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Characterization of a pothos (*Scindapsus aureus*) virus with unusual properties

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Abstract

A virus for which the name of pothos latent virus (PoLV) is proposed, was isolated by inoculation of sap from symptomless plants of *Scindapsus aureus*. PoLV had isometric particles *c.* 30 nm in diameter, a monopartite genome consisting of a non polyadenylated, single-stranded RNA molecule *c.* 4,300 nucleotides in length, constituting *c.* 17% of the particle weight, and a single type of coat protein subunit with a M_r of *c.* 40,000 Daltons. The biological properties (host range reactions) of PoLV resembled those of *Tombusviridae* for it infected most of the artificial hosts locally, inducing symptoms recalling those elicited by several species of the above family. Like tombus- and carmoviruses, PoLV had two subgenomic RNAs which, however, differed in size from those of both genera. The dsRNA pattern was also distinctly different. Cytopathological features recalled those of tombusviruses except for the lack of multivesicular inclusion bodies. PoLV was serologically related to, but distinct from two *Carmovirus* (i.e., galinsoga mosaic and Ahlum waterborne viruses) and three *Tombusvirus* species (i.e., eggplant mottled crinkle, Sikte waterborne and Lato river viruses). Thus, PoLV had properties somewhat intermediate between those of *Tombusvirus* and *Carmovirus* genera but bridged the two taxa through the serological relationship with some of their species. The taxonomic position of PoLV is still undetermined. It must await the results of molecular investigations now underway.

Introduction

From roots of rooted cuttings of *Vitis rupestris* growing in hydroponic culture, a virus with isometric particles was transmitted by inoculation of sap to herbaceous hosts in which it induced symptoms unlike those elicited by the most common grapevine viruses, i.e. grapevine fanleaf and other European and American nepoviruses, grapevine trichovirus A and B [Martelli, 1993]. Despite repeated attempts, this virus could not be isolated from leaves or cortical tissues of any of many tested rootlings, nor could it be observed inside the cells of thin sectioned root tissues. However, it was readily recovered from pellets obtained after high-speed centrifugation of samples of the hydroponic solution. The conclusion was drawn that the virus was a surface root contaminant and a search for its source was carried out. As specified later, some 20 different species of ornamental plants growing in the same hydroponic bench as grape rootlings were individually

checked for the presence of the virus, which was identified, and isolated, only from apparently symptomless plants of *Scindapsus aureus* (pothos, family *Araceae*). This paper describes the properties of this virus, for which the name of pothos latent virus (PolV) is proposed, and discusses its taxonomic position in the light of the results of investigations in which a PoLV isolate was compared with species of the *Tombusvirus* and *Carmovirus* genera.

Materials and methods

Virus isolates and herbaceous host range. The PoLV isolate used in this study was that originally obtained from grapevine roots and found to be indistinguishable from other isolates recovered later from pothos plants. The virus was transmitted by macerating root tissues in 0.1 M phosphate buffer pH 7.2 and rubbing the slurry on celite-dusted leaves of herbaceous hosts. It was comparatively studied with authentic isolates of galinsoga mosaic carmovirus (GaMV) [Behncken et al., 1982] and cymbidium ringspot tombusvirus (CyRSV) [Hollings et al., 1977]. All viruses were maintained in *Nicotiana benthamiana* plants grown in a temperature-controlled glasshouse at 22–24 °C. For host range studies, PoLV was manually inoculated to 29 different plant species and cultivars belonging to six botanical families.

Virus purification. All three viruses were purified basically as described by Gallitelli et al. [1985] from systemically infected *N. benthamiana*. PoLV was also purified from *N. clevelandii*, or locally infected *Gomphrena globosa*. Tissues were homogenized in 2.5 vol of extraction buffer (0.1 M sodium acetate pH 5.2, containing 0.1% 2-mercaptoethanol), filtered and centrifuged at low-speed (6,000 × g for 10 min) prior to adjusting the solution to a final concentration of 1.1% NaCl and 8% polyethylene glycol MW 6,000. The precipitate was recovered by low-speed centrifugation 6,000 × g, the pellets were dissolved in resuspension buffer (0.02 M sodium acetate pH 6.0) and submitted to a cycle of low- and high-speed (120,000 × g for 45 min) centrifugation. The pellets were resuspended, layered on sucrose density gradient columns prepared by freezing and thawing a 25% sucrose solution in 0.02 M sodium acetate buffer, and centrifuged at 24,000 rpm in a Beckman SW 27.1 rotor for 2.5 h. Gradient tubes were scanned with a ISCO ultraviolet absorbance monitor and peaks corresponding to virus fractions collected and concentrated by centrifugation at 120,000 × g for 2 h.

Purified PoLV preparations were centrifuged at equilibrium for 18 h at 36,000 rpm in a Beckman SW.41 rotor in CsCl and Cs_2SO_4 solutions in resuspension buffer with initial density of 1.354 g/cm^3 and 1.283 g/cm^3, respectively.

Properties of purified virus. Viral nucleic acids were extracted from gradient-purified particles of the three viruses with a standard SDS-phenol method [Diener and Schneider, 1968] and electrophoresed in 1.2% agarose gels in TBE buffer (89 mM tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) after denaturation with 50% formamide at 65 °C for 5 min. Reference markers were genomic RNAs of CyRSV (4733 nt) [Grieco et al., 1989] and GaMV (*c.* 3.900 nt) [Gallitelli et al., 1985]. Total nucleic acids (TNA) were obtained from *N. benthamiana* plants as described by White and Kaper [1989].

