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Cytopathology of *Pigeonpea sterility mosaic virus* in pigeonpea and *Nicotiana benthamiana*: similarities with those of eriophyid mite-borne agents of undefined aetiology

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Summary

Pigeonpea sterility mosaic virus (PPSMV) is transmitted by the eriophyid mite, *Aceria cajani*, and is very closely associated with sterility mosaic disease (SMD) of pigeonpea (*Cajanus cajan*) in the Indian subcontinent. Antiserum produced to purified PPSMV preparations detected a virus-specific 32 kDa protein in sap of SMD-affected pigeonpea plants by ELISA and Western blotting. PPSMV was transmitted mechanically in sap of SMD-affected pigeonpea leaves to *Nicotiana benthamiana*. Ultrastructural studies of symptom-bearing leaves of two pigeonpea cultivars, (ICP8863 and ICP2376) and *N. benthamiana* infected with PPSMV, detected quasi-spherical, membrane bound bodies (MBBs) of *c*. 100-150 nm and amorphous electron-dense material (EDM). These structures were distributed singly or in groups, in the cytoplasm of all cells, except those in conductive tissues. Fibrous inclusions (FIs), composed of randomly dispersed fibrils with electron lucent areas, were present in the cytoplasm of palisade cells and rarely in mesophyll cells of the two pigeonpea cultivars but were not detected in infected *N. benthamiana* plants. In the PPSMV-infected pigeonpea cultivars and *N. benthamiana,* immuno-gold labelling, using antiserum to PPSMV, specifically labelled the MBBs and associated EDM, but not the FIs. The MBBs and associated inclusions are similar in appearance to those reported for plants infected with the eriophyid mite-transmitted High Plains virus and the agents of unidentified aetiology associated with rose rosette, fig mosaic, thistle mosaic, wheat spot chlorosis and yellow ringspot of budwood. The nature of these different inclusions is discussed.

Key words: Electron microscopy, immuno-gold labelling, ultrastructure

Introduction

Eriophyid mites transmit the agents of several economically important diseases of crop plants (Oldfield & Proeseler, 1996). All characterised eriophyid mite-transmitted viruses have flexuous rod shaped particles and belong to either the genus *Rymovirus, Tritimovirus* (in the family *Potyviridae*) or *Alexivirus* (Van Regenmortel *et al*., 2000). An exception is the recently characterised *Blackcurrant reversion virus* (family *Comoviridae*, genus *Nepovirus*) that has isometric particles (summarised by Jones, 2000). However, for several diseases caused by other eriophyid mite-transmitted agents, especially those affecting dicotyledonous plants, no disease agents have been isolated and characterised (Oldfield & Proeseler, 1996). In 1931, one such disease, named sterility mosaic (SMD), was identified affecting pigeonpea (*Cajanus cajan*) in the Indian subcontinent (Reddy *et al*., 1998). Affected plants showed mosaic symptoms on leaves (Fig. 1A) and ceased flowering but a few SMD-tolerant pigeonpea cultivars showed only chlorotic ringspots without significant effects on flowering (Fig. 1B). SMD has subsequently emerged in all the pigeonpea growing countries in the Indian subcontinent and has gained considerable importance because of its devastating effect on pigeonpea yield (Kannaiyan *et al*., 1984). In nature, the causal agent of SMD is transmitted by the eriophyid mite, *Aceria cajani*, but it is not transmitted through seed, pollen or soil (Reddy *et al*., 1998). The mite vector is highly host-specific and, because of this, the natural host range of SMD is restricted to pigeonpea and a few of its wild relatives (Kumar *et al*., 2001*b*). Experimentally, SMD is transmitted by grafting (Ghanekar *et al*., 1992). All previous studies on the SMD causal agent indicated that it is likely to be a virus or virus-like agent (Ghanekar *et al*., 1992; Nene, 1995) but attempts to isolate and characterise the putative virus have not been successful (Reddy *et al*., 1994; Singh *et al*., 1999).

Recently, using a new purification method, slender highly flexuous filamentous virus-like particles (VLPs) of *c*. 3-10 nm in diameter were isolated from SMD-affected pigeonpea (Kumar *et al*., 2000,

