

Electron Microscopy of Structures Present in Embryonic Cells of Plants Infected with *Plum Pox Virus*

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Abstract

POLÁK J., JOKEŠ M., DUCHÁČOVÁ M., HAUPTMANOVÁ A., KOMÍNEK P. (2008): **Electron microscopy of structures present in embryonic cells of plants infected with *Plum pox virus***. Plant Protect. Sci., **44**: 81–84.

Electron microscopy was used to detect the presence of virus particles or inclusions in growth tips and parenchymatic cells of leaves of plum, apricot and peach trees artificially infected with *Plum pox virus* (PPV). Typical pinwheels were found in ultrathin sections of leaves of PPV infected plums, apricots and peaches. Filamentous particles or their aggregates approximately 750 nm long were found in ultrathin sections of growth tips of plum, apricot, and peach shoots with a diameter of 0.5 mm. Pinwheels were never present in embryonic cells. No virus particles were found in ultrathin sections of growth tips of PPV infected plum, apricot and peach shoots of 0.2 mm in diameter. Embryonic cells of growth tips up to 0.2 mm in diameter are PPV free. PPV particles are present in growth tips at a distance 0.2–0.5 mm from the top; the virus is probably multiplied in this part of the growth tips.

Keywords: sharka; herbaceous and woody hosts; meristematic cells; growth tips; PPV aggregates; ultrathin sections

Plum pox virus (PPV) is a member of the Potyviridae family, genus Potyvirus. It has been known for over 80 years that potyviruses induce the formation *in vivo* of cytoplasmic and nuclear inclusions which are observable by light microscope (KUNKEL 1922; KASSANIS 1939). Electron microscopy has shown that these inclusions are aggregates of virus particles with host cell components (EDWARDSON 1974; MARTELLI & RUSSO 1977).

PPV induce pinwheels and laminated aggregates in the cytoplasm of infected cells (EDWARDSON *et al.* 1984). Several reports have been published on the presence of PPV inclusions in cells of herba-

ceous and woody hosts. PPV particles and pinwheel structures were observed in parenchymatic cells of *Chenopodium foetidum*, *Nicotiana glutinosa*, *Prunus domestica* cv. Green Gage, and *Prunus domestica* cv. Italian Prune experimentally infected with PPV (GOLINOWSKI & GARBACZEWSKA 1980). PLEŠE *et al.* (1969) observed intracellular lamellar inclusions of PPV. BOVEY (1971) and MACOVEI (1971) described lamellar inclusions of the pinwheel type in leaves of *Nicotiana clevelandii*, apricot, prune and peach trees infected with PPV; ABDEL-GHAFFAR *et al.* (1998) revealed the presence of pinwheel and laminated inclusions in cells

Supported by the Ministry of Agriculture of the Czech Republic, Projects No. 1B44051 and MZe 0002700603.

of *N. clevelandii* infected with the PPV-El Amar strain. No reports were found on the presence of PPV in embryonic plant tissue.

Recently, our laboratory investigated the experimental conditions for recovering of fruit trees of the genus *Prunus* from *Plum pox virus*. Trees of plum, apricot and peach cultivars were artificially infected with PPV-M and PPV-D strains by grafting. The presence of PPV in inoculated trees was verified serologically, and different procedures of virus elimination were applied. *In vitro* cultures and growth tips of different length were used. Electron microscopy was used to detect the presence of virus particles or inclusions in the growth tips. The presence of PPV particles in parenchymatic cells of leaves of trees infected with PPV was verified by electron microscopy at the same time. The results obtained are presented in this original paper.

MATERIAL AND METHODS

Plant material, virus inoculation. Two year old trees of plum, apricot and peach were artificially infected by PPV. Grafts of apricot infected with PPV-M or PPV-D were used for bud inoculation. Plum *Prunus domestica* L. cv. Švestka domácí was inoculated with PPV-D, while cv. Čačanská lepotica was inoculated with PPV-M. One apricot tree *P. armeniaca* L. cv. Karola was inoculated with PPV-D, and another tree of cv. Karola with PPV-M. In peach *P. persica* L., one tree of cv. Redhaven and one of cv. Earliglo were inoculated with PPV-D, and one tree of cv. Earliglo with PPV-M. Leaves

of infected trees showed typical PPV symptoms. Three months after virus inoculation the presence of PPV in the inoculated trees was confirmed by ELISA. A healthy tree of plum, cv. St. Julien, was used as a control.

Preparation of samples, electron microscopy of ultrathin sections. Samples from shoot apices and leaves of PPV infected trees were used for electronmicroscopical studies. Growth tips of apricot cv. Karola, peach cvs. Redhaven and Earliglo, plum cvs. Čačanská lepotica and Švestka domácí in diameter of 0.2 mm and 0.5 mm, and samples 0.5 mm in size from leaves of the same cultivars were taken for investigation in June. Samples from the healthy plum cv. St. Julien were collected simultaneously. All specimens were fixed with 2% phosphate-buffered glutaraldehyde at pH 7.0 for 1.5 hours. Tissues were dehydrated in an ethanol-propylene oxide series, embedded in LR White, placed transversely in blocks. Thin sections were prepared with an ultramicrotome Ultracut E fy Reichert and stained with uranyl acetate and lead citrate. Samples were examined with Philips transmission electron microscope 208 S.

Preparation of in vitro samples for the detection of PPV in growth tips by RT-PCR. Murashige-Skoog medium (MS) was used for cultivation of growth tips 0.2 and 0.5 mm in diameter. Auxins, 2,4-D (0.1 mg/l), agarose (6.5 g/l), saccharose (25 g/l), and myo-inositol (0.1 g/l) were added to the MS medium on which the growth tips were cultivated for 2 weeks at 21°C. The growth tips were then transferred onto the basic MS medium with auxins, 2,4-D (0.1 mg/l), cytokinins BAP (1.2 mg/l), aga-