The type of nucleic acid and its strandedness were determined by submitting nucleic acid preparations to enzymatic digestion with DNase-free pancreatic RNase (2 µg/ml) in high- (2 × SSC buffer) or low-salt (0.02 × SSC buffer) conditions (1 × SSC buffer: 0.15 M sodium chloride and 0.015 M trisodium citrate), or with RNase-free DNase (2.5 µg/ml) in 0.01 M magnesium chloride.

Coat protein subunits were obtained by boiling for 5 min purified virus preparations in the presence of 1.5% SDS and 3.5% 2-mercaptoethanol in 0.035 M tris-HCl buffer pH 7.0. Dissociated coat proteins were electrophoresed in 12.5% polyacrylamide slab gels in Laemmli's (1970) discontinuous buffer system, and stained with Coomassie brilliant blue. A MW-SDS-70L kit (Sigma Chemical Co. St. Louis) with markers with mol. wt from 14,200 (α-lactoalbumin) to 66,000 daltons (bovine serum albumin) was used as reference.

Double-stranded RNAs. Double-stranded RNAs were recovered from *N. benthamiana* plants infected by PoLV, GaMV and CyRSV by phenol

extraction and differential purification through cellulose CF11 columns [Dodds, 1993]. After digestion with RNase-free DNase (60 µg/ml) in 10 mM magnesium chloride for 30 min at 37 °C and DNase-free pancreatic RNase (0.025 µg/µl) in 2 × SSC for 30 min at 37 °C, dsRNAs were separated by electrophoresis in 1% agarose gel.

Serology. An antiserum to PoLV was raised in a rabbit by injecting purified virus (1 mg/ml nucleoprotein per injection) at weekly intervals, once intramuscularly, after emulsification in Freund's incomplete adjuvant, and twice intravenously. Antiserum collection began a week after the last injection and the titre was determined in gel double-diffusion.

PoLV preparations were tested in immunodiffusion: (i) at Bari, with antisera to the following tombusviruses, CyRSV, tomato bushy stunt (TBSV), artichoke mottled crinkle (AMCV), cucumber necrosis (CNV), carnation Italian ringspot (CIRV), grapevine Algerian latent (GALV), Lato river (LRV), Moroccan pepper (MPV), petunia asteroid mosaic (PAMV), pelargonium leaf curl (PLCV), eggplant mottled crinkle (EMCV), Sikte waterborne (SWBV) and carmoviruses, GaMV, carnation mottle (CarMV), cucumber leaf spot (CLSV), and melon necrotic spot (MNSV); (ii) at Braunschweig, with antisera to CyRSV, SWBV and EMCV tombusviruses, GaMV and Ahlum waterborne (AWBV) carmoviruses.

Immunoelectron microscopy (IEM) tests [Milne and Luisoni, 1977] were made by exposing PoLV, GaMV, LRV and CyRSV particles to PoLV antiserum diluted 1:10, 1:20, and 1:100 and PoLV particles to antisera to SWBV and EMCV diluted 1:1.

Standard DAS-ELISA [Clark and Adams, 1977] was used for the search of the natural host of PoLV. IgGs were fractionated from PoLV antiserum by protein A-sepharose columns [Clark and Bar-Joseph, 1984] and conjugated with alkaline phosphatase.

Molecular cloning. cDNA was synthesized as described by Gubler and Hoffman [1983] using artificially polyadenylated PoLV RNA [Sippel, 1973] primed by oligo(dT). cDNA thus obtained was ligated to *SmA I*-cut pUC18 plasmid which was used for transforming competent *Escherichia coli* DH5α cells [Sambrook et al., 1989]. Identification of cDNA clones containing poly(A) tracts were made by ^{32}P oligo(dT) hybridization according to Barry et al. [1989]. Northern blots and hybridization assays were made according to Sambrook et al. [1989].

Electron microscopy. Purified virus preparations were mounted in 2% aqueous uranyl acetate before observation with a Philips 201C electron microscope. For thin sectioning, tissue fragments excised from ELISA-positive pothos rootlets and symptomatic leaves of systemically PoLV-infected *N. benthamiana* and locally infected *Chenopodium quinoa* were processed according to standard procedures [Martelli and Russo, 1984]. Samples were double fixed in 4% glutaraldehyde and 1% osmium tetroxide, dehydrated in graded ethanol dilutions and embedded in Spurr's resin. Thin sections were stained with uranyl acetate ad lead citrate before observation. Controls consisted of tissues sampled from ELISA-negative pothos rootlets and healthy *N. benthamiana* and *C. quinoa* plants.

Transmission to pothos. Ten two-leaf cuttings from an ELISA-negative pothos plant were individually placed in small containers with tap water in a glasshouse, and the leaves were manually inoculated with purified virus preparations.