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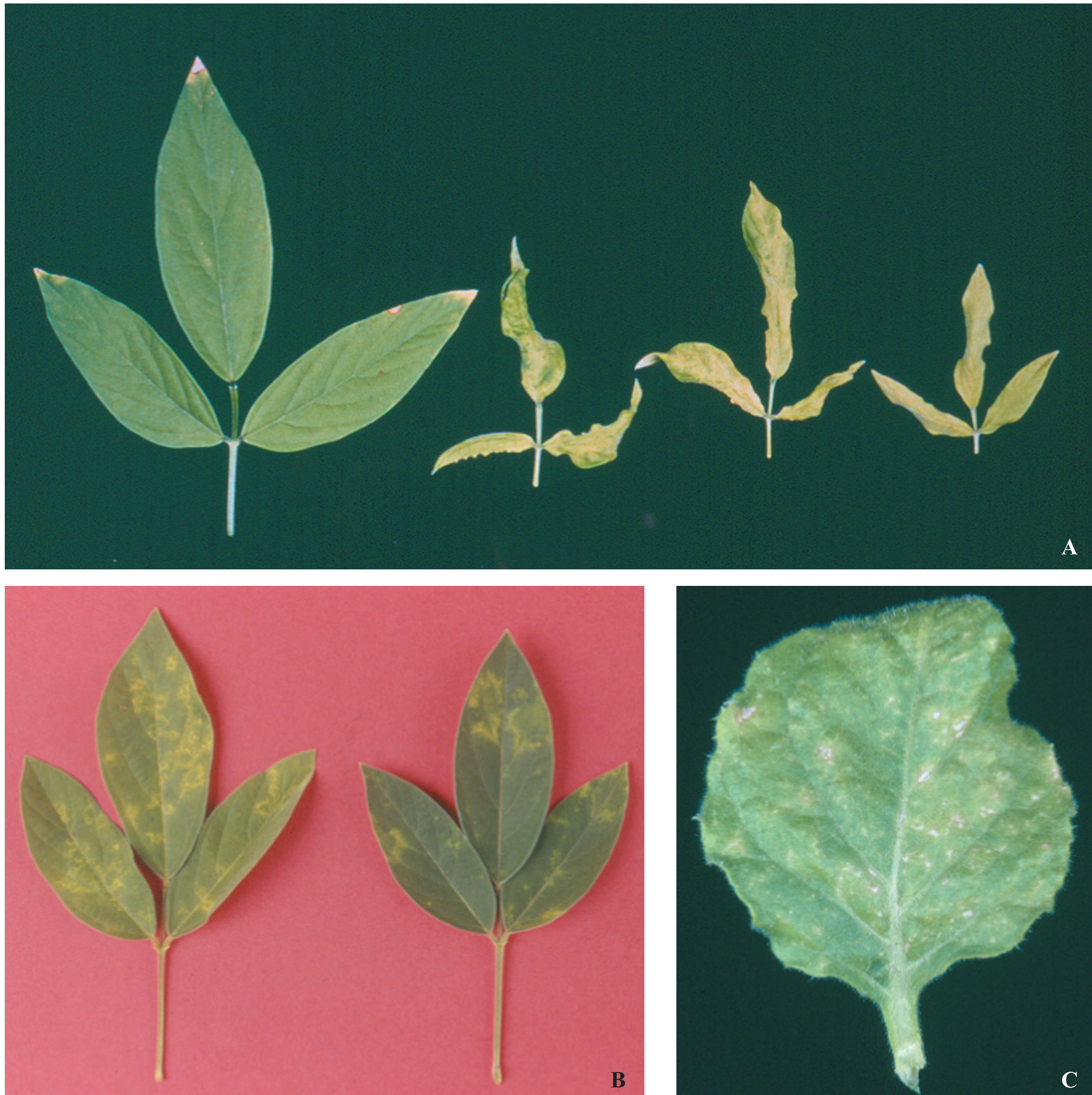

Fig. 1. Leaf symptoms of PPSMV-infected, (A) pigeonpea cv. ICP8863 showing severe mosaic, healthy leaf on left, (B) cv. ICP2376 showing chlorotic ringspots, and (C) *N. benthamiana* showing mild chlorosis and necrotic spots.

2001*a*, unpublished data). The VLPs are believed to be those of the agent of SMD, provisionally named *Pigeonpea sterility mosaic virus* (PPSMV). The particles are difficult to purify and are highly unstable *in vitro*, but they were partially characterised and a polyclonal antiserum to PPSMV VLP preparations produced. Using this antiserum in ELISA and Western blotting has shown the specific association of PPSMV with SMD. The purified VLP preparations of PPSMV contained a major virus-specific polypeptide of 32 kDa and up to 6 RNA species of *c*. 1.1-3.5 kb. The nucleotide sequence of some cDNA clones made to PPSMV RNA and the analysis of the virus-specific 32 kDa protein by matrix-assisted laser desorption ionisation-time of flight (MALDI-ToF), showed no significant sequence matches to any known viral sequences in database searches (Kumar *et al*., 2001*a*, unpublished data).

Although the purified PPSMV VLP preparations were not infective to plants, PPSMV was transmitted experimentally to *Nicotiana benthamiana* by mechanical inoculation of fresh leaf sap extracts of SMD-affected pigeonpea. Systemically infected leaves of *N. benthamiana* plants developed mild chlorosis and some necrotic spots (Fig. 1C; Kumar *et al*., 2001*a*).

