm at 10 dpi. Cyt – cytoplasm; CW – cell wall; Vac – vacuole. Bar = 500 nm, (**b**) Deformation and necrotization of chloroplasts of bundle sheath cell at 42 dpi. CH – chloroplast. Bar = 500 nm.

infected cells at both 10 and 42 dpi. MDMV-Sp particles were definitely more numerous even at 10 dpi. The virion concentration was highest in SCMV-infected cells.

At 42 dpi the number of virions increased. The arrangement of virions in configurations was reported by Lesemann (1995), who noted that potyvirus particles tend to attach to cell membranes, for example to the tonoplast or to cylindrical inclusion arms. Our observations are in agreement with those. MDMV-Sp particles were associated with a single membrane in cylindrical form, similarly to those Francki et al. (1985) described in cells infected with *Bean yellow mosaic virus* (BYMV).

MDMV-Sp particles occurred in very interesting configurations: tightly packed uniseriate sheets of virus particles running at various angles, usually helically shaped. Virus particles were seen in cross sections of these structures. Only Francki et al. (1985) presented similar structures.

The characteristic large coils located between the cell wall and plasmalemma of all three virus isolates studied, found already at 10 dpi and very numerous at 42 dpi, were described by Krass and Ford (1969) in MDMV-A infection, and by Murant and Roberts (1971) investigating *Parsnip mosaic potyvirus* (ParMV). They called such coils "micro-inclusion bodies", which, in their opinion, were associated with cell-to-cell transport of virions. They did not identify these coils as virion aggregates. Because

we located the coils in front of the plasmodesmata, we suggest that they were aggregates of particles that could not pass through the plasmodesmata to neighboring cells. Parallel particle aggregates have also been observed in thickened cell walls of tobacco cells infected with *Potato virus* Y (PVY) (Garbaczewska et al., 1996). In studies of MDMV-Sp, Achon et al. (1996) did not report such observations.

CYTOPLASMIC INCLUSIONS (CIs)

Cytoplasmic cylindrical inclusions (CCIs)

CCIs consisting of pinwheels, scrolls, lamellar aggregates and short curved lamellar aggregates are one of the main characteristics of the potyviruses. Differences in CCI morphology have been used to assign potyviruses to the four subgroups (Edwardson et al., 1984). Our findings on CCI morphology are completely consistent with data presented by Lesemann et al. (1992); in cells infected with MDMV isolates they found only pinwheels and scrolls, and in cells infected with SCMV they noted lamellar aggregates in addition to pinwheels and scrolls.

Our observations of the structures and locations of CCIs produced by all investigated virus isolates are in accord with the reports of other authors (Francki et al., 1985; Lesemann et al., 1988; Roberts et al., 1998). The number of pinwheels and scrolls increased with duration of infection, particularly in the case of MDMV-Sp. At 10 dpi the pinwheels appeared as bundles perpendicular to the cell wall in front of the plasmodesmata. Later they tended to move from the cell wall towards the central part of the cell, where they changed into scrolls. These results confirm the work of other authors (Purcifull and Edwardson, 1967; Roberts et al., 1998) who showed that the pinwheels become scrolls by rolling of the pinwheel arms in later stages of infection. In their studies of MDMV-Sp and MDMV-Spl, Achon et al. (1996) observed short, curved lamellar aggregates in addition to pinwheels and scrolls. Referring to a paper by Edwardson et al. (1984), Lesemann et al. (1992) stated that it is not clear whether MDMV and SrMV generate short, curved lamellar aggregates. In cells infected with SCMV-P1 the pinwheels and scrolls were more numerous in the early observations at 10 dpi. We may speculate that this phenomenon is due to earlier rolling of pinwheels arms into scrolls, and probably faster degeneration of both forms than in MDMV infection. The high number of lamellar aggregates at 42 dpi might result from the arrangement of pinwheels, as suggested by Edwardson (1974).

Our observation of CCIs in sieve elements is consistent with Zechmann et al.'s (2003) suggestion that they are imported into sieve elements from neighboring cells such as companion cells.

AMORPHOUS INCLUSIONS (AIs)

Of special interest in our comparative study is the finding of large, amorphous, irregular inclusions exclusively in MDMV-Sp-infected mesophyll cells. Sometimes two such inclusions were noted in one cell.

AIs were already observed by Edwardson (1974) in cells infected with *Pepper mottle virus* (PeMV) and by Martelli and Russo (1976) in cells infected with *Watermelon mosaic virus* (WMV). Martelli and Russo (1976) found that this type of inclusion contained proteins and RNA. De Mejia et al. (1985a) isolated AIs from cells infected with PeMV and WMV-1, a strain of *Papaya ringspot virus* (PRSV-W), and produced specific antisera against purified AI proteins. In a continuation of their studies, de Mejia et al. (1985b) provided evidence that AI protein is related to the *Tobacco vein mottling virus* (TVMV) aphid transmission helper component protein.

The role of AIs has not been definitively explained. Surprisingly, this type of inclusion does not occur commonly in potyvirus-infected plant cells. In comparative studies of the cytopathology of JGMV, MDMV, SCMV and SrMV isolates, Lesemann et al. (1992) found that the appearance of AI was associated only with SrMV infection. Our finding of AIs in MDMV-Sp-infected cells is the first reported observation of such an association. Achon et al. (1996) did not mention AIs connected with MDMV-Sp or MDMV-Sp1 infection.

OTHER CYTOPATHOLOGICAL CHANGES

Many authors have demonstrated that the vesicles produced in response to virus infection were generated from ER (Krass and Ford, 1969; Lawson et al., 1971; Francki, 1985; Calder and Ingerfeld, 1990). According to Lesemann et al. (1999), Picorna-like plant viruses like Potyviridae and Comoviridae induce clusters of free vesicles in the cytoplasm which appear to contain viral dsRNA and which derive from buds of ER-like membranes.

We confirmed that the vesicles were derived from buds originating from ER membranes. They contained electron-dense material, often in the form of fine fibrils.

An important observation was cell wall thickening in all virus infections studied. Martelli and Russo (1977) extensively studied modifications of cell walls under virus infection, and distinguished two types of alterations. The patterns we found indicate that cell wall thickening was associated with a defense mechanism, preventing or attenuating cell-to-cell transport of the virus. This interpretation is consistent with the views of Gill and Chong (1979) and Hinrichs-Berger et al. (1999).

The reaction of mitochondria to a virus infection usually consists in enlargement (Lesemann et

al., 1979) and clumping (Lesemann, 1988) as well as an increase in the number of mitochondria in infected cells (Russo and Martelli, 1969).

Our observations of MDMV-Sp-infected cells are consistent with those presented by Russo and Martelli (1969), who studied changes in the structure of *Gomphrena globosa* L. cells infected with *Beet mosaic virus* (BMV). They described important modifications of mitochondria, including pleomorphic organelles and fragmentation of a whole organelle into many small separate units.

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