Cylindrical Inclusions in Coriander Leaf Cells Infected with Parsnip Mosaic Virus

By A. F. MURANT AND I. M. ROBERTS
Scottish Horticultural Research Institute, Invergowrie, Dundee

(Accepted 14 September 1970)

SUMMARY

Cytoplasmic inclusions (X-bodies) in coriander leaf cells infected with parsnip mosaic virus contained much endoplasmic reticulum and many Golgi bodies, together with cylindrical inclusions which, depending on the plane of sectioning appeared as bundles or pinwheels with attached laminated aggregates. The pinwheels differed from those associated with several other filamentous viruses because the radiating plates forming their arms were exceptionally long and flexuous. These plates were frequently attached to the plasmalemma or to elements of the endoplasmic reticulum. In its effects on cells, parsnip mosaic virus thus resembles other members of the potato virus Y group.

INTRODUCTION

Cytoplasmic inclusions (X-bodies) in plant cells infected with flexuous filamentous viruses often contain complex cylindrical inclusions composed of curved plates radiating from a central axis; when seen in cross-section they resemble pinwheels. The detailed structure of such inclusions was described by Edwardson (1966), Purcifull & Edwardson (1967) and Edwardson, Purcifull & Christie (1968), who found that they were not composed of aggregated virus particles, as had been thought previously (Yamaguchi, Kikumoto & Matsui, 1963; Hayashi, Matsui & Yamaguchi, 1965; Weintraub & Ragetli, 1966). They seem to contain protein (Shepard, 1968; Weintraub & Ragetli, 1968) which is, however, serologically unrelated to the protein of the virus particles (Shepard & Shalla, 1969). Edwardson (1966) suggested that the formation of such cylindrical inclusions was characteristic of cells infected with viruses belonging to the potato virus Y group (Brandes & Bercks, 1965).

Parsnip mosaic virus, cryptogram */*//*:E:E:S:Ap (PMV: Murant, Munthe & Goold, 1970), resembles members of the potato virus Y group in particle morphology (slightly flexuous filaments, 755 x 14 nm.), mode of transmission (aphid-borne, brief acquisition feeds) and properties in vitro (thermal inactivation point, 55 to 58°; dilution end point about 10^{-3}; sedimentation coefficient, 149 S). This paper reports electron microscope studies which show that PMV also resembles members of the potato virus Y group in producing cylindrical inclusions in plant cells.

METHODS

The isolate of PMV described by Murant et al. (1970) was used. It was transmitted by manual inoculation of sap to young coriander (Coriandrum sativum L.) plants having one true leaf; Celite (Johns Manville, Ltd.) was used as the abrasive. Leaf samples were taken from the upper uninoculated leaves 7 days later, before the first appearance of symptoms,
and also on the 11th and 14th days, when the leaves showed veinal necrosis symptoms. Leaf pieces were embedded according to the ‘modified’ method of Harrison, Stefanac & Roberts (1970), and sectioned on a LKB Ultrotome I ultramicrotome. Sections were mounted on collodion-coated grids and poststained with uranyl acetate and lead citrate. They were

Fig. 1. Inclusions in cells from a systemically infected leaf of coriander 11 days after the plants were inoculated, (a) pinwheels (PW) and laminated aggregates (LA), (b) bundles (B) associated with the plasmalemma on both sides of the cell wall (CW) separating adjacent cells, (c) pinwheel structures (PW) at the cell wall (CW).
Parsnip mosaic virus-induced inclusions examined in a Siemens Elmiskop I electron microscope at 80 kV using a 50 μm objective aperture.

RESULTS AND DISCUSSION

In leaf samples taken 7 days after the plants were inoculated, before the appearance of visible symptoms of infection, the cell organelles appeared normal but the cytoplasm in many cells was concentrated unusually into masses, often close to the nucleus and protruding into the central vacuole. In these areas of cytoplasm the amounts of endoplasmic reticulum, vesicles, Golgi bodies and mitochondria were much greater than in other parts of the same cells or in cells from comparable healthy tissue.