Although the taxonomic relationship of PPSMV to other viruses is as yet unclear, the morphology of

the VLPs in purified preparations and the number and sizes of its apparent nucleoprotein components has some similarities to members of the genus *Tenuivirus* and to the recently reported High Plains virus (HPV) (Jensen *et al*., 1996; Falk & Tsai, 1998). PPSMV and HPV are each transmitted by eriophyid mites, and infected plants contain a virus-specific 32 kDa protein and up to six RNA species (Jensen *et al*., 1996; Kumar *et al*., 2001*a*, unpublished data; Mirabile *et al*., 2001). However, the two viruses are serologically unrelated and differ in host range and in the vector mite species involved in transmission (Kumar *et al*., 2001*b*; Jensen *et al*., 1996). Ultrastructural studies of plants affected with HPV detected large ovoid bodies *c*. 100-200 nm in diameter (Ahn *et al*., 1996, 1998). These bodies were reported to have a double membrane and were therefore termed, 'double membrane bound-bodies' (DMBs). It is difficult to unequivocally identify two distinct membrane layers in the electron micrographs in these and in our studies reported here. Because we make frequent reference and comparisons with this earlier information we refer to them throughout this paper in a generic way as membrane-bound bodies (MBBs).

Similar MBBs were also detected in tissues of plants infected with the uncharacterised mite-transmitted agents of several other diseases such as, fig mosaic, rose rosette, thistle mosaic, wheat spot mosaic and yellow ringspot of budwood (Bradfute & Nault, 1969; Appiano, 1982; Roberts & Jones, 1997; Ahn *et al*., 1996, 1998). Some workers have suggested that these MBBs are novel virus-like particles and this was strengthened when *in situ* immuno-gold labelling (IGL) using antiserum to HPV preparations heavily labelled the MBBs present in HPV-infected tissues (Jensen *et al*., 1996; Ahn *et al*., 1996, 1998).

In this paper, we report the ultrastructural effects of PPSMV in its natural host, pigeonpea, and in an experimental host, *N. benthamiana,* and report the occurrence of quasi-spherical MBBs in these infected plants, and their labelling with antiserum to PPSMV in immuno-gold labelling studies. The viral nature of the PPSMV MBBs is discussed in the light of these findings.

Materials and Methods

Plant material and virus inoculation

For ultrastructural studies, two pigeonpea cultivars were used; the highly SMD-susceptible cv. ICP8863 that shows severe mosaic symptoms and sterility, and the SMD-tolerant cv. ICP2376 that shows only chlorotic ringspot symptoms and little or no sterility. Pigeonpea seedlings at the primary leaf stage (11-15 days old) were inoculated with *A. cajani* cultures maintained in growth cabinets using the leaf stapling technique (Nene & Reddy, 1976). Inoculated plants were kept in growth cabinets maintained at 27°C with 55% humidity during the day and 18°C and 35% humidity during the night.

Symptomatic leaves of SMD-affected pigeonpea were extracted in three volumes of 2% nicotine solution and the inoculum rubbed immediately onto corundum-dusted leaves of *N. benthamiana* plants that were then kept in an insect-proof glasshouse maintained at 18-26°C.

Antiserum production

A polyclonal antiserum to PPSMV was raised in a rabbit by immunising with purified PPSMV preparations made from infected pigeonpea (Kumar *et al*., 2001*a*, unpublished data). Immunoglobulin G (IgG) from four bleeds of this polyclonal antiserum was precipitated with ammonium sulphate as described by Van Regenmortel (1982) and assessed for reactivity in IGL experiments. To minimise the non-specific reaction of these IgGs to healthy plant proteins, IgG dilutions were cross-absorbed for 6-7 h with healthy pigeonpea protein, prepared as described by Da Rocha *et al*. (1986).

Electron microscopy

Fresh leaves from infected pigeonpea cv. ICP8863 showing severe mosaic (Fig. 1A), cv. ICP2376 showing chlorotic ring spots (Fig. 1B), and *N. benthamiana* showing veinal chlorosis and necrotic spots (Fig. 1C), were harvested from two different batches of infected material and sliced immediately into small pieces (*c*. 1 mm × 4 mm) with a new scalpel blade and transferred into fixative solution (5% glutaraldehyde in 0.1 M PIPES, pH 8). These pieces of tissue were processed immediately or, for samples taken in India, left in fixative in transit (4-6 days) and the embedding procedure completed upon receipt.

The tissues sampled for thin sectioning studies were from symptom-bearing areas of *c*. 45 day-old pigeonpea cv. ICP8863 but from *c*. 80 day-old cv. ICP2376 because this cultivar develops symptoms to PPSMV only slowly. PPSMV is also slow to multiply in *N. benthamiana* plants and can be detected usually only a relatively long time after inoculation (~45 days post inoculation (dpi)). Samples from this herbaceous host were therefore collected *c*. 80 dpi. Leaves from healthy pigeonpea and *N. benthamiana* plants of similar age were processed in parallel as controls.

Tissue samples were processed for ultrathin sectioning as described by Fasseas *et al*. (1989). As post-fixation in osmium tetroxide and uranyl acetate affects antigenicity (Bendayan & Zollinger, 1983; Roberts, 1994) this step was omitted for the tissue samples used for IGL experiments. Embedded tissues were sectioned with glass knives, heat

stretched (Roberts, 1970) and mounted on pyroxylin-filmed nickel grids (100 mesh). Sections were post-stained with 5% aqueous uranyl acetate followed by lead citrate and examined in a JEOL 1200 EX or Philips CM10 transmission electron microscope operating at 80 kV.