Results

Host range and symptomatology. PoLV had a moderately wide host range, infecting 12 out of 28 different herbaceous plant species and cultivars in five botanical families. *N. benthamiana* and *N. clevelandii* were the only hosts systemically infected, both reacting with mottling of the leaves followed by necrosis and death of the plant in about three weeks. *Gomphrena globosa, Chenopodium amaranticolor, C. quinoa, C. murale, C. foetidum, N. cavicola* and *Petunia hybrida* developed chlorotic/necrotic local lesions not followed by systemic invasion. *Ocimum basilicum* reacted with black necrotic lesions very similar to those typically induced by tombusviruses (Fig. 1) [Martelli, 1981], whereas

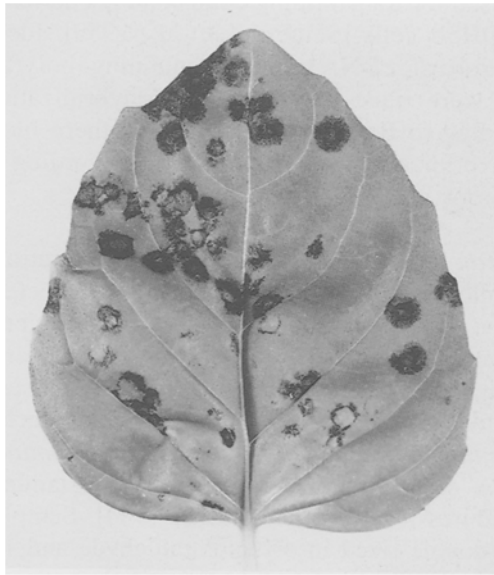

Fig. 1. Necrotic local lesions induced by PoLV in inoculated leaves of basil (*Ocimum basilicum*).

Phaseolus vulgaris cv. Bountiful and La Victoire produced minute necrotic lesions.

Cucumis sativus, Cucurbita maxima, C. pepo, D. stramonium, N. debneyi, N. glutinosa, N. occidentalis, N. megalosiphon, N. rustica, N. trigonophylla, N. undulata, N. tabacum cv. Samsun, White Burley and Xanthi, and *Vigna unguiculata* did not develop visible symptoms, nor they were latently infected, as ascertained by back inoculations and/or ELISA.

Properties of purified virus. PoLV was readily purified in high quantity (up to 80 mg/kg of tissue) from all hosts, regardless of whether they were systemically (*N. benthamiana* and *N. clevelandii*) or locally (*G. globosa*) infected. Purified preparations sedimented as a single band both in sucrose density gradients and at equilibrium in CsCl or Cs_2SO_4. Buoyant density in CsCl was 1.36 g/cm^3. Purified preparations contained isometric particles *c.* 30 nm in diameter, with a somewhat rounded outline and a knobby surface (Fig. 2). These preparations were infective when inoculated to assay hosts and exhibited u.v. absorption spectrum of nucleoproteins with E_{max} = 260 and E_{min} = 242 nm, and E_{260}/E_{280} = 1.65.

A single protein species with an estimated M_r of *c.* 40,000 Daltons was observed in gels loaded with dissociated virus particle preparations. PoLV coat protein subunits migrated at a rate intermediate between that of GaMV (*c.* 36,000 Daltons) and CyRSV (*c.* 43,000 Daltons) (Fig. 3A).

Virus particles contained *c.* 17% of nucleic acid which was resistant to DNase but was degraded by pancreatic RNase in high- and low-salt conditions and was therefore identified as single stranded RNA. Nucleic acid extracts from purified virions yielded a single RNA species with an estimated size of *c.* 4,300 nt, which, in agarose gel electrophoresis migrated at a rate intermediate between that of CyRSV (4,733 nt) and GaMV (*c.* 3,900 nt) RNAs (Fig. 3B).

Nucleic acid extracts from virions and infected *N. benthamiana* tissues were infective.

Double stranded RNAs. Electrophoretic patterns of dsRNAs extracted from hosts infected with GaMV, PoLV and CyRSV consisted of three bands migrating at different rates (Fig. 4A). The topmost of these bands likely represented the double stranded form of full-size genomic RNA, whereas the two lower bands were thought to be the double stranded forms of subgenomic RNAs 1 and 2 [Russo et al., 1994]. The full-size genomic dsRNA of PoLV (Fig. 4A, lanes b and d) had a size intermediate between that of the comparable dsRNAs of CyRSV (Fig. 4A, lane c) and GaMV (Fig. 4A, lane a). Also subgenomc dsRNAs of the three viruses were readily differentiated from one another because of the electrophoretic migration rates, on whose basis the following relative sizes were estimated: *c.* 1.7 and 1.4 kb for GaMV (Fig. 4A, lane a); *c.* 2.1 and 0.9 kb for CyRSV (Fig. 4A, lane c); and *c.* 2.0 and 0.8 kb for PoLV (Fig. 4A, lanes b and d).

Molecular hybridization. Several plasmids containing artificially synthesized poly(A) tails and DNA complementary to the 3′ end sequences of PoLV genomic RNA were obtained, which ranged in size between 0.2 and 0.9 Kb. A probe of *c.* 0.8 Kb, denoted pPoLV, which contained the putative movement protein cistron but did not extend into the coat protein gene (M. Russo, personal communication), was used for Northern blot assays. This probe did not react with TNAs from *N. benthamiana* plants either healthy (Fig.

