Virus-like Particles in Tomato Plants Affected by the Yellow Leaf Curl Disease

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SUMMARY

The ultrastructural changes of phloem parenchyma cells of tomato plants affected by yellow leaf curl disease are described. The nuclei were severely altered, the chromatin was reduced to a few peripheral clumps and the nucleoplasm contained ring-like electron opaque structures and massive aggregates of virus-like particles. These particles were rounded, measured 15 to 17 nm in diam. and occasionally appeared to occur in pairs. Based on these findings, the presence of a geminivirus in the tomato tissues examined is inferred.

Yellow leaf curl is a major disease of tomato in the south-eastern part of the Mediterranean basin and neighbouring countries (Yassin & Nour, 1965; Cohen & Nitzany, 1966; Nour El-Din et al. 1969; Mazyad et al. 1979). Affected plants are stunted, have small, chlorotic, puckered leaves and produce virtually no fruit. Similar disorders have been reported from India (Verma et al. 1975), Nigeria (Lana & Wilson, 1976) and Latin America (Debroter et al. 1963; Matyis et al. 1975).

The tobacco white fly Bemisia tabaci Genn. was found to be the vector of all these diseases: the disease agents, however, are not reported to be sap-transmissible except in three cases: one each from Brazil (Matyis et al. 1975), Venezuela (De Uzcategui & Lastra, 1978) and Lebanon (Makkouk & Shehab, 1978).

The virions of Brazilian golden mosaic of tomato appeared in the electron microscope as 'Siamese twin, isometric particles, the individual components being 12 to 13 nm in diameter' (Matyis et al. 1975). This was one of the earliest records of a novel type of plant virus, the geminiviruses (Harrison et al. 1977), some members of which have been shown to contain single-stranded (ss) DNA (Goodman, 1977).

Other geminiviruses, such as the agents of bean golden mosaic (Galvez & Castaño, 1976), euphorbia mosaic (Matyis et al. 1975) and tobacco leaf curl (Osaki et al. 1979) diseases, are transmitted by whiteflies and induce peculiar ultrastructural changes in infected phloem cells (Kim et al. 1978; Osaki et al. 1979). This prompted us to investigate the ultrastructure of tomato yellow leaf curl virus (TYLCV) infections.

Samples were collected from tomato plants 30 days after they had been inoculated through B. tabaci with an Israeli isolate of TYLCV, when the typical symptoms of TYLCV infections were evident. Tissue samples consisted of small fragments from petioles, main veins and secondary veins excised in a drop of 4% glutaraldehyde in 0.05 M-cacodylate buffer, pH 7.0. The samples were kept for 2 h under slight vacuum at room temperature in the same fixing solution, then rinsed in a change of buffer and transported to Bari. After postfixation for 2 h at 4 °C with 1% (w/v) osmium tetroxide, the samples were stained overnight at 4 °C in 0.5% uranyl acetate, dehydrated in graded ethanol dilutions and embedded in Spurr's medium. Sections were double stained with uranyl acetate and lead citrate before examination with a Philips 201C electron microscope. Comparable samples from healthy tomato leaves served as controls.

In infected tissues, cytological modifications were observed in cells of the vascular bundles.
Fig. 1. An infected phloem parenchyma cell of tomato showing an altered nucleus (N) which contains a large vacuolated ring-shaped inclusion (FR = fibrillar ring). The nucleoplasm is filled with virus-like particles and the chromatin is much reduced. W = cell wall.

of petioles and main veins. The major changes occurred in the nuclei of phloem parenchyma cells. Altered nuclei were easily recognized because of their uniformly granular texture, reduction of chromatin to a few, small peripheral clumps and apparent absence of nucleoli. Some nuclei, however, contained one or more intensely electron opaque ring-shaped structures varying in size from 0.4 to 3 μm (Fig. 1). On closer examination, these rings appeared to be made up of an amorphous or finely fibrillar matrix in which a few small
Fig. 2. (a) Higher magnification of part of Fig. 1. A portion of the fibrillar ring (FR) is visible. It is surrounded by clumps of virus-like particles (V) scattered in the nucleoplasm. (b) Small aggregates of virus-like particles in the nucleoplasm of a degenerating nucleus. Apparently paired particles are encircled or indicated by arrow heads.

granules were interspersed (Fig. 2a). The periphery of the rings was lined with rounded electron-dense bodies, 15 to 17 nm in diam., resembling virus particles (Fig. 2a).

Similar virus-like particles were plentiful in the nucleoplasm of all modified nuclei, filling most of the organelle. They were not detected in the cytoplasm of infected cells nor in seemingly normal nuclei. In the nucleoplasm, the particles were scattered at random or occurred in small clumps; they were not organized in orderly arrays. In certain areas, where a better resolution was achieved, some of the virus-like particles appeared to be paired (Fig. 2b).

Many striking similarities exist between the ultrastructural changes induced by TYLCV in tomato plants and those associated with infections by members of the geminivirus group.
(i) Localization of virus particles. The intranuclear occurrence of virus particles is a distinctive feature of all geminiviruses investigated so far at the ultrastructural level (Esau & Hoefert, 1973; Bock et al. 1974; Kim et al. 1978; Francki et al. 1979; Osaki et al. 1979). Virions appear first in the nucleus where they remain confined until the late stages of infection (Esau, 1977; Kim et al. 1978). (ii) Type of nuclear modifications. Depletion of chromatin and granular appearance of the nucleoplasm have been observed in nuclei of cells infected by beet curly top (Esau, 1977; Esau & Magyarosy, 1979), bean golden mosaic (Kim et al. 1978) and chloris striate mosaic (Francki et al. 1979) viruses. Furthermore, intranuclear ring-shaped inclusions have been detected in association with infections by beet curly top virus in three different hosts (Esau & Hoefert, 1973; Esau, 1977; Esau & Magyarosy, 1979), by golden mosaic virus in bean (Kim et al. 1978) and by tobacco leaf curl virus in Lonicera japonica Thunb. (Osaki et al. 1979). These structures were called ‘fibrillar rings’ by Kim et al. (1978) who suggested that they might be a possible diagnostic feature for viruses with ssDNA, such as geminiviruses. (iii) Size and shape of virus particles. In thin-sectioned cells, particles of all geminiviruses show the same size and outward appearance as the virus-like particles found in TYLCV-infected samples (Bock et al. 1974; Esau, 1977; Kim et al. 1978; Francki et al. 1979; Osaki et al. 1979).

Hatta & Francki (1979) have convincingly demonstrated that geminivirus particles are usually found in pairs, which therefore also occur as doublets in situ. Some of the structures shown in Fig. 2(b) (arrows and encircled areas) are indeed remarkably similar to profiles of geminate particles of chloris striate mosaic virus as they appear in thin section (Hatta & Francki, 1979).

Based on these similarities, it seems plausible to conclude that a geminivirus is responsible for the ultrastructural changes observed in phloem parenchyma cells of tomato plants affected by yellow leaf curl.

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