Immuno-gold labelling

Immuno-gold labelling of thin sections was done essentially as described by Fasseas *et al*. (1989). Serial sections were blocked for 1 h with IGL buffer (0.05 M PIPES buffer, pH 7.5 containing 0.5% bovine serum albumin, 0.5% Tween-20 and 0.01% sodium azide). Grids were then transferred onto 25 µl drops of PPSMV IgG diluted 1:100 in IGL buffer and incubated overnight (16-18 h) at room temperature. Grids were washed with IGL buffer and incubated for 5 h on 15 nm colloidal gold conjugated anti-rabbit IgG (AuroProbe, Amersham Life Sciences, Buckinghamshire, UK). After washing with 0.05 M PIPES buffer, pH 7.5 followed by washes with distilled water, the grids were stained with uranyl acetate and lead citrate as above and examined in the electron microscope. To confirm the specificity of the IGL to PPSMV antigen, three kinds of experimental controls were used: (i) immuno-gold labelling with antiserum to PPSMV of sections from leaves of healthy pigeonpea and *N. benthamiana*, (ii) treatment of sections of PPSMV-infected tissues with an antiserum to a heterologous virus (*Tobacco mosaic virus*, TMV) and (iii) treatment of the sections with the gold conjugate alone.

Results

General cell structure

The cellular leaf structure of healthy and PPSMV-infected pigeonpea showed some distinctive features. The central vacuoles of some palisade cells were often filled with electron dense material similar in appearance to that sometimes found both in healthy tissues and in tissues of blackcurrant infected with *Blackcurrant reversion virus* and raspberry infected with Rubus yellow net and Raspberry vein chlorosis viruses (I M Roberts, unpublished data). This material occurred as a single large mass or as several smaller clumps in a single cell. It was also observed in smaller amounts in mesophyll cells, but never in the cells of conductive tissue. In PPSMV-infected pigeonpea, the cell cytoplasm was granular and chloroplasts were often deformed by large starch grains and there were large intercellular spaces typical of leaves of this age. All the other organelles appeared normal.

In cells of healthy and PPSMV-infected *N. benthamiana*, the chloroplasts were also deformed by large starch granules and there were large intracellular spaces. The cytoplasm was sparse and appeared granular, and many cell organelles were deformed. However, it is likely that some of these structural features could be due to the age of the plants sampled.

Cytopathology of infected cells

Electron microscopy of ultrathin sections detected two types of inclusions in cells of PPSMV-infected pigeonpea (cvs ICP8863 and ICP2376) plants: (i) quasi-spherical membrane-bound bodies (MBBs) of *c*. 120-150 nm in diameter (Figs 2, 3, 5) frequently found in association with amorphous electron-dense material (EDM) (Fig. 4), and (ii) fibrous inclusions (FIs) (Fig. 3) which were regularly found adjacent to the cell nucleus. In infected *N. benthamiana*, MBBs and EDM, but not FIs, were observed (Figs 2, 4). None of these structures were observed in healthy plants.

In size, shape and appearance, the MBBs observed in the two pigeonpea cultivars and in *N. benthamiana* were similar (Fig. 2). The MBBs were found singly only in low numbers throughout the cytoplasm but were more commonly found in groups (Figs 2, 3, 5). Serial sections conclusively showed that these MBBs were discrete, quasi-spherical structures, although a few appeared as dumb-bells (Fig. 5). No crystalline or paracrystalline arrays of MBBs were observed. MBBs were present in all cell types except those of the vascular bundles, and were rarely observed in bundle sheath parenchyma cells. The MBBs were often found lying close to the plasmalemma, but this may reflect their position relative to the paucity of the cell cytoplasm. The bounding membrane of each MBB was sometimes obscure or incomplete and we found no convincing evidence that they had a double membrane (Fig. 5). The central area of some MBBs was semi-electron opaque and some were filled with EDM (Figs 2, 4, 5). In some cells, partially formed MBBs, elongated vesicles or dumb-bell shaped structures were seen (Figs 2, 5). In some cells, patches of EDM were found closely associated with groups of MBBs (Fig. 5) and with membranes that appeared to be derived from the ER. Although in a few cells, EDM was observed on its own in the cytoplasm (Fig. 5B), this may be because the association with the MBBs may be out of the plane of the section. In a few cells of PPSMV-infected *N. benthamiana,* a sponge-like network of membrane-bound electron-dense material was found that had the same IGL specificity as the MBBs seen elsewhere (Fig. 4).