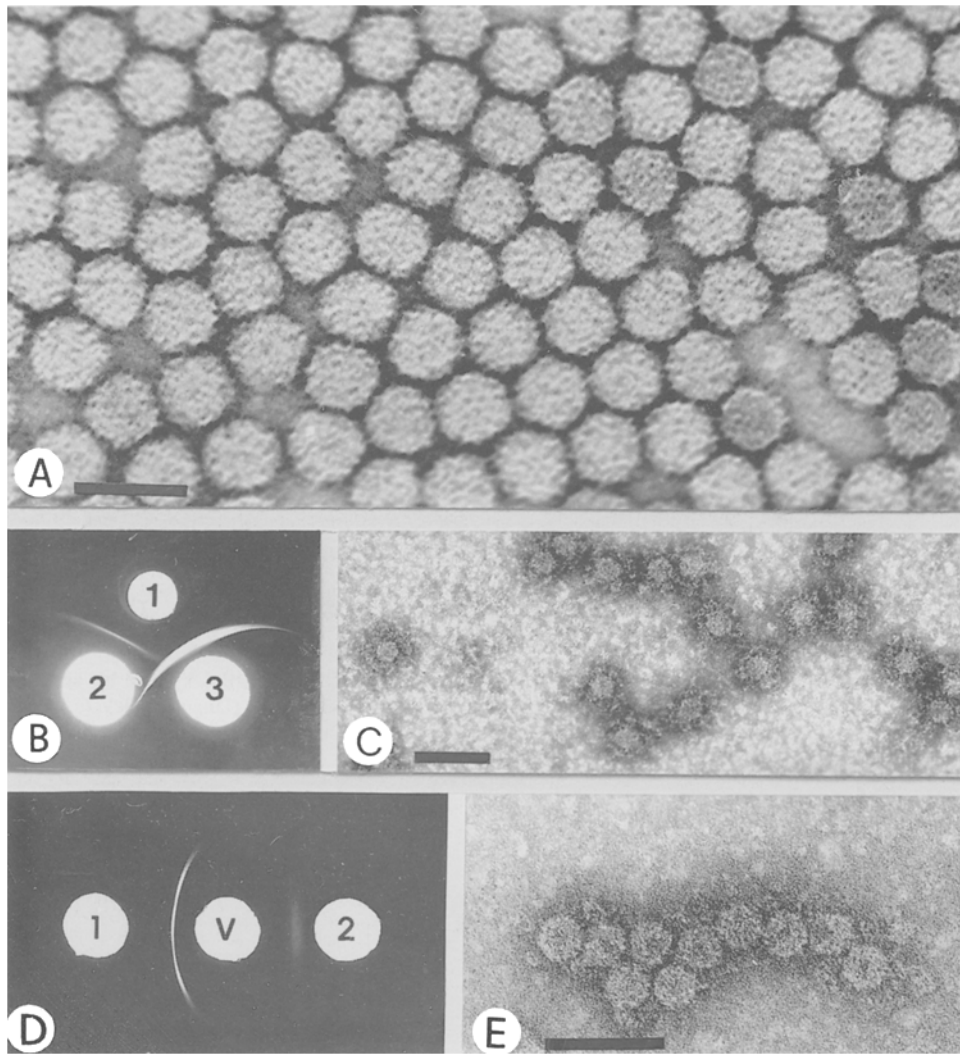

Fig. 2. A. Purified PoLV particles mounted in uranyl acetate Bar = 50 nm. B. Evidence that PoLV (well 3) is serologically related to, but distinct from, GaMV (well 2), a carmovirus. Well 1 contains PoLV antiserum. C. GaMV particles decorated by undiluted PoLV antiserum. Bar = 100 nm. D. Evidence that PoLV is distantly serologically related to EMCV, a tombusvirus. Well V contains a purified preparation of PoLV, well 1 and well 2, antisera to PoLV and EMCV, respectively. E. PoLV particles decorated by the antiserum to SWBV, a tombusvirus, diluted 1:1. Bar = 100 nm.

4B, lane a) or infected with GaMV or CyRSV (not shown), but recognized TNAs from plants infected with PoLV (Fig. 4B, lane b) and RNA extracted from purified PoLV particles (Fig. 4B, lane c). In both extracts three bands were present, denoted G, 1, and 2 in Fig. 4B, which gave specific hybrization signals with the PoLV cDNA probe. Since the two fast migrating RNA species contained sequences that are 3′ coterminal with PoLV genomic RNA, they are likely to represent subgenomic RNAs.

Serology. The antiserum to PoLV had a titre of 1:1024 and did not react visibly with healthy plant extracts. By contrast, it reacted with the homologous antigen and purified preparations of GaMV with precipitin lines that formed a spur at the junction (Fig. 2B). Comparable precipitin patterns

