Virus-like Particles and Cellular Changes in Plants Infected with Sweetpotato Viruses

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Studies with the transmission electron microscope were used to detect and attempt to identify viruses infecting sweetpotato (*Ipomoea batatas*) and other *Ipomoea* species. Flexuous-rods, short curved-rods, and spherical virus-like particles were observed in cells of symptomatic plants. Also, various cytopathic changes such as crystals, vesicles, fibril structures, and cylindrical inclusions were observed. The present study showed that some of these cytopathic changes were associated with some viral groups, which might be helpful in diagnosis.

Keywords : Crinivirus, Cytopathology, Geminivirus, Potyvirus, Sweetpotato viruses

Electron microscopy has played an important role in the development of plant virology, contributing significantly to the understanding of molecular biology and pathology of viruses and to the development of virus taxonomy and diagnostics. Electron microscopy studies have revealed wide differences in particle morphology and size of viruses, which are of great help in classification and virus detection. Moreover, with the electron microscope, virologists have determined that virus particles may occur in most plant tissues, such as *Tobacco mosaic virus* (TMV), or in specific tissues only such as phloem-limited *Citrus tristeza virus* (CTV). Electron microscopy studies have also helped to trace the viruses in their insect vectors (Bos, 1983).

There are many sweetpotato (*Ipomoea batatas* (L.) Lam) virus diseases described in the literature and various virus groups have been identified as the causal agents (Clark and Moyer, 1988; Moyer and Salazar, 1989). However, the etiology of several sweetpotato diseases has not been determined and therefore reliable detection procedures for some of them have not been developed (Moyer and Salazar, 1989).

Viruses have been presumed for many years to cause

several important diseases of sweetpotato, but the first extensive characterization of a sweetpotato virus was conducted with *Sweetpotato feathery mottle virus* (SPFMV) (Moyer and Cali, 1985; Moyer and Kennedy, 1978). Recently, two sweetpotato viruses, the United States isolate of *Sweetpotato leaf curl virus* (SPLCV-US) (Lotrakul et al., 1998) and *Sweetpotato chlorotic stunt virus* (SPCSV) (Winter et al., 1992) have been well characterized and their cytopathic effects reported. Although virus etiology is currently an area of considerable research activity, several sweetpotato viruses have yet to be isolated and characterized successfully (Chavi et al., 1997; Gibson et al., 1998). This could be due in part to the lack of information about inter-relationship between different virus groups and the role of viruses on host plant showing synergism.

Studies of sweetpotato tissues from infected plants using electron microscope can provide information that could be useful for virus identification. The purpose of this investigation was to identify the virus or viral group from various *Ipomoea* tissues infected with different sweetpotato viruses.

Materials and Methods

Plant materials and source of viruses. The sweetpotato heirloom cultivar White Bunch and the African cultivar Wagabolige (PI595888) were obtained from the USDA (sweetpotato germ plasm repository) Plant Genetics Resources Unit at Griffin, GA, propagated by cuttings and maintained in screen cages in a greenhouse. White Bunch was reported to be mix-infected with SPFMV and SPCSV by Pio-Ribeiro et al. (1996). Since *I. aquatica* is not a host for most SPFMV strains (C. A. Clark, personal communication), scions of White Bunch were grafted to *I. aquatica* in order to eliminate SPFMV before using it as source of a SPCSV for further studies. A single-whitefly-transmitted isolate of SPCSV maintained on *I. nil* cv. Scarlet O'Hara (SOH) was used in most experiments. Cuttings of Wagabolige were planted in the field, and later scions from field plants grafted to *I. setosa* (C. A. Clark, personal communication).

The sweetpotato breeding line W-285 showing leaf curl

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symptoms was obtained from the USDA-ARS Vegetable Laboratory, Charleston, SC. Scions from W-285 were grafted to *I. aquatica*, *I. cordatotriloba*, SOH, and *I. setosa*.