In the cytoplasm of palisade cells of each pigeonpea cultivar, FIs composed of randomly dispersed fibrils, and often associated with the cell nucleus, were seen (Fig. 3). The fibrils in FIs were of indeterminate length and showed no crystalline or paracrystalline aggregation patterns, but appeared to be disjointed or twisted with large electron lucent

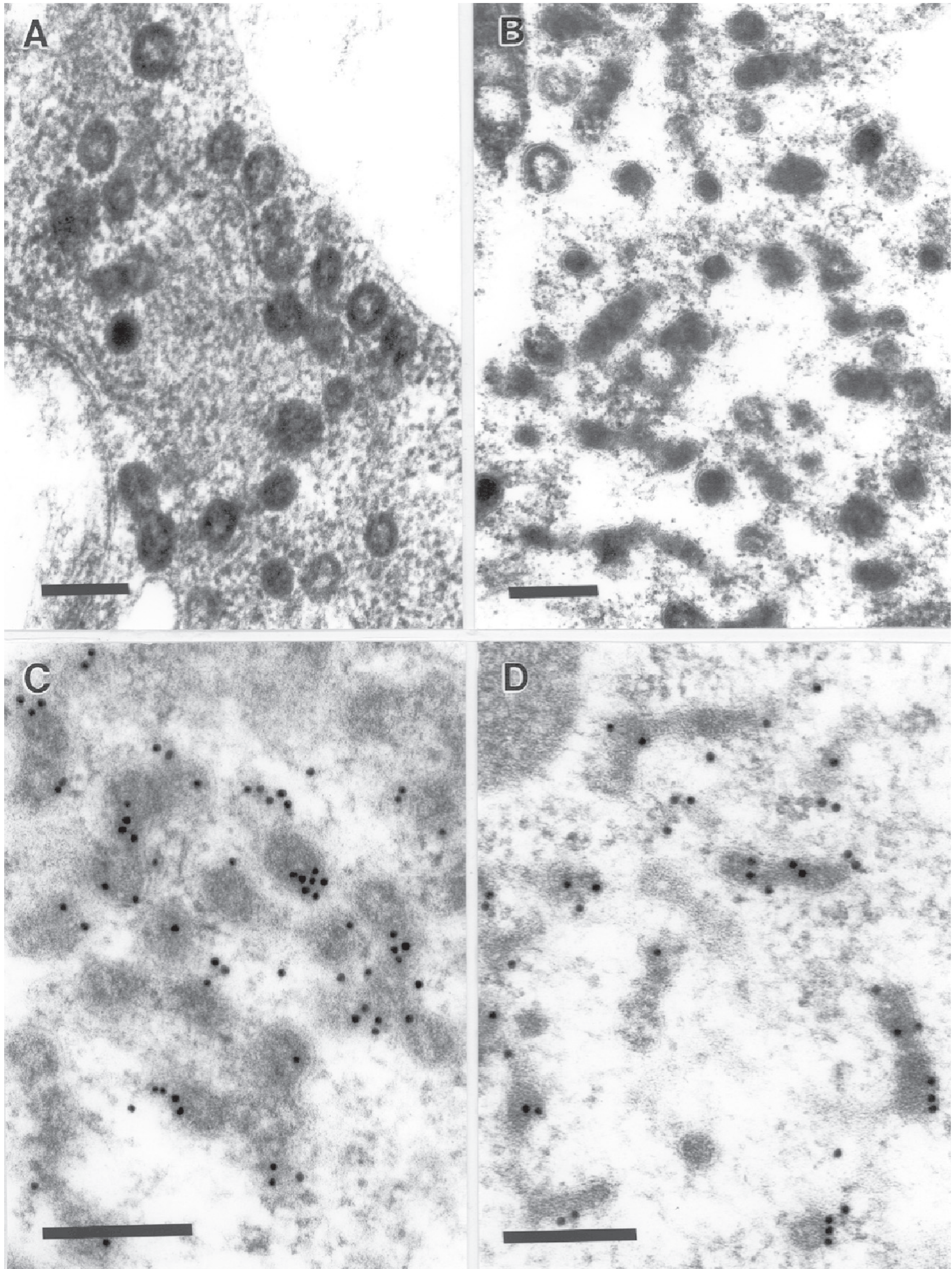

Fig. 2. Electron micrographs of sections showing groups of MBBs in palisade cells of PPSMV-infected (A, C) pigeonpea, and (B, D) *N. benthamiana*. Sections in 'A' and 'B' were post-fixed with osmium tetroxide. Sections 'C' and 'D', were fixed in glutaraldehyde alone and immuno-gold labelled using anti-PPSMV IgG. Note the labelling of the MBBs. Bars = 200 nm.