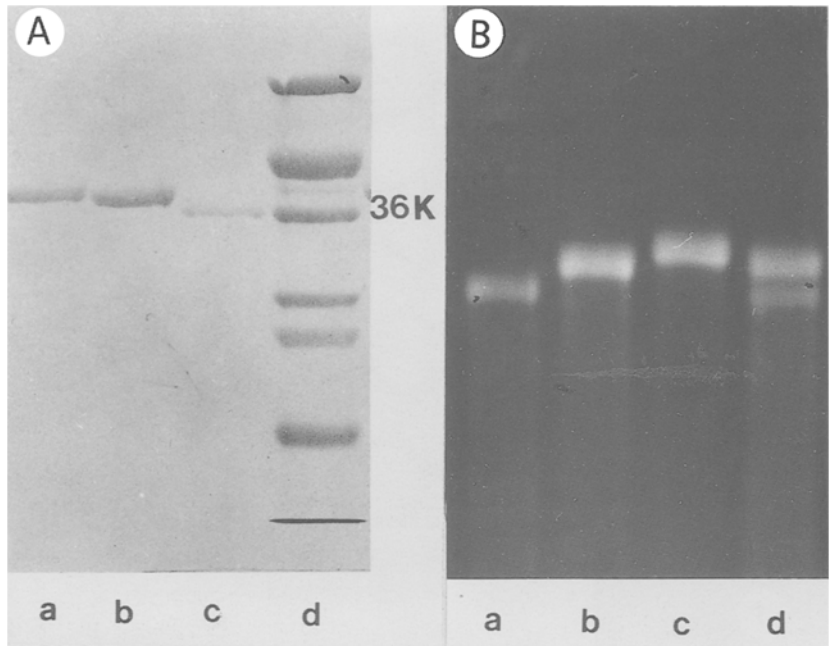

Fig. 3. A. Electropherogram of dissociated coat protein preparations of CyRSV (lane a), PoLV (lane b), and GaMV (lane c). Mol. wt markers in lane d. B. Electropherogram of genomic RNAs of GaMV (c. 3,900 nt) (lane a), PoLV (c. 4,300 nt) (lane b), CyRSV (4,733 nt) (lane c), and of a mixture of GaMV and PoLV (lane d). PoLV coat protein subunits and RNA show a migration rate intermediate between that of GaMV and CyRSV.

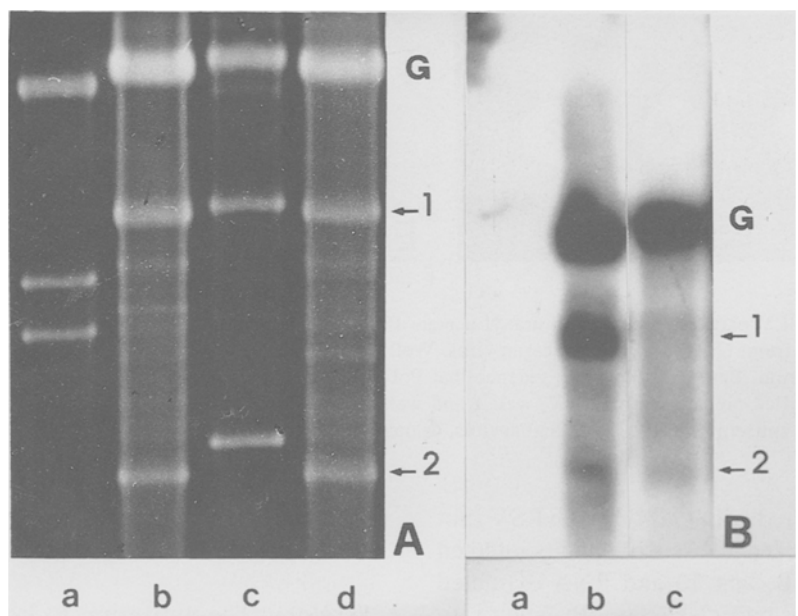

Fig. 4. A. Electropherogram of dsRNAs extracted from *N. benthamiana* infected by GaMV (lane a), PoLV (lanes b and d), and CyRSV (lane c). The double stranded forms of genomic (G) and two subgenomic RNAs are visible. 1 and 2 indicate the subgenomic RNAs of PoLV. B. Northern blot of PoLV nucleic acids extracted from infected *N. benthamiana* (lane b) and virions (lane c). All RNA bands hybridized with a probe containing the 3' terminal sequences of PoLV genomic RNA (G), indicating that the two smaller RNA species (1 and 2) are subgenomic.

were obtained when an antiserum to GaMV (titre 1:512) was used against PoLV and GaMV antigens. Very faint precipitin lines developed when PoLV preparations were exposed to antisera to LRV [Gallitelli et al., 1989], AWBV, SWBV [Li et al., 1992] and EMCV [Makkouk et al., 1981] (Fig. 2D). In immunomicroscopy tests, the PoLV antiserum decorated PoLV particles to the highest dilution tested (1:100) and GaMV particles to a dilution to 1:20 (Fig. 2C), but did not decorate CyRSV particles. On the other hand, both EMCV and SWBV antisera induced a light but distinct decoration of PoLV particles (Fig. 2E). No visible precipitin lines were formed when PoLV preparations were allowed to react in immunodiffusion with antisera to any of the other tombusviruses and carmoviruses tested.

Cytopathology. Thin sectioned tissue samples from ELISA-positive, but not ELISA-negative, pothos rootlets contained uniformly electron dense rounded bodies with a regular outline and a diameter of *c.* 24 nm, interpreted as profiles of virus particles. Virions were scattered throughout the cytoplasm, sometimes forming discrete aggregates and, in certain cells, they lined the tonoplast from which droplets of darkly-staining material, possibly lipids, were protruding. Many of the virus-containing cells had a 'washed out' appearance and a thinned ground cytoplasm (Fig. 5A).

Infected cells of *N. benthamiana* and C. quinoa showed the same type of ultrastructural modifications. The ground cytoplasm was dense with virus particles which often invaded the central vacuole (Fig. 5B). Virions were either scattered at random, or appressed in more or less compact accumulations, or regularly arranged in a crystalline lattice (Fig. 6). Sometimes virus clusters or crystals were within extensions of the tonoplast that protruded into the vacuole (Fig. 6). The cytoplasm contained also accumulations of membranes and groups of vesicles likely deriving from proliferation of the endoplasmic reticulum. Localized cell wall thickening and callose deposits were occasionally seen, more often in *C. quinoa* local lesions.

