Luteovirus-like Particles Associated with Subterranean Clover Red Leaf Virus Infection

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SUMMARY

Small isometric virus-like particles have been detected in thin sections of phloem transfer cells of subterranean clover plants infected with subterranean clover red leaf virus (SCRLV). Virus-like particles similar in size and appearance but serologically distinct from those of potato leafroll virus, were also detected in purified preparations from SCRLV-infected plants. The morphology of the particles and their distribution in infected cells is consistent with SCRLV being a member of the luteovirus group, as previously suggested by its biological properties.

Subterranean clover red leaf virus (SCRLV) was described by Kellock (1971) as the causal agent of a serious disease of subterranean clover (Trifolium subterraneum L.) in Australia. However, no virus particles appear to have been isolated or observed in cells of infected plants. The virus has been transmitted persistently by its aphid vector, Aulacorthum solani (Kltb.) to a number of leguminous plants (Kellock, 1971; Johnstone, 1978; Ashby et al., 1979). Based on disease symptomatology and vector relationships, the virus has tentatively been assigned to the luteovirus group (Matthews, 1979). Furthermore, Ashby et al. (1979) suggested that SCRLV may be related to soybean dwarf virus (SDV) and filaree red leaf virus, which have been respectively classified as a member and a possible member of the luteovirus group (Matthews, 1979). In this paper, we report the presence of luteovirus-like particles in partially purified preparations from, and in thin sections of, phloem transfer cells of SCRLV-infected subterranean clover plants.

SCRLV was maintained on T. subterraneum cv. Bacchus Marsh or Mt. Barker by transmission with A. solani. Pieces of leaf tissue, which included small veins and roots with vascular tissue, were excised from healthy and diseased plants. They were fixed, dehydrated, embedded and sectioned for electron microscopy (Hatta & Francki, 1981). Half the tissue pieces were incubated with pancreatic ribonuclease (RNase) after aldehyde fixation to digest RNA in the ribosomes (Hatta & Francki, 1981), whereas the other samples received no enzyme treatment. Unless ribosomal RNA is digested, it is difficult to distinguish particles of viruses such as those of luteoviruses, from cytoplasmic ribosomes (Hatta & Francki, 1981).

Examination of thin sections of RNase-treated tissues of SCRLV-infected plants revealed the presence of densely stained particles about 23 nm in diam. in many of the phloem transfer cells (Gunning & Steer, 1975). Cells containing these particles were often located next to cells devoid of densely stained particles (Fig. 1a) indicating that the enzyme treatment was effective in digesting RNA of the ribosomes but not the densely stained structures which were presumed to be virus particles. The susceptibility of RNA in ribosomes in this type of cell was demonstrated by comparing the ultrastructure of cells from healthy plants prepared with and without RNase treatment (Fig. 1 b, c). Densely stained virus-like particles were never detected in the cytoplasm of cells from healthy subterranean clover plants.

Virus-like particles were detected only in transfer cells of leaves from SCRLV-infected plants. They were easier to detect and appeared to be more numerous in cells of older leaves with red leaf symptoms. The particles were scattered throughout the cytoplasm of the infected cells (Fig. 1a, 2a) and sometimes reached very high concentrations, especially in older cells.
Fig. 1. Thin sections of leaf phloem transfer cells from SCRLV-infected (a) and healthy (b, c) subterranean clover plants. Cells in (a) and (b) are from tissues which had been treated with RNase after aldehyde fixation, whereas those in (c) are from untreated tissue. The cell on the left in (a) shows numerous densely stained virus-like particles scattered throughout the cytoplasm, whereas the cell on the right is devoid of such particles. The cell shown in (b) has no darkly stained particles due to RNase treatment, whereas that in (c) contains numerous darkly stained ribosomes in the cytoplasm. CW, Ingrowth of cell wall; M, mitochondrion; ST, sieve tube; Ch, chloroplast; Va, vacuole. Bar markers represent 0.5 μm.
Fig. 2. Thin sections of leaf phloem transfer cells from SCRLV-infected subterranean clover plants are shown in (a, b). The cells are from RNase-treated tissue showing virus-like particles scattered in the cytoplasm (a), the vacuole (small arrow in a) and the nucleoplasm (b). CW, Cell wall; Va, vacuole; Nm, nuclear membrane; N, nucleoplasm; V, virus-like particles. Bar markers represent 0.5 μm. Virus-like particles isolated from SCRLV-infected subterranean clover plants are shown in (c) and after mixing with a preparation of RCNMV in (d) (arrow points to a particle from SCRLV-infected plants in d, whereas the remaining particles are those of RCNMV). Bar marker represents 100 nm. Virus-like particles isolated from PLRV-infected *P. floridana* plants are shown in (e to g). A partially purified preparation in (e) shows two types of particles, some similar to those in (c) and some smaller and rounder ones (arrows). The same preparation as in (e) is shown in (f) after having been trapped on grids with anti-PLRV serum, and in (g) after having been trapped and decorated with the same antiserum. Bar marker represents 100 nm.
In some of the cells, particles were also detected in the nucleus (Fig. 2b) and in vacuoles (Fig. 2a). A few unidentified cells of the root vascular tissues also contained RNase-resistant virus-like particles. Transfer cells containing virus-like particles often contained small vesicles with stranded material (larger arrows in Fig. 2a) similar to those observed in barley yellow dwarf virus (BYDV)-infected oat leaf cells (Gill & Chong, 1975). However, the appearance of plastids, mitochondria and microbodies in leaf and root cells containing virus-like particles was normal.