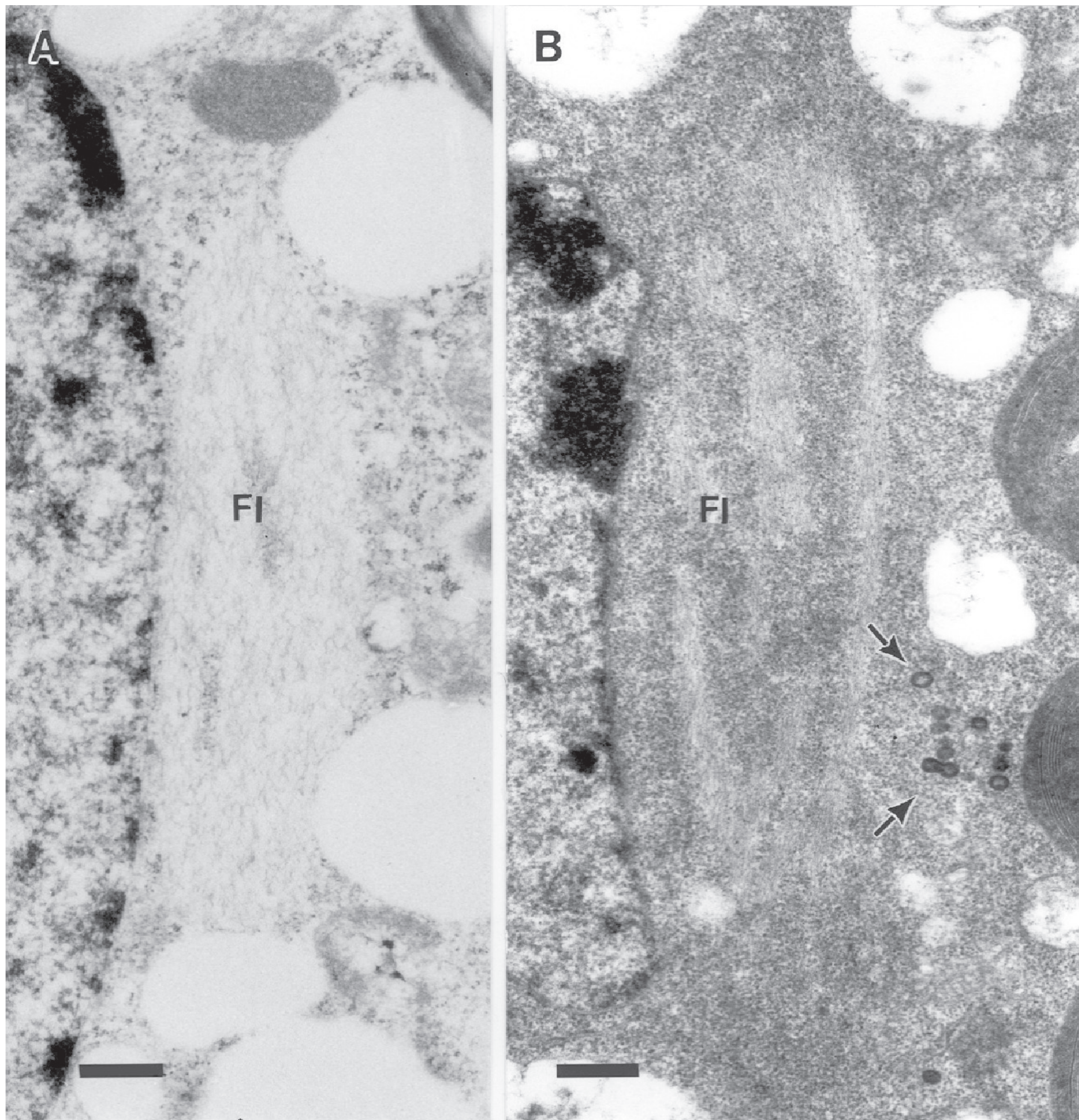

Fig. 3. Electron micrographs of sections of PPSMV-infected pigeonpea showing FIs. (A) leaf tissue fixed in glutaraldehyde alone and (B) leaf tissue post-fixed in osmium tetroxide showing an FI with MBBs (arrow) nearby. Bars = 500 nm.

areas between them (Fig. 3A). The FIs were not membrane bound and occurred in various sizes, sometimes as large as the cell nucleus and also occurred as small discrete patches interspersed in the cytoplasm. The FIs could be differentiated from the rest of the cytoplasm by the absence of ribosomes. FIs and MBBs were found in the same cell (Fig. 3B). The FIs were most common in palisade cells, less common in mesophyll cells and were not found in epidermal or vascular cells. These inclusions were most clearly seen in tissues fixed in glutaraldehyde alone (Fig. 3A), and were less obvious in tissues post-fixed with osmium tetroxide (Fig. 3B). FIs were not detected in any of the sections of PPSMV-infected *N. benthamiana* plants examined.

Immuno-gold labelling of ultrathin sections

PPSMV IgG purified from four different bleeds gave similar binding specificities; for most of the assays described here, IgG obtained from bleed-2 was used. Cross-absorption of the PPSMV antiserum dilution with an extract of healthy pigeonpea markedly improved the label specificity by decreasing the amount of background gold. Examination of the thin

Fig. 4. Electron micrograph of a section of a PPSMV-infected *N. benthamiana* leaf showing (A) a sponge-like network in the condensed cytoplasm of a mesophyll cell, and (B) a higher magnification of the area marked in (A). Note the specific IG labelling of the electron-dense material and the bulbous regions in some parts of the network (arrows). Bars = (A) 1 µm, (B) 200 nm.