Except for nuclei and peroxisomes, which retained an apparently normal structure, other major organelles were variously deranged. Chloroplasts were rounded or variously misshapen, and exhibited abnormalities of the lamellar system but no peripheral vesiculation (Fig. 6). Mitochondria showed dilation and reduction of the number of cristae (Fig. 5D) and often contained virus particles. When present, virions were grouped in an electron lucent area more or less in the center of the mitochondria, which had apparently intact outer envelopes (Fig. 5B, C).

No multivesicular bodies [Martelli and Russo, 1984; Russo et al., 1987; Martelli et al., 1988] were observed in any of the hosts studied. However, unusual cytopathological structures were frequently seen, which consisted of single-membraned rounded to ovoid bodies, the largest of which had about the size of a mitochondrion. These structures were lined with vesicular evaginations of the bounding membrane that measured up to 120 nm in diameter and contained a network of finely fibrillar material. The center of these bodies was either electron lucent, or contained amorphous electron dense material resembling a lipid droplet (Fig. 5E).

Search for the host of PoLV. With IgGs from the antiserum to PoLV an ELISA kit was prepared with which a survey for the presence of the virus in the ornamentals growing in the same hydroponic bench as pothos plants was carried out. A total of 478 samples taken from leaves of the following 20 species belonging to 9 botanical families were examined: *Dracaena deremensis (Agavaceae); Aglaonema pseudo-bracteatum, Dieffenbachia amoena, D. picta, Monstera deliciosa, Scindapsus aureus, Syngonium podophyllum (Araceae); Zebrina pendula (Commelinaceae); Codiaeum variegatum (Euphorbiaceae); Calathea makoyana, Marantha erytrhroneura, M. leuconera (Marantaceae); Ficus benjamina, F. elastica, F. radicans (Moraceae); Peperomia caperata, P. magnoliaefolia, P. scadens (Piperaceae); Pteris cretica (Polypodiaceae); Pilea cadieri (Urticaceae).*

None of the above hosts was ELISA-positive except for some *S. aureus* rooted cuttings, which prompted an extension of the survey to all pothos rootlings grown in hydroponic culture and to other plants of the same species growing elsewhere. Of 195 *S. aureus* from hydroponics, 43 (22.6%) were ELISA-positive, but none of 39 samples collected outside the glasshouse.

