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The distribution and propagation of *Zucchini yellow mosaic virus* (ZYMV) within Styrian pumpkin plants

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Abstract. The distribution and propagation of *Zucchini yellow mosaic virus* (ZYMV) within Styrian pumpkin plants (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* GREB.) was studied for one week starting 6 hours after ZYMV-inoculation using transmission electron microscopy (TEM). By negative staining, virus particles were found within the cotyledons and its petioles four days after the inoculation and within the whole plant seven days after the infection. Two weeks after ZYMV-inoculation the cytoplasm of infected leaf and stem cells showed typical features of ZYMV like cylindrical inclusions and proliferated endoplasmatic reticulum. No ZYMV-induced modifications were found within root cells.

Keywords: *Zucchini yellow mosaic virus* (ZYMV), Styrian pumpkin, *Cucurbita pepo* L., negative staining, transmission electron microscopy, TEM.

Introduction

Zucchini yellow mosaic virus (ZYMV) is a *Potyvirus* and infects a large variety of economically important cucurbit plants worldwide like zucchini squash (*Curcubita pepo*), cucumber (*Cucumis sativus*), cantaloupe (*Cucumis melo*), watermelon (*Citrullus lanatus*) and various species of pumpkin (WANG & al. 1992, DESBIEZ & LECOQ 1997). Since 1997, severe epidemics of ZYMV-disease in Styria (Austria) have caused of yearly crop losses in Styrian pumpkin (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* GREB.) production of up to 60 % (WEBER 1998, RIEDLE-BAUER & al. 2002). Symptoms caused by ZYMV on Styrian pumpkin plants are yellowing, leaf deformations, stunting, color alterations and deformations of the fruits which make them unmarketable. The leaves develop a yellow mosaic and often show dark green blisters.

Within the cells of ZYMV-infected plants like zucchini squash, cucumber etc. the virus induces scroll elements of cylindrical inclusions (pinwheels) which do not contain virions, vesicles containing fibrillous material and cytoplasmic inclusions of proliferated endoplasmatic reticulum (LISA & al. 1981, LESEMANN & al. 1983, DESBIEZ & LECOQ 1997). In the present study the distribution and propagation of ZYMV within two weeks of old Styrian pumpkin plants was studied starting 6 hours after inoculation over a period of one week. Additionally the impact of ZYMV on the ultrastructure was documented two weeks after the inoculation when plants developed severe symptoms of ZYMV-disease. The movement of viruses similar to ZYMV within plants is well documented. It is assumed

that after the infection the virus goes through replication and moves symplastically through plasmodesmata from cell to cell until it reaches sieve elements. Systemic infection of the host is then performed by the virus through the phloem transport system (CARRINGTON & al. 1996, DERRICK & NELSON 1999, CHENG & al. 2000). However little information is available about the propagation and distribution of ZYMV within Styrian pumpkin plants. Therefore the transmission electron microscope (TEM) was used to study the distribution and propagation of ZYMV over a period of one week starting 6 hours after inoculation and to document ultrastructural alterations induced by the virus within Styrian pumpkin plants.

Material and Methods

Plant material

Styrian pumpkin seeds (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* GREB.) received from Saatzucht Gleisdorf (Plant Breeding Company, Gleisdorf, Austria) were germinated on a humid Perlite cloth. One week old seedlings were replanted in pots filled with soil and transferred into separated growth chambers with a photoperiod of 12 hours (PhAR 400–700 nm). Day and night temperatures were 22 °C and 18 °C, respectively, the relative humidity was 70 %. The plant material was kept at 100 % relative water content. Cotyledons were infected with the sap of ZYMV-infected plant material before the first foliage leaves emerged. Therefore 1g of infected Styrian pumpkin material (leaves or mesocarp) was homogenated in 1 ml of a 1 %-K_2HPO_4 solution (pH 6.5). After applying celite (Cebeda, CSR) to the homogenate, the inoculum was spread out on the cotyledons of the seedlings. Mock inoculation was performed on control plants by spreading out the buffer with celite but without the inoculum onto the cotyledons.

Negative staining

Starting 6 hours after inoculation, negative staining was performed on at least three plants every day over a period of one week using different plant parts (cotyledons, leaves, stems and roots) in order to study the distribution and propagation of ZYMV within the plant. Therefore crude sap of infected Styrian pumpkin material was applied on top of a carbon coated copper grid (400-mesh) for five minutes, and subsequently the grid was washed in a 0.06 M phosphate buffer. Then the depositions on the grid were stained with 2 %-phosphotungstic acid in phosphate buffer (pH 6.5) for two minutes. The grids were then air-dried and observed with the TEM.

Chemical fixation

To study the impact of ZYMV on the ultrastructure of Styrian pumpkin plants, infected plant material showing severe symptoms of ZYMV-disease was prepared for TEM. Therefore leaves and roots of infected and control Styrian pumpkin plants were harvested separately two weeks after ZYMV-inoculation. Small pieces of the samples were fixed in 3 %-glutaraldehyde in 0.06 M phosphate buffer at pH 7.2 for 90 minutes at room temperature (RT). Postfixation was carried out in 1 % osmium tetroxide in 0.06 M phosphate buffer pH 7.2 for 90 minutes at RT. Dehydration was performed in increasing concentrations of acetone (50 % to 100 %) and propylene oxide before the samples were embedded in Agar 100 epoxy resin. Ultrathin sections were post-stained with lead citrate and uranyl acetate before they were observed in a Philips CM10 TEM.