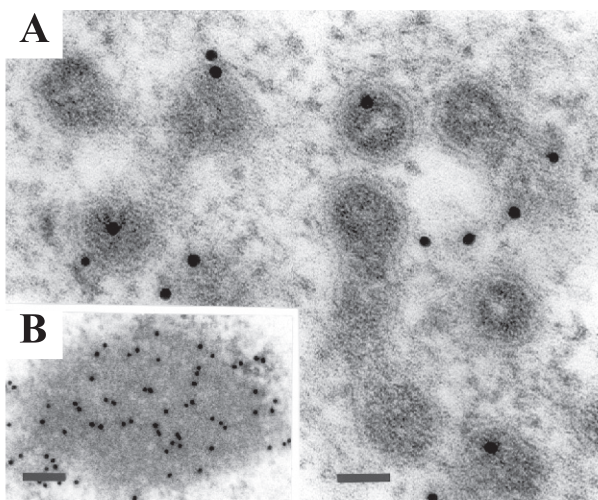

Fig. 5 (left). Immuno-gold labelled sections of PPSMV-infected pigeonpea showing (A) a clearly defining membrane surrounding, or partially surrounding MBBs and specific labelling of these bodies, and (B) a discrete mass of EDM showing specific IG labelling. Bars = (A) 50 nm, (B) 100 nm.

sections of infected pigeonpea (cvs ICP2376, ICP8863) and *N. benthamiana* immuno-gold labelled using PPSMV IgG showed that gold particles were specifically localized on the MBBs and associated EDM (Figs 2, 4, 5). The EDM observed in infected cells, whether present together with MBBs, or alone, was more heavily labelled with gold particles than were the MBBs (Fig. 5). The FIs found

in infected pigeonpea were not labelled by PPSMV IgG but some non-specific labelling of the nucleus and cell wall was observed. Control experiments using heterologous antiserum, treatment of sections with gold conjugate alone, and IGL experiments on sections from healthy plants, produced only sparse, randomly scattered gold particles binding to sections.

Discussion

Our ultrastructural studies of the two pigeonpea cultivars infected with PPSMV by viruliferous mites and of *N. benthamiana* plants infected by mechanical inoculation with sap extracts from SMD-affected pigeonpea detected MBBs and EDM very similar to those reported in plants infected with other mite-transmitted agents (Ahn *et al*., 1996, 1998). The very similar cytopathology of the two pigeonpea cultivars (the SMD-susceptible cv. ICP8863 and the SMD-tolerant cv. ICP2376) infected with PPSMV indicates that the different symptomatology in these two cultivars following infection with PPSMV is due to host factors as suggested from earlier studies (Reddy *et al*., 1998; Kumar *et al*., 2001*b*). Immunogold labelling using antiserum to PPSMV indicated that the MBBs and EDM found in infected pigeonpea and *N. benthamiana* contain the 32 kDa antigen that is present in purified PPSMV VLP preparations and in leaf sap extracts from PPSMV-infected plants (Kumar *et al*., 2001*a*, unpublished data).

The detection of MBBs and EDM in PPSMV-infected *N. benthamiana*, that are indistinguishable in structure and specific labelling from those found in infected pigeonpea (Fig. 2), confirms the mechanical transmission of PPSMV to this herbaceous plant. This is a very significant finding because several decades of work on eriophyid-mite transmitted agents of unknown aetiology have suggested that these agents are not transmitted mechanically and have a restricted host range. HPV is reported to be transmitted mechanically in sap to moist maize seed by vascular puncture (Loui & Seifers, 1998; Forster *et al*., 2001), but this disease agent seems restricted in host range to species in the Graminae (Forster *et al*., 2001). By contrast, the mite-transmitted agent of fig mosaic was transmitted from its natural host, fig (*Ficus carica*: Moracea), to periwinkle (*Caranthus roseus*: Vinceae) by its mite vector, *Aceria ficus,* and the cells of the infected periwinkle plants contained MBBs indistinguishable from those present in infected fig (Credi, 1998). More recently, Rohozinski *et al*. (2001) reported the mechanical transmission to *Nicotiana* species of a virus-like agent from multiflora rose affected with rose rosette disease, another disease caused by a virus-like agent transmitted by eriophyid mites (Amrine *et al*., 1988; Epstein & Hill, 1995). However, in their ultrastructural studies of infected *Nicotiana* plants they failed to detect the MBBs that are known to be present in rosette-diseased multiflora rose (Gergerich *et al*., 1989; Ahn *et al*., 1996) suggesting that the agent in their experimental host may not be that inducing rosette disease. Furthermore, measurements of the isometric particles they reported in crystalline arrays in cells of infected *Nicotiana* plants (Rohozinski *et al*., 2001), measure 10 nm or less in diameter, very close to that expected for peroxisome catalase. If these are indeed catalase crystals, it is possible that the absence of a bounding membrane around them is due to cell autolysis.