Partially purified preparations of virus-like particles were obtained from SCRLV-infected plants showing red leaf symptoms as follows. The tissues were extracted in 0.1 M-phosphate buffer pH 7.4, containing 0.1% 2-mercaptoethanol, 0.01 M-EDTA and 1% Triton X-100. The extracts were subjected to differential and sucrose density-gradient centrifugation. Fractions containing virus-like particles were recovered, concentrated by ultracentrifugation, negatively stained in 2% uranyl acetate and examined in a JEM 100CX electron microscope.

Virus-like particles isolated from SCRLV-infected plants had hexagonal outlines and smooth surfaces (Fig. 2c). They were smaller than red clover necrotic mosaic virus (RCNMV) (Fig. 2d) and had a different shape and surface appearance. Taking the diam. of RCNMV particles as 34-2 nm (T. Hatta & R. I. B. Francki, unpublished results), the diam. of particles from SCRLV-infected plants was calculated to be 30.4 nm. The size and appearance of the virus-like particles were indistinguishable from those of viruses such as southern bean mosaic virus, tobacco necrosis virus and velvet tobacco mottle virus.

In order to see if SCRLV has particles similar to those of a luteovirus, we compared them to particles isolated from Physalis floridana Rydb. infected with potato leafroll virus (PLRV). PLRV has been temporarily excluded from the luteovirus group because its nucleic acid was erroneously reported to be DNA (Sarkar, 1976). However, it has now been established that PLRV has single-stranded RNA of similar mol. wt. to that of BYDV and hence should be included in the luteovirus group (Rowhani & Stace-Smith, 1979; Mehrad et al., 1979; Takanami & Kubo, 1979). Two types of virus-like particles were observed in preparations from PLRV-infected P. floridana extracts (Fig. 2e) purified by a similar procedure to that used on SCRLV-infected subterranean clover. One type of particle was indistinguishable from particles isolated from SCRLV-infected plants, whereas the other type of particles (arrows in Fig. 2e) were smaller, rounder and sometimes allowed stain penetration. The larger particles were identified by their reactions with anti-PLRV serum by immunoelectron microscopy (Rao et al., 1981). They were trapped on specimen grids (Fig. 2f) and decorated (Fig. 2g) with anti-PLRV serum. Similar particles isolated from SCRLV-infected subterranean clover failed to be trapped or decorated by this antiserum. From these experiments we conclude that SCRLV and PLRV have very similar particles which are, however, serologically distinct. The identity of the smaller particles is obscure but they resemble particles of carnation cryptic virus (Lisa et al., 1981) and hence may be particles of a cryptic virus infecting our line of P. floridana seed.

Plants infected with luteoviruses which have been examined by electron microscopy have been found to contain virus particles in the vascular tissues, principally in the phloem. Those examined include PLRV (Kojima et al., 1969), beet western yellows virus (Esau & Hoefert, 1972), BYDV (Gill & Chong, 1975) and SDV (Tamada, 1975). We have also examined thin sections from plants infected with BYDV and PLRV as well as those infected with carrot red leaf virus, which also appears to be a luteovirus (Waterhouse & Murant, 1981). In all these plants we detected RNase-resistant particles with similar morphology and cellular distribution to those observed in SCRLV-infected plants (K. W. Jayasena et al., unpublished results). This, together with the observations that virus-like particles isolated from SCRLV-infected subterranean clover are indistinguishable from PLRV particles when viewed in uranyl acetate-stained preparations, support the biological data suggesting that SCRLV is a luteovirus.
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REFERENCES


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