Acta virol. **30** : 519—521, 1986

TUBULAR STRUCTURES IN CELLS OF CUCUMOVIRUS-INFECTED *NICOTIANA GLUTINOSA* LEAVES

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Received March 13, 1986

Summary. — Peculiar tubular structures about 250 nm in diameter were found in cells of *Nicotiana glutinosa* leaves infected with a cucumovirus strain isolated from field-grown tobacco in southwest Slovakia.

Key words: *Cucumovirus*; *electron microscopy*; *leaf cells*; *tubular structures*

In the autumn of 1983, field-grown tobacco (*Nicotiana tabacum*) plants at the village Báč in southwest Slovakia were found showing a green mosaic and attempts were made to identify the causative virus. In the course of these investigations, peculiar tubular structures were observed in cells of *Nicotiana glutinosa* leaves infected with the virus in question. The results are reported below.

Virus isolation and identification

Leaf samples from the naturally infected tobacco plants were kept frozen at —20 °C until used in experiments on mechanical inoculation of a series of test plants. For this purpose the frozen leaves were homogenized in a ratio of 1 : 5 with 0.1 mol/l phosphate buffer, pH 7.0. The results were as follows:

Nicotiana tabacum L. cv. Xanthi nc.: green mosaic
N. glutinosa L.: green mosaic, with leaf deformations of the puker type
Physalis floridana L.: ditto
Cucumis sativus L. cv. Mladoboleslavská: chlorotic spots, vein clearing, mosaic
Phaseolus vulgaris L.: cv. Alfa and Bountiful: mosaic on secondary leaves
Pisum sativum L. Juran: mosaic, bending of the apex, browning of the stem
Lycopersicum esculentum Mill: mosaic, leaf deformation, filiform leaves
Chenopodium amaranticolor Coste et Reyn.: chlorotic local lesions inoculated leaves
Trifolium pratense L. cv. Start: no infection.

The symptoms on *Nicotiana tabacum* and *N. glutinosa* remained the same when the plants were inoculated with material from single lesions on *C. amaranticolor*.

In further experiments, the virus, labelled NT 2, was transmitted by three aphid species in the non-persistent manner (5 min acquisition feeding, 24 hr test feeding). The virus was transmitted from and to tobacco species (*N. tabacum*, *N. glutinosa*) by *Macrosiphon euhorbiae* (clone NT from tobacco grown in the locality Báč) and from and to *Pisum sativum* by *Aphis craccivora* (clone TP from red clover in Bratislava) and *Acyrthosiphon pisum* (clone VL, Juríik *et al.* 1980).

Attempts to purify the virus by routine techniques failed so far. Crude sap from infected tobacco (*N. tabacum*, *N. glutinosa*) and pea plants diluted with 0.1 mol/l phosphate buffer, pH 7.0, reacted in double diffusion precipitation tests in 0.1 % agarose with antiserum against cucumber mosaic virus (CMV) strain E (Havránek, 1978).

Based on these results it appears justified to consider the NT 2 isolate as a cucumovirus but a definite identification would require detailed serological investigations. In the host plants tested, the NT 2 isolate was symptomatologically different from most CMV strains isolated in Czechoslovakia (see, e. g., Havránek 1971; Musil *et al.*, 1979, 1983). In some plants (cucumber, tomato, pea, bean) the symptoms resembled those caused by C-CMV isolated from cucumber (Havránek, 1971), while in *N. glutinosa* the typical symptoms of NT 2 infection (Fig. 3) were similar to those observed in CMV infection of *N. glutinosa* by, e. g., Betto *et al.* (1964) and Horváth *et al.* (1975). The low stability of NT 2 isolate may prove to be a serious obstacle in attempts at its definite identification.

Ultrastructure of infected N. glutinosa mesophyll cells

Samples from dark green "elevated" areas of leaves of N. glutinosa were fixed at three weeks after inoculation with NT 2 virus (by *M. euphorbiae* aphids) in 2.5 % glutaraldehyde in 0.2 mol/l cacodylate buffer pH 7.2 for 1 hr at 4 °C, washed in the same buffer and post-fixed for 3 hr at room temperature in a 1 % OsO_4 solution in the same buffer. The fixed materials were dehydrated through an ethanol series and embedded in Spurr low viscosity medium. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope at 80 kV.

Cells from dark green areas were vacuolated like older normal plant cells, showing a thin cytoplasm layer. The cytoplasm contained occasional single isometric virus particles 30—31 nm in diameter (Fig. 1). In the vacuoles, there occurred various aggregates of tubular structures which in cross sections appeared to possess a cylindric character. The length of the tubular structures varied and could not be definitely determined due to the irregular arrangement of the tubules. The tubular structures had a diameter of about 250 nm. Their surface was not smooth but appeared finely granular (Fig. 2). The tubular structures were observed repeatedly not only in different plants from the same experimental series but also in *N. glutinosa* plants from different experiments and thus appear to be a characteristic feature of NT 2 virus infection of *N. glutinosa*. In view of their localisation and morphology, the tubular structures observed in the present experiments apparently are not

identical with those described by Gerola *et al.* (1964) in CMV-infected *N. glutinosa* leaves.

Acknowledgement. Thanks are due to Dr. P. Havránek, Vegetable Crops Research Institute Olomouc, for E-CMV antiserum, to Mr. L. Szalai and Mrs E. Cimprichová for technical assistance and Mr. N. Dokoupil and Mr. P. Kvíčala for photography.

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Explanation to Figures (Plates LIX—LXI):

Fig. 1. Part of a NT 2 virus infected *N. glutinosa* mesophyll cell with a central vacuole containing tubular structures about 250 nm thick (T). Occasional single virions 30—31 nm in diameter in the cytoplasm × 99 000.

Fig. 2. Vacuole of a NT 2 virus infected *N. glutinosa* mesophyll cell containing numerous tubular structures about 250 nm thick. Cross sections suggest a cylindric character of the tubules. × 82 000.

Fig. 3. Symptoms of NT 2 virus infection in *N. glutinosa* at 6 weeks after inoculation (right); healthy *N. glutinosa* leaf at left.