![Fig. 2. Two pinwheels linked (arrows) through the formation of laminated aggregates (LA).](image)

In leaf samples taken 11 and 14 days after the plants were inoculated, when symptoms of PMV infection were well developed, these areas of cytoplasm contained many cylindrical inclusions, in addition to the organelles mentioned above. Depending on the plane of sectioning, these were seen as ‘pinwheels’, ‘bundles’ or ‘laminated aggregates’ (Fig. 1a to c) similar to those defined by Edwardson (1966), Purcifull & Edwardson (1967) and Edwardson et al. (1968). However, the pinwheels, although similar in basic structure to those in cells infected by other viruses in the potato virus Y group, differed in that the radiating plates forming their arms were exceptionally long and flexuous (Fig. 1a, b; 2). They resembled most closely the pinwheels induced by lettuce mosaic virus (Edwardson, 1966; Goethals, Horvath & Meyer, 1969) and bean yellow mosaic virus (Edwardson, 1966; Weintraub & Ragetli, 1966; Kamei, Honda & Matsui, 1969). Cells infected with watermelon mosaic virus (Edwardson et al. 1968) and several other viruses (Edwardson et al. 1970) contain ‘tubes’ or ‘circular inclusions’ which result from a scroll-like coiling of the pinwheel plates. No such tubes were seen in PMV-infected cells, nor were the plates curved into incomplete tubes, as reported for western celery mosaic, another virus affecting umbelliferous plants (Purcifull & Shepard, 1967).
Fig. 3. (a) Association of pinwheel arms with elements of the endoplasmic reticulum (arrows), (b) at higher magnification to show the relationship of a plate to the membranes of the endoplasmic reticulum (arrow).

Fig. 4. (a) Association of densely staining rods, possibly virus particles (V), with part of a pinwheel structure, (b) 'micro-inclusion body' (MI) situated near the cell wall and opposite the attachment of bundles (B) to the plasmalemma of the adjacent cell.
The plates were always single at the hubs of the pinwheels, but distally they were often associated with other plates to form laminated aggregates. Frequently a laminated aggregate was formed from the arms of two separate pinwheels. In this way complexes of cylindrical inclusions were formed (Fig. 1a; 2).

Bundles or laminated aggregates were frequently attached at one end to the plasmalemma (Fig. 1b) or to elements of the endoplasmic reticulum (Fig. 3a), often so as to suggest that they may be formed from or at these cell membranes (Fig. 3b). Associations with the plasmalemma occurred only in certain regions of the cell, but in adjacent cells they commonly occurred on either side of the intervening cell wall (Fig. 1b). A similar association of cylindrical inclusions with plasmalemma and endoplasmic reticulum was reported for pokeweed mosaic virus by Kim & Fulton (1969) and for maize dwarf mosaic virus by Krass & Ford (1969). The latter authors also described circular to ovoid structures which they termed 'micro-inclusion bodies', situated between the plasmalemma and the cell wall. A similar structure in a coriander cell infected with PMV is shown in Fig. 4b.

Chloroplasts, mitochondria and nuclei appeared to be relatively normal in tissues sampled at 11 or 14 days but there were some signs of disintegration of their bounding membranes. Possible virus particles were rare; they were associated with the pinwheels (Fig. 4a) but could not be identified with certainty: in this respect PMV resembles tobacco etch virus (Edwardson et al. 1968). We found no aggregates or masses of virus particles such as those described by Hayashi et al. (1965) and Kim & Fulton (1969). This paucity of virus particles in thin sections is perhaps not surprising because the infectivity dilution end point of newly systemically infected coriander tissue is usually only between $10^{-2}$ and $10^{-3}$. However, the abundance of cylindrical inclusions in such cells is correspondingly more remarkable, particularly if they play any role in virus synthesis and are not merely by-products of virus infection.

The discovery of cylindrical inclusions in PMV-infected cells provides additional evidence of its similarity to other viruses in the potato virus Y group, and further justifies its classification with them.

REFERENCES


A. F. MURANT AND I. M. ROBERTS


(Received 8 June 1970)