Scions of sweetpotato samples collected from farms from various areas in the United States were grafted onto *I. setosa*. Grafted plants reacted with chlorosis, chlorotic spots, leaf curl, leaf malformation, mild mottle, mosaic, and vein clearing. Some samples were selected, because they were negative or gave weak positive reactions in preliminary tests using the enzyme-linked-immunosorbent assay (ELISA) with polyclonal antibodies for the severe russet crack strain of SPFMV (SPFMV-SRC) (supplied by J. Moyer, North Carolina State University). However, these samples reacted positive with a monoclonal antibody for *Potato virus Y 1* (PTY 1) (Agdia, Elkhart, Indiana) that reacts with most potyviruses. The selected field-collected sweetpotatoes were designated LSU-1, LSU-2, LSU-3, LSU-4, and LSU-5. Virus isolates were obtained for LSU-2 and LSU-5 after single local lesion transfers through *Chenopodium quinoa*. Additionally, a single aphid probe was used to obtain an isolate of LSU-1. SOH and *I. setosa* mechanically inoculated with SPFMV-C were used as control for infection of SPFMV.

Beach morning glory (*I. pes-caprae*) cuttings showing mild mottle were collected from a beach near Tampa (Pinellas County) FL. *I. setosa* was grafted with scions from the beach morning glory and reacted with leaf distortion and crinkling. The putative virus causing these symptoms was mechanically transmitted to *I. setosa* and SOH.

Uninoculated SOH and *I. setosa* seedlings routinely grown in the greenhouse were used as controls.

Tissue preparation for transmission electron microscopy. Tissue samples of about 2 mm in width and 5 mm in length (veins and adjacent mesophyll) from leaves of plants infected with the previously listed viruses were collected for electron microscopy studies. Generally, samples were obtained from infected plants 2-4 weeks after inoculations. Samples were fixed in 4% glutaraldehyde containing 0.05 M cacodylate buffer (pH not adjusted) for 2 h at room temperature, followed by three washings (20 min each) with the same buffer. After post-fixation in 1% osmium tetroxide for 2 h, samples were washed briefly with distilled water, and stained overnight in 0.5% uranyl acetate at 4°C. The following day, samples were dehydrated by graded dilution series of ethanol up to 100% at room temperature. Infiltration was achieved through 50% (v/v) LR (London Resin) White Resin medium grade (Electron Microscopy Sciences, Fort Washington, PA) in 100% ethanol and two times in 100% LR White Resin; each step was 30 min in duration. Blocks were polymerized by exposure to a temperature at 60°C overnight. Ultra-thin sections (about 70 nm) were cut with a glass knife and collected on Formvar film-coated copper grid (100 square mesh, Electron Microscopy Sciences, Fort Washington, PA), then stained in lead citrate for 15 sec, and observed using a transmission electron microscope (100CX, JEOL) operated at 80 kV.

Fig. 1. Thin section of a phloem parenchyma cell of *Ipomoea nil* cv. Scarlet O'Hara infected with a whitefly-transmitted isolate of *Sweetpotato chlorotic stunt virus* from the cultivar White Bunch. Virus-like particles are seen in the cytoplasm (A). Vesicles containing fibrils are aggregated in the cytoplasm (B) and higher magnification of vesicles containing fibrils. CW, cell wall; DM, double membrane; N, nucleus; Np, proliferated nuclear membrane; Vp, virus-like particles; Vc, vacuole; Vs, vesicles.

Results

SPCSV-WB. SOH inoculated with a whitefly-transmitted isolate of SPCSV from White Bunch (SPCSV-WB) showed leaf distortion and general or interveinal chlorosis. Infected tissues of SOH yielded cells (phloem parenchyma) with flexuous, rod-shaped virus-like particles of approximately 12 nm in diameter of undetermined length (Fig. 1A). Also, infected tissues had cellular changes that were similar to those induced by most members of the family

Closteroviridae (Brunt et al., 1996; Francki et al., 1985). Many membrane-enclosed circular and oval-shaped vesicles of approximately 50-100 nm in diameter were observed in parenchyma cells (Fig. 1B). A higher magnification of these vesicles revealed that they all contained densely stained fibrils (Fig. 1C). In most cases, the vesicles occurred in electron-lucent vacuoles of various sizes, which were devoid of other cytopathic components. In vacuoles, the vesicles often were aligned in a single row throughout the internal surface, or spread inside of a vacuole. Both the

Fig. 2. Thin section of a phloem parenchyma cell of *I. cordatotriloba* grafted with a scion from the sweetpotato breeding line W-285. Aggregated virus-like particles are seen in the nucleoplasm (A) and a fibril body is shown in the nucleus (B). Aggregation of virus-like particles (C) is seen and higher magnification of aggregates of virus-like particles (D). Thin sections showing filamentous virus-like particles in the phloem parenchyma cells of *I. cordatotriloba* (E) and *I. setosa* (F) grafted with a scion from the sweetpotato breeding line W-285. CW, cell wall; FB, fibril body; N, nucleus; Nm, nuclear membrane; No, nucleolus; VA, aggregated virus-like particles; VI, individual virus-like particles; Vp, virus-like particles.