An attempt was made to isolate PoLV from

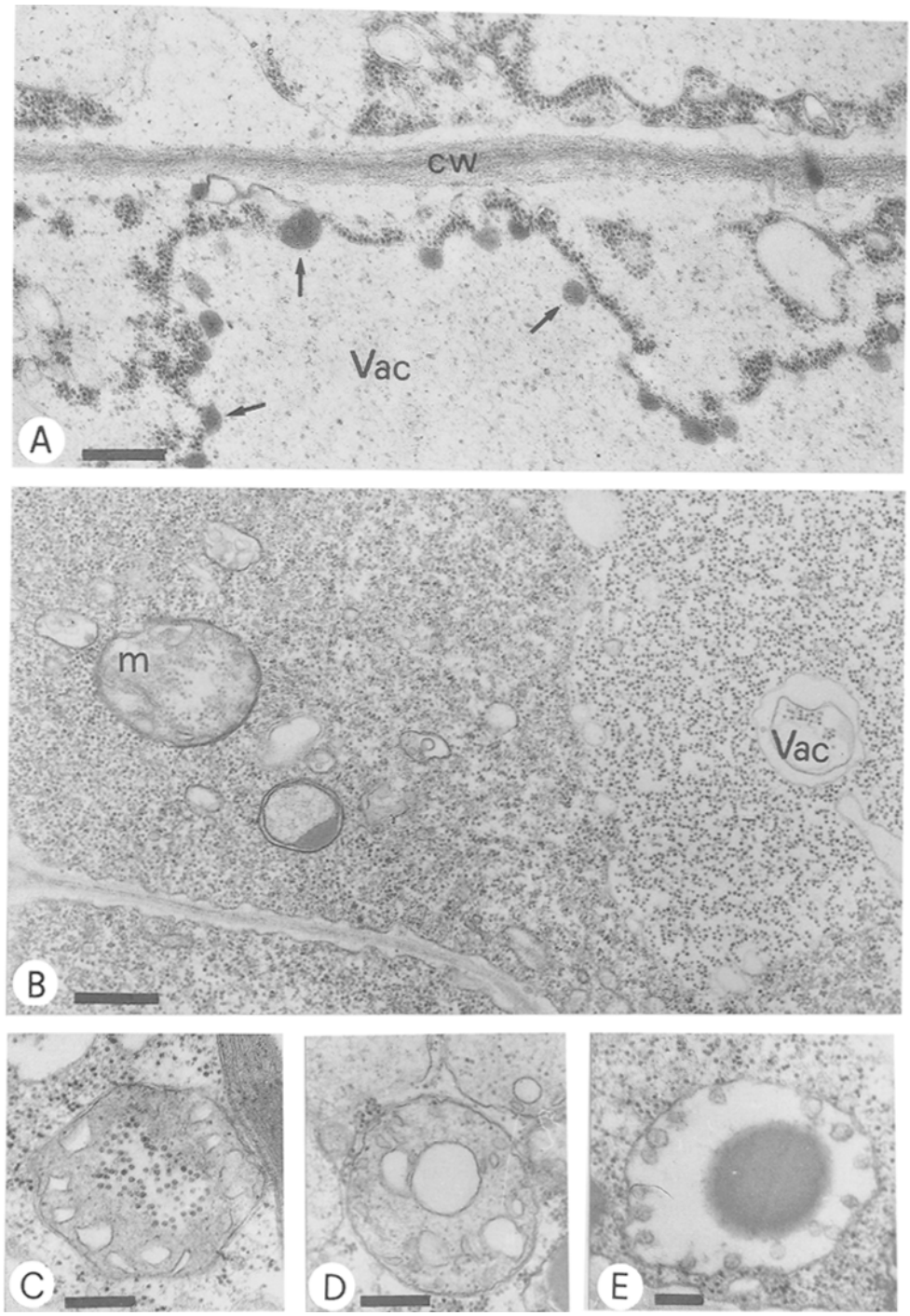

Fig. 5. A. Thin sectioned cortical cells of a pothos rootlet with natural PoLV infection. Virus particles are plentiful and are mostly aligned along the cytoplasmic side of the tonoplast. Arrows point to droplets of lipid-like material protruding into the vacuole (Vac). CW = cell wall. Bar = 200 nm. B. Mesophyll cells of *N. benthamiana* systemically infected by PoLV. The cytoplasm and vacuole (Vac) are dense with virus particles, which are also inside an altered mitochondrion (m). Bar = 500 nm. C. Detailed view of a virion-containing mitochondrion. Note the apparently intact bounding membrane. Bar = 200 nm. D. An altered mitochondrion with dilated cristae. Bar = 200 nm. E. A membranous body containing a droplet of lipid-like material and many vesicles protruding from the bounding membrane. Bar = 200 nm.

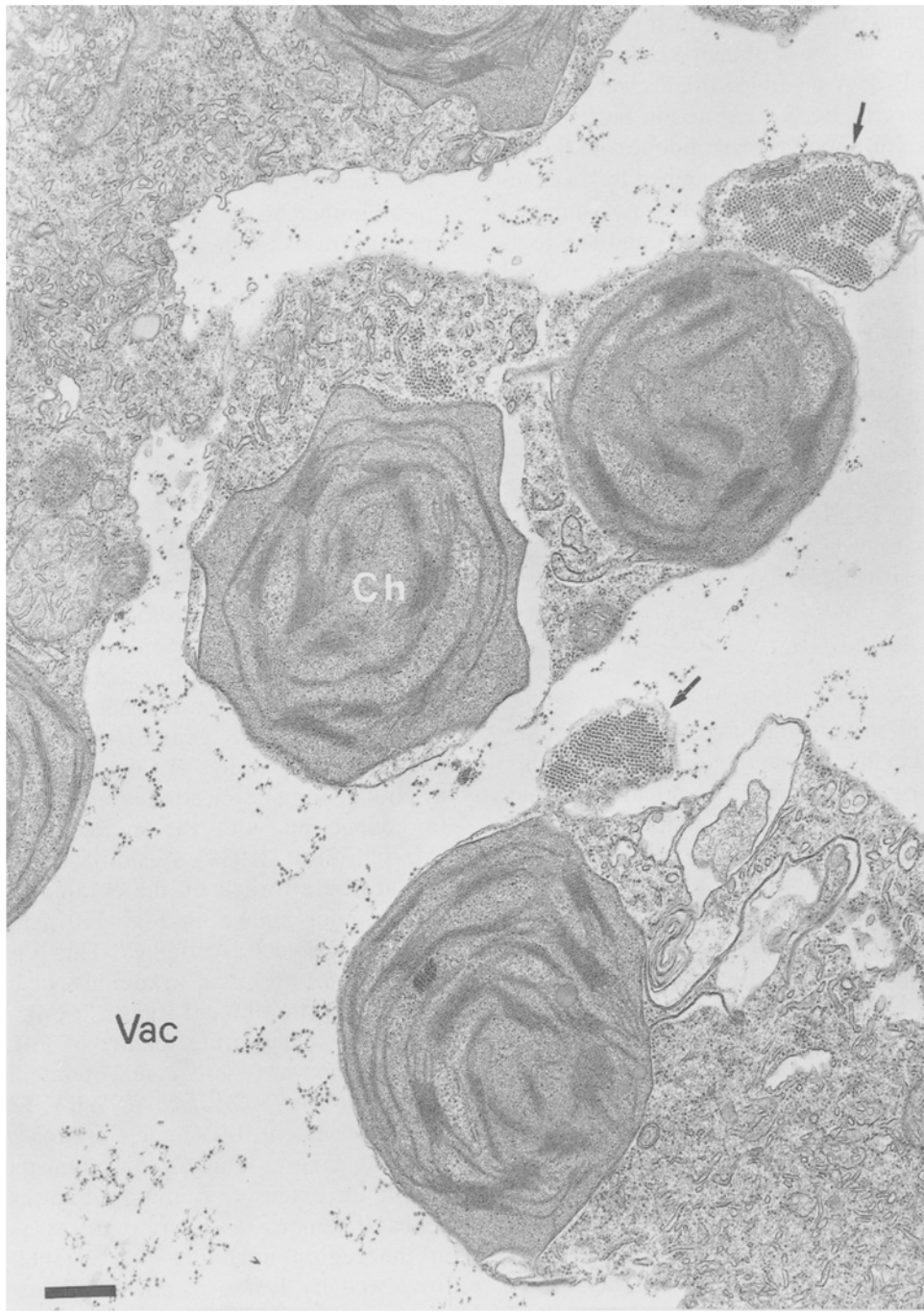

Fig. 6. A mesophyll cell of *C. quinoa* locally infected with PoLV. The cytoplasm shows membrane proliferation and chloroplasts (Ch) are misshapen and with a deranged tylakoid system. Virus particles are in the ground cytoplasm and in crystalline arrays within cytoplasmic extensions (arrows) protruding into the vacuole (Vac). Bar = 500 nm.