In pigeonpea, in addition to MBBs and EDM, FIs were found but these were not labelled with PPSMV antiserum (Fig. 3). FIs were not detected in infected *N. benthamiana,* but it is possible that in this host FIs may be growth stage-specific or, because of the poor state of the *N. benthamiana* tissue due to age, they are not identifiable. The FIs were not observed in cells of plants infected with other eriophyid mite-transmitted agents of unknown aetiology (Ahn *et al*., 1996, 1998). FIs have some similarities to the amorphous semi-electron opaque inclusions found abundantly in plant cells infected with tenuiviruses (Ammar *et al*., 1985; Espinoza *et al*., 1991, 1992) and that contain a non-capsid virus protein (NCP). In tenuivirus and tospovirus infections, NCP inclusions can occur in various forms depending on the developmental stage of plants (Espinoza *et al*., 1992; Kitajima *et al*., 1992). It is possible therefore that the FIs in PPSMV-infected plants may be a NCP of PPSMV. However, it is necessary to characterise more fully the PPSMV genome to assess this possibility further.

In leaf sap extracts and in purified preparations of PPSMV and HPV, no structures comparable to MBBs were detected. However, thin filamentous VLPs *c*. 5-10 nm were observed in some highly purified preparations but these were not infective to plants (Ahn *et al*., 1998; Kumar *et al*., 2001*a*, unpublished data). It is possible that these VLPs may represent tightly packed structural components, such as nucleoproteins, that are released from ruptured MBBs during the purification process. If this is so, then it may explain the requirement for detergent to purify the VLPs. Alternatively, the VLPs may be fragments of the FI seen in the cytoplasm of infected pigeonpea cells. However, this later possibility seems less likely because the FIs were not labelled with antiserum to PPSMV, that was produced by immunisation with purified PPSMV VLPs, nor were they detected in PPSMV-infected *N. benthamiana*. It is therefore unclear if either the MBBs or the filamentous VLPs represent the infective particles of PPSMV. Our other studies have shown that PPSMV infectivity is short-lived in sap (15 min) (Kumar *et al*., 2001*a*, unpublished data). Possibly, the lack of infectivity observed with purified

preparations could be due either to the length of time the VLPs are *in vitro*, or that intact MBBs, that are destroyed in the purification process, may be required for infectivity.

Intracellular inclusions induced by plant viruses can be unique to particular viruses or virus genera and have been used as valuable tools in virus identification (Edwardson *et al*., 1993). Inclusions can consist of virus particles, cell organelles, and virus-related material such as amorphous, granular, fibrillar or other proteinaceous structures with a conspicuous morphology (Francki *et al*., 1985). The MBBs found here, and in plants infected with HPV and other eriophyid mite-transmitted agents of unknown aetiology, are unusual and have a distinct morphology (Ahn *et al*., 1998). Viroplasmic-like structures, such as proliferated rough ER and EDM that are common in viroplasms of many plant and animal viruses, were found in association with MBBs in infected cells. It is perhaps noteworthy that this sponge-like network of EDM resembles the cisternal ER, although there is no direct evidence of an association of the MBBs and the ER in our sections. The EDM observed in tissues infected with PPSMV, HPV and thistle mosaic are like those reported in plants infected with some tospo-, caulimo- and phytoreo-viruses (Shepherd, 1976; Stern *et al*., 1977; Francki *et al*., 1985; Ahn *et al*., 1996, 1998; Kitajima *et al*., 1992). Very occasionally, some sections suggested that the MBBs may occur by budding from cell membranes (Figs 2, 5). If this is so, then they resemble the immature particles of animal and insect poxviruses and of the immature particles of *Tomato spotted wilt virus* (TSWV) at an early stage of infection (Milne, 1970; Francki *et al*., 1985; Kitajima *et al*., 1992).

Our ultrastructural studies reported here, together with those of others, would indicate a close similarity of PPSMV with HPV and the agents involved in fig mosaic, rose rosette, thistle mosaic, wheat spot mosaic and yellow ringspot of budwood (Bradfute & Nault, 1969; Appiano, 1982; Roberts & Jones, 1997; Ahn *et al*., 1996, 1998), each of which is transmitted by eriophyid mites. This is supported further by the similarities in biochemical properties of purified preparations of the VLPs of PPSMV and HPV that suggest that these viruses are distinct from those assigned to current virus genera (Jensen *et al*., 1996; Mirabile *et al*., 2001; Kumar *et al*., 2000, 2001*a*, unpublished data). Nevertheless, for PPSMV, the morphology of its VLPs in purified preparations, the number and sizes of its nucleoprotein components, and some ultrastructural features induced in plants following infection, are similar to viruses in the genus *Tenuivirus* and possibly the genus *Tospovirus* (Ammar *et al*., 1985; Espinoza *et al*., 1992; Kitajima *et al*., 1992). However, PPSMV differs from them serologically, in host range and transmission (Kumar *et al*., 2001*b*; Falk & Tsai, 1998). Only the unequivocal identification of the infectious particle of PPSMV and the complete characterisation of the PPSMV genome will resolve the relationships, if any, between PPSMV and member species of these, and possibly other, virus genera.

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