vacuoles and the vesicles were bounded by single membrane. The number of vesicles in the vacuole was variable. The vacuole seemed to be aggregated together at specific regions in the cytoplasm and these aggregates were double membrane bounded. Also, the membrane surrounding the area of vacuole aggregation seemed to be associated with the nuclear membrane. The side of the nuclear membrane near the aggregation proliferated and some parts of the nuclear membrane were fused with the membrane surrounding the vacuole (Fig. 1B). Virus-like particles were rarely found in the cytoplasm, and were not observed in the vesicles.

Virus-like particles and inclusions from the sweetpotato breeding line W-285. Three different types of virus-like particles were observed in *I. cordatotriloba* that was graft inoculated with scions from W-285 showed leaf curling and vein yellowing. First, virus-like particles and fibrillar inclusions were observed in the nucleus of phloem parenchyma cells (Fig. 2A and 2B). Crystalline arrangements of virus particles were not observed. The fibrillar inclusions were approximately 4 μm in diameter. These structures consisted of fine, densely packed fibrils and contained many pore-like regions. The size of these pores was variable. Virus-like particles were not observed in the fibril body but were

Fig. 3. Thin section of mesophyll tissue is showing cylindrical inclusions in the cytoplasm of *I. nil* cv. Scarlet O'Hara (A) infected with the LSU-1 virus and *I. setosa* (B) infected with the common strain of *Sweetpotato feathery mottle virus*. Aggregation of virus-like particles might effect on malformation of vacuole (C) and virus-like particles are along the tonoplast (D). Virus-like particles with inclusion bodies are showing in the cytoplasm of a phloem parenchyma cell of *I. setosa* infected with the common strain of *Sweetpotato feathery mottle virus* (E). Crystalline inclusions are showing in the nucleus of a glandular trichome from healthy *I. nil* cv. Scarlet O'Hara (F). Bd, bundle; Ci, circle; CrI, crystalline inclusion; CW, cell wall; EpC, epidermal cell; Lp, loop; Mt, mitochondria; N, nucleus; Nm, nucleus membrane; PW, pinwheel; Sc, scroll; TC, trichome cell; Tp, tonoplast; Vc, vacuole; Vp, virus-like particles.

not observed in healthy tissues. These inclusions which are similar to those induced by members of the family *Geminiviridae*, were not frequently detected in *I. cordatotriloba*. Twin or geminate particles were not found in leaf dip preparation or fixed tissues.

Filamentous particles also were observed in the nucleus of vascular parenchyma cells (Fig. 2C and 2D). Three or more such particles were aggregated into bundles or many individual particles were scattered through a viroplasm-like area. The length of individual particles was about 200-270 nm, but the particle length from bundles was about 120-150 nm. No viral-like inclusion bodies were found in the cytoplasm. Due to the unusual morphology and appearance of those particles, it was difficult to determine the virus group.

A third type of particle was observed only occasionally in the nucleus of phloem parenchyma cells. They were filamentous and aggregated near the nucleoli (Fig. 2E and 2F). These virus-like particles were not found in the cytoplasm. The particle length was about 1,000-1,500 nm. The width of particles was not determined, but its morphology was similar to that of most flexuous-rod viruses.

In general, the cells from infected plants contained nuclei that had a nuclear membrane that was folded or broken (Data not show). These three different types of particle or viral induced inclusion bodies were not observed in non-inoculated *I. cordatotriloba* or other *Ipomoea* species examined.