leaves of ELISA-positive and ELISA-negative *S. aureus* plants using seven different buffers. Successful recovery was obtained only when leaf tissues from ELISA-positive samples were ground in the presence of ELISA extraction buffer (PBS pH 7.4 plus 2% polyvinylpyrrolidone and 0.05% Tween 20). One virus isolate obtained by mechanical inoculation from pothos leaves was compared biologically (host range responses) and serologically with the viral isolate originally recovered from surface-contaminated grapevine roots and found to be indistinguishable from it.

Transmission to pothos. Inoculated pothos leaves did not show visible symptoms of infection. Four weeks after inoculation, however, extracts from leaf blades of eight of ten pothos cuttings gave strong positive ELISA reactions with PoLV antiserum. The virus was also detected in the petioles of ELISA-positive leaves of two cuttings.

Discussion

The results of the present investigation provide experimental evidence that the virus object of this paper infects pothos plants systemically without inducing visible symptoms. On this basis, the suggested name pothos latent virus appears appropriate.

It is also reasonable to assume that PoLV is a soil-borne virus that, similarly to many other such viruses, is adapted to the aquatic environment [reviewed by Koenig, 1992] from which it can be acquired by the host through the roots. Although it was not ascertained whether PoLV infections are mediated by a fungal vector, a direct ingress in the roots without the intervention of a vector would not be surprising, this being a trait of the family *Tombusviridae* [Martelli et al., 1988; Morris and Carrington, 1988; Koenig, 1986, 1992], with species of which PoLV appears to be related. That certain viruses can be picked up directly and efficiently by plants grown in hydroponic culture was recently confirmed with maize white line mosaic virus, an unclassified soil-borne virus with unknown vector [Louie et al., 1992].

PoLV shares several features with species of the *Tombusvirus* and *Carmovirus* genera, such as: (i) shape, size, and outward aspect of the particles; (ii) monopartite ssRNA genome without a poly(A) tail, accounting for *c.* 17% of the particle weight; (iii) presence of two subgenomic RNAs; (iv) biological (i.e., tendency to remain localized in infected hosts) and epidemiological behaviour; (v) invasiveness of host tissues when it spreads systemically; (vi) production of an exceedingly high number of virus particles. It also resembles tombusviruses for the reaction specifically induced in *O. basilicum.*

However, PoLV differs from tombus- and carmoviruses because of the size and electrophoretic pattern of genomic and subgenomic RNAs and the size of coat protein subunits, at least with respect to the two representative of the above genera (CyRSV and GaMV) with which it was compared.

Although PoLV has some ultrastructural features frequently observed in tombusvirus infections, i.e., crystalline viral inclusions, presence of virus particles within mitochondria, bubble-like evaginations of the tonoplast containing virus particles, it lacks multivesicular bodies which are regarded as a major characterizing feature of the *Tombusvirus* genus [Russo et al., 1987]. Multivesicular bodies are thought to be the site of viral genome replication, which may take place in connection with the membranous vesicles [Martelli et al., 1988]. A similar function could perhaps be afforded by the cytoplasmic vesiculated bodies shown in Fig. 5E, whose origin, however, was not ascertained. This type of structure was not observed in tombus- or carmovirus infections [Martelli and Russo, 1984].

The most intriguing feature of PoLV was its straight forward serological relatedness with species of the *Tombusvirus* (SWBV, EMCV and, to a lesser extent, LRV) and *Carmovirus* (GaMV, AWBV) genera. This is most unusual because despite the extensive similarity in the coat protein genes of tombus- and carmoviruses, particularly in the region encoding the S (shell) domain [reviewed by Russo et al., 1994], as yet, convincing intergeneric relationships have not been observed. Although some years ago turnip crinkle carmovirus (TCV) was reported as being related with several tombusviruses [Jaegle and Van Regenmortel, 1985], the alleged relationship was exceedingly distant (serological differentiation index between 10 and 13) and could be appre-

ciated only by ELISA. By contrast, no serological cross reactivity was found between melon necrotic spot carmovirus (MNSV) and any of several tombusvirus species notwithstanding the fact that MNSV coat protein is more similar to that of tombusviruses than that of carmoviruses [Riviere et al., 1989].

Examples of spurious serological cross-reactivity between viral species belonging to different genera are occasionally reported in the literature. However, one such case in which a monoclonal antibody to alfalfa mosaic alfamovirus (AMV) was claimed to recognize cucumber mosaic cucumovirus (CMV) [Hagjimorad et al., 1990] was recently dismissed as an artefact [Hajimorad et al., 1994].

In conclusion, it appears that PoLV represents a hitherto unreported example of a virus with properties intermediate between *Tombusvirus* and *Carmovirus*, the two present genera of family *Tombusviridae*, which it bridges through a clear-cut serological relationship with some species of both. This condition makes the taxonomic allocation of PoLV uncertain, and requires additional information which is now being gathered with molecular studies.

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