Results

Filamentous, elongated virus particles were detected by negative staining for the first time only in symptom free cotyledons and its petiole 4 days after ZYMV-inoculation. The particles were 750 nm in length and 11 nm in width (Fig. 1b). The virus did not spread out of the cotyledons until the 7th day after the infection when it was found in the cotyledons, stems, the first leaves and in roots. No visible symptoms of ZYMV-disease were observed on the plants at this time. However two weeks after inoculation infected plants developed severe symptoms of ZYMV-disease (yellowing, stunting, blistering, leaf-deformations). At this time the cytoplasm of infected leaf and stem cells contained a large amount of cylindrical inclusions, which were found to be organized as long tubular bundles if cut longitudinally and as scrolls and pinwheels if cut transversely. Besides that, proliferated endoplasmatic reticulum (ER) was observed frequently throughout the infected cells of leaves and stems (Fig. 1a, c). Virus particles were not found in plasmodesmata. No virus-induced modifications were observed within root cells although virus particles were detected there by negative staining.

In control plants no virions, cylindrical inclusions nor virus-induced modification were found.

Discussion

Viruses move within the plant from cell to cell (short distance movement) and after entering the vascular system over long distances to systemically infect the whole plant (Carrington & al. 1996, Cheng & al. 2000). Whereas the virus moves from cell to cell in rather slow rates of approximately one cell per 2 hours (Mise & Ahlquist 1995, Szécsi & al. 1999), vascular movement occurs at rates of centimeters per hour (Nelson & van Bel 1998).

In the present study the distribution and propagation of ZYMV within Styrian pumpkin plants was investigated, over a period of one week starting 6 hours after inoculation, by negative staining using TEM. Four days after inoculation, the virions were detected within the cotyledons and their petioles. Seven days after the infection the virus was detected in all parts of the plants, including roots. These results lead to the assumption that after the virus reaches the phloem of the host it moves up and down within the plant at the same time. Our results are similar to what has been reported recently for *Tobacco mosaic virus*, which has been found moving up and down within the phloem of the stem seven days after the infection of the 2nd leaf (Cheng & al. 2000).

Two weeks after ZYMV-inoculation the cytoplasm of infected Styrian pumpkin leaf and stem cells showed severe ultrastructural changes like cylindrical inclusions and proliferated ER, which were similar to what was found in other ZYMV-infected cucurbit plants (Lisa & al. 1981, Lesemann & al. 1983, Desbiez & Lecoq 1997). Virus particles, which had the same average size described in the literature for ZYMV (Lisa & al. 1981) were detected by negative staining in leaves, cotyledons, stems and roots depending on the infection state. However, no ZYMV-induced ultrastructural modifications occurred within root cells. It seems that ZYMV-related ultrastructural changes within the plant are restricted to stem and leaf cells only.

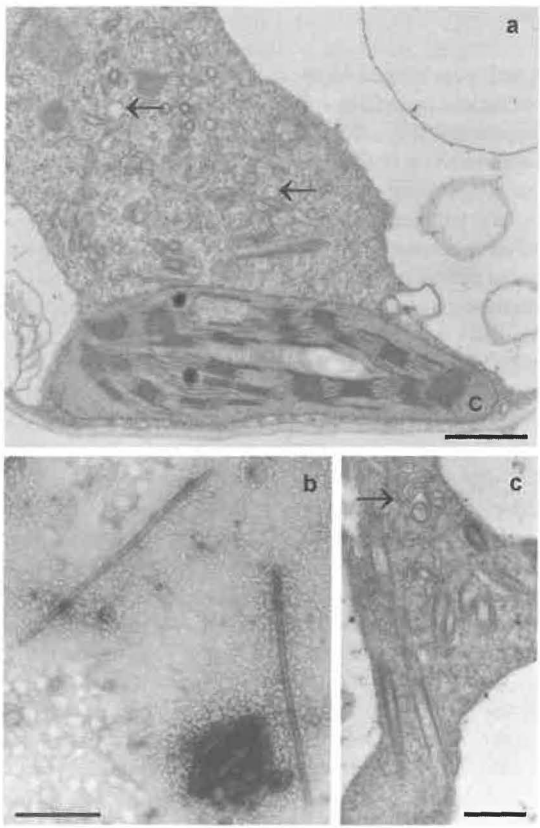

Figure 1: Transmission electron micrographs. Bars: a = 1 μm; b = 250 nm, c = 500 nm. a) *Zucchini yellow mosaic virus* (ZYMV)- infected mesophyll cell two weeks after the inoculation showing a chloroplast (C), cylindrical inclusions and proliferated endoplasmatic reticulum (arrows) within the cytoplasm. b) Particles of ZYMV detected in the sap of infected leaf-material by negative staining one week after inoculation. c) Infected mesophyll cell two weeks after the ZYMV-inoculation showing cylindrical inclusions in various formations including pinwheels (arrow).

Conclusion

After entering the host ZYMV needs about one week to infect the whole plant. Although the virus needs quite a long time to spread through the first infected leaf, it moves quite fast after it enters the phloem to systemically infect the whole plant. This leads to the conclusion that a rapid movement and distribution of viruses within plants is limited by the short distance movement (cell to cell) rather than the long distance movement through the phloem transport system.

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