Potyvirus-like particles and potyvirus inclusions. Inclusion bodies, which consisted of bundles, circles, pinwheels, and scrolls, were observed in the mesophyll tissues (Fig. 3A and 3B), and also in the epidermal and vascular tissues (data not shown) of plants of SOH and *I. setosa* that were graft inoculated with scions from four selected sweet-

potatoes from the field. However, the loop inclusions such as those produced by SPFMV-C (Fig. 3B), were not observed in the mesophyll tissues of plants of SOH (Fig. 3A). In addition, virus-like particles might affect the shape of vacuoles because cytoplasmic strands containing virus-like particles protruded into the central vacuole (Fig. 3C) and parallel filamentous particles assumed to be viruses were located along the tonoplast and extended along the surface of the vacuole membrane (Fig. 3D). These particles could be incorporated in the cytoplasmic strands that extended into the central vacuole. Aggregates of virus and cross sections of virus aggregates were present throughout the cytoplasm of cells containing inclusion bodies (Fig. 3C). They were flexuous rods of about 12 nm in diameter and ranged between 500 and 800 nm in length. Virus particles usually occurred in the cytoplasm as aggregates of various sizes. Occasionally, a few free SPFMV-like particles were also observed (Fig. 3E). Neither inclusion bodies nor particles were found in the cell organelles of infected plants. Inclusions or abnormal structures were not present in the cytoplasm of cells of non-inoculated plants, except for crystalline inclusions that occurred occasionally in the nucleus of leaf hairs and trichomes of healthy SOH (Fig. 3F).

Isometric virus-like particles from the sweetpotato cv. Wagabolige. *I. setosa* grafted with field collected scions of Wagabolige induced symptoms of chlorosis, vein banding, mottling, and chlorotic spots (C. A. Clark, personal communication). Isometric virus-like particles were found in the cytoplasm of companion cells and vascular parenchyma cells (Fig. 4A and 4B). The particles were spherical 40-55 nm in diameter, and most of them appeared as empty shells with a central cavity (15-25 nm in diameter). Most particles were aggregated with irregular arrangement in the cyto-

Fig. 4. Thin section of a companion cell of *I. setosa* grafted with a scion from the sweetpotato cv. Wagabolige. Spherical virus-like particles are present in the cytoplasm (A). Higher magnification of the spherical virus-like particles (B). Some particles were present in a tubule-like structure. CW, cell wall; FM, fibril material; Pp, periplasm of plasma membrane; Tu, tubule-like structure containing virus-like particles; Vc, vacuole; Vp, virus-like particles.

Fig. 5. Thin section of a mesophyll cell of *I. setosa* mechanically inoculated with leaves of a beach morning glory (*I. pes-caprae*) showing spherical virus-like particles. (A) Spherical virus-like particles with a single membrane in the cytoplasm. (B) Spherical virus-like particles present in the chloroplast. Flexuous virus-like particles are showing in the cytoplasm of a mesophyll cell of *I. setosa* mechanically inoculated with leaves of a beach morning glory (*I. pes-caprae*) (C). G, grana; N, nucleus; S, stroma; Vc, vacuole; Vp, virus-like particle.

plasm, and a few particles were scattered randomly. However, there were groups of 2-4 particles that seem to be inserted in tubule-like structures, and some of these structures were bounded by single membrane to form a vesicle (Fig. 4B). Particles were not observed in some vesicles. Another cytological change consisted in enlargement of portions of the cell membrane and fibrillar materials

scattered in the periplasm (Fig. 4A). Similar materials were scattered around the aggregates of virus-like particles in the cytoplasm. Virus-like particles were not found in cell organelles. Viroplasms were not found in the cytoplasm.

Flexuous-rod and isometric virus-like particles from beach morning glory. Analysis of thin sections from *I. setosa*, showing leaf distortion and crinkling, after being grafted with scions from beach morning glory, *I. pes-caprae*, showed two different virus-like particles. Spherical virus-like particle was observed, about 90-100 nm in diameter (Fig. 5A and 5B). Some particles were found in the cytoplasm of mesophyll cells bounded by a single membrane (Fig. 5A). Others were found in the stroma of chloroplasts of mesophyll cells, and were not membrane bounded (Fig. 5B). The other types of filamentous virus-like particles were observed in the cytoplasm of epidermal and mesophyll cells (Fig. 5C). These particles were found consistently in tissues from plants showing symptoms and were about 12-16 nm in diameter and 500 to 1,200 nm in length and usually found aggregated. However, neither virus-induced inclusions nor cytological changes were observed.

Inclusions in non-inoculated *I. nil* cv. SOH. In thin sections of non-inoculated SOH plants, crystalline inclusions were found in the nucleus of epidermal, secretory glands, and parenchyma cells. Those inclusions were rectangular crystals with loose electron-dense centers like an axis. Some crystalline structures were scissor-like with a diagonal end (Data not show).

Discussion

Pio-Ribeiro et al. (1996) reported the infection of the sweetpotato cultivar White Bunch with the crinivirus, SPCSV. The cytopathology of SOH infected with SPCSV-WB observed in the present study was similar to the typical cellular changes induced by the members of the family *Closteroviridae* (Coffin and Coutts, 1993; Francki et al., 1985) and supports the findings of Pio-Ribeiro et al. (1996). Cytopathic changes included the presence of virus particles and vesicles in phloem cells. Sometimes, virus particles present in sieve element and phloem cells were similar to P-protein, but the occurrence of membranous, fibril-containing vesicles in the cytoplasm is the most important diagnostic feature of infections by members of the genus *Crinivirus* (Coffin and Coutts, 1993; Franki et al., 1985).

Based on the presence or absence of vesicles, there are two different subgroups within the family *Closteroviridae*. Subgroup I induces vesicles and subgroup II does not

(Coffin and Coutts, 1993). Most member of the genus *Crinivirus* belongs to the subgroup I, and they induce vesicles containing fibrils, which have been suggested to be virus-specific RNA (Franki et al., 1985). These vesicles are referred to BYV (*Beet yellow virus*) type (Coffin and Coutts, 1993; Larsen et al., 1991). The origin of the BYV-type vesicles has not been explained because of the lack of association with any particular cell organelle (Esau and Hoefert, 1981; Larsen et al., 1991). In the case of *Diodia vein chlorosis virus* (DVCV), Larsen et al. (1991) suggested that the vesicles associated with this closterovirus were derived from the vacuole membrane. In addition, mitochondrial involvement in the origin of virus-induced vesicles associated with closterovirus-like particles has been reported for grapevine leaf roll disease (Kim et al., 1989). Vesicles in infected plants with SPCSV are formed apparently by invagination or budding of a small portion of the vacuole membrane into the lumen (Winter et al., 1992). According to results of this investigation, vesicles and vacuoles associated with the SPCSV-WB appear to be associated with the nuclear membrane. Therefore, it appears that virus-induced vesicles associated with infection of some members of the *Closteroviridae* have various origins depending upon the particular virus. If so, it would then be possible to use the involvement of cell organelles as diagnostic indicators for the classification of members within this family.

Fibril bodies were found in the nucleoplasm of *I. cordatotriloba* cells infected with one of the viruses obtained from the sweetpotato breeding line W-285. They were similar to those induced by *Bean golden mosaic virus* (BGMV) and other members of the family *Geminiviridae* (Franki et al., 1985; Kim and Flores, 1979; Lastra and Gil, 1981). Virus-like particles occurred only in the nucleus and appeared as numerous clumps or aggregates of virus-like particles. The geminivirus nature of these virus-like particles was confirmed by Lotrakul et al. (1998), and Lotrakul and Valverde (1999). The morphology of the fibril bodies was different from fibril ring-shape inclusions that had been described as a diagnostic feature for whitefly-transmissible geminiviruses (Franki et al., 1985). Based on the results of this investigation and Lotrakul et al. (1998 and 1999), the geminivirus infecting W-285 could belong to a new group of the *Geminiviridae* inducing different fibril ring structures in the nucleus and having unique molecular properties.

Other *I. cordatotriloba* tissue that was graft inoculated with W-285 contained short and curved-rod shaped particles in the nucleus. As previously noted, the finding of virus particles in the nucleus was a good indication that the virus may belong to a DNA virus group. Among the DNA plant viruses, the ultrastructure of plant tissues infected with

members of the family *Geminiviridae* and *Caulimoviridae* have been extensively studied (Brunt et al., 1996; Franki et al., 1985; Kim et al., 1978). The presence of virus particles in the nucleus is related to the location and mode of viral replication (Kong et al., 2000). The short-curved particles may not be a member of the family *Geminiviridae* or *Caulimoviridae*, since their morphology was different. The *Badnaviridae* is another DNA plant virus, group with virions that are bacilliform in shape, 25-35 nm in diameter, 140-400 nm in length, without an envelope (Brunt et al., 1996; Franki et al., 1985). The virus-like particles found in *I. cordatotriloba* were shorter in length than that of the known badnaviruses. Yamashita et al. (1984) reported the infection of sweetpotato by a badnavirus in Japan named *Sweetpotato leaf curl virus* (a name that conflicts with SPLCV). They described a virion of 18 nm in width and 80-200 nm in length, found in both the nucleus and the cytoplasm of infected cells. The virus-like particles reported here occurred only in the nucleoplasm. However, the virion shape and size were similar to the badnavirus described in Japan. It is likely that the short and curved-rod shaped particles found in *I. cordatotriloba* belong to a member of the *Badnaviridae* based on morphology of the virus-like particles and the location within the cell.

Virus particles of two closteroviruses, BYV and *Nandida stem pitting virus* (NSPV) have been reported to be present both in the cytoplasm and in the nuclei of infected plants (Ahmed et al., 1983; Brunt et al., 1996; Franki et al., 1985). However, these two viruses can be differentiated with the type of inclusions they induce. Instead of BYV-type vesicles, NSPV forms unique tubular-coil structures in the cytoplasm, which are probably composed of virus particles (Franki et al., 1985). The thread-like, long and flexuous-rod shaped virus-like particles found in the nuclei of *I. setosa* plants grafted with W285 could belong to a member of the family *Closteroviridae*. Virus-like particles were occasionally found in the nuclei, but not in the cytoplasm of infected plants. However, other cytological changes commonly found in infections by members of the *Closteroviridae* such as, vesicles, proliferation of the endoplasmic reticulum, or fibrils and vesicles in the mitochondria were not observed (Ahmed et al., 1983; Franki et al., 1985). Therefore, these virus-like particles could belong to a member of new virus group, but further studies such as serology, vector transmission, and virus purification have to be conducted in order to confirm this.

Cytopathology studies using the electron microscope have been useful to diagnose infections by potyviruses. Viruses of the family *Potyviridae* induce various types of virus associating inclusions in the cytoplasm of host plants. Those inclusions include bundles (longitudinal sections of

cylindrical inclusions), scrolls (appear as circles or coils in cross sections), loops (cylindrical inclusions, not organized into pinwheels and bundles), pinwheels (cross-sections of cylindrical inclusions), tubes (scrolls in longitudinal-section), and laminated aggregates (Edwardson and Christie, 1996; Franki et al., 1985). Cylindrical inclusions have been observed in all kind of plant cell tissues including epidermal, mesophyll, and vascular bundles, but they occur more frequently in epidermal and mesophyll cells (Edwardson and Christie, 1996). Hence, the occurrence of those inclusion bodies in plant cells confirms that the host plants are infected with members of the family *Potyviridae*. However, there are two exception, *Sweetpotato mild mottle virus* (SPMMV) (Hollings et al., 1976) and *Sweetpotato yellow dwarf virus* (SPYDV) (Brunt et al., 1996; Clark and Moyer, 1988) are two whitefly-transmissible viruses that induce cylindrical inclusions in sweetpotato.

Most viruses obtained from the field-collected sweetpotatoes induced various types of cylindrical inclusions. These inclusions were found frequently and easily. It is possible that the source of cylindrical inclusions in the plants examined were the result of infections by various strains of SPFMV, since this virus is very common in the United States (Clark and Moyer, 1988). Also, based on ELISA assays for SPFMV, almost all of field-collected samples reacted positively with polyclonal antiserum to SPFMV-SRC (C. A. Clark, personal communication). All types of cylindrical inclusion were found and they consisted of pinwheels, bundles, loops, scrolls, and short usually curved laminated aggregates. Edwardson and Christie (1996) classified potyviruses according to the type of cylindrical inclusions induced, and SPFMV induced all types of cylindrical inclusions, including loops.

In the case of selected LSU viruses (LSU-1, LSU-3, LSU-4, and LSU-5), cylindrical inclusions were found rarely in infected *I. setosa*, and the type of inclusions was different than those reported to occur in SPFMV-infected plants. Cells of plants infected with these viruses contained bundles, pinwheels, and scrolls, but loops were not found. In comparison of cylindrical inclusions induced by SPFMV-C and selected LSU viruses, the types of cylindrical inclusions and frequency were different. Therefore, the LSU viruses may be different strains of SPFMV, or they may be distinct members of the *Potyviridae* inducing different types of inclusions from SPFMV. Cylindrical inclusions were not found in plants infected with LSU-2.

Several other viruses of the genus *Potyvirus* that infect sweetpotatoes have been reported; *Sweetpotato vein mosaic virus* (SPVMV) from Argentina, *Sweetpotato latent virus* (SPLV) from Taiwan, and *Sweetpotato virus G* (SPVG) from China (Brunt et al., 1996; Clark and Moyer, 1988;

Edwardson and Christie, 1996). Therefore, it is possible that a distinct potyvirus, or mixed infections of potyviruses could be inducing the inclusions found in cells of plants infected with the LSU viruses. Further analysis of the LSU viruses will be necessary to determine their identity, using tests such as serology and PCR followed by sequencing.

Caulimovirus particles occur both in the cytoplasm and nucleus, and amorphous viroplasms containing virus particles can be observed in the cytoplasm (Conti et al., 1972; Franki et al., 1985). Caulimovirus like-particles, 40-55 nm in diameter similar to those observed by Atkey and Brunt (1987), were found only in the cytoplasm of cells of *I. setosa* inoculated with scions of Wagabolige. The particles were spherical with electron-lucent centers of about 20 nm in diameter. These virus-like particles were similar to that of a caulimovirus found in sweetpotato from Peru (L. F. Salazar, personal communication). In a number of cases, caulimovirus particles have been observed occasionally inside plasmodesmata (Franki et al., 1985), and it has been proposed that the virus is transported from cell to cell through plasmodesmata. Even though, plasmodesmata containing virus-like particles were not found in the examined tissues, the tubule-like structures found in this investigation could be involved in virus transport (Fig. 4B). It is possible that these tubule-like structures could have originated from the plasma membrane, or were parts of plasmodesmata pulled out from the plasma membrane during the virus movement. Although the virus-like particles may belong to a caulimovirus because of the morphology, more tests such as aphid transmission, PCR, and analysis of nucleic acids are necessary to confirm this.

Two types of virus-like particles were found in tissues of *I. setosa* mechanically and graft inoculated with the diseased beach morning glory, spherical and rod shaped. The flexuous-rod shaped virus-like particles were of approximately 12-14 nm in width and 500-1,000 nm in length. Some particles were up to 1,200 nm in the length. The cells did not contain viral induced inclusions. Viruses of the family *Closteroviridae* have maximum modal length, up to 2,200 nm, are not mechanically transmitted, and some members induce BYV-type vesicles (Brunt et al., 1996). The cells observed from infected plants did not contain BYV-type vesicles, and infected plants did not show the yellowing symptoms typically induced by members of the *Closteroviridae* (Brunt et al., 1996). The information regarding the morphology, cytopathology, and symptoms associated with this filamentous particle were not sufficient to determine the virus group. However, this virus was mechanically transmitted, and induced symptoms including leaf crinkling on *I. setosa*. Therefore, it is possible that it could be a previously unreported virus. Further studies

including host range, serology, vector transmission, virus purification, and analysis of nucleic acids are needed to determine the identity of this virus(es).

The spherical virus-like particles found in tissues of *I. setosa* mechanically inoculated with the beach morning glory sample were of about 90-100 nm in diameter. Some consisted of membrane-bounded spherical particles found in the cytoplasm, while others were not membrane-bounded and were present inside the chloroplast. It was not clear whether the cell organelle membrane bounded the virus-like particles. If the spherical particles were assembled inside chloroplasts, these particles could be membrane-bounded during budding out from the chloroplast. These membrane-bounded spherical virus-like particles in the cytoplasm were similar to those reported in plants infected with *Tomato spotted wilt virus* (TSWV), except that TSWV particles are normally grouped as two or more within the cytoplasmic membranous cisternae (Francki and Hatta, 1981). In the case of the spherical beach morning glory virus, single particles were membrane-bounded. Only rod shaped virus-like particles and virus-like phytoferretin have been reported in the chloroplast (Francki et al., 1985). The spherical virus-like particles found in the chloroplast of *I. setosa* are larger than the virus-like phytoferretin. As in the case of the flexuous rod shaped virus-like particles, further studies are needed in order to determine the identity of the virus(es) from beach morning glory.

Tobacco etch virus (TEV) induces cytopathic changes consisting of crystalline inclusions in the nucleus of host cells (Franki et al., 1985). The crystals in the nucleus of healthy *I. nil* and *I. aquatica* examined in this study were similar to those caused by an infection with TEV. However, there have been previous reports of similar inclusions in healthy *Ipomoea* species (Weintraub et al., 1968). The possibility that the crystals found in the nuclei of *I. nil* could be caused by a seed-borne virus prevalent in *Ipomoea* species, but not transmissible mechanically, by grafting, or vectors cannot be eliminated. This finding is important to avoid false diagnosis using transmission electron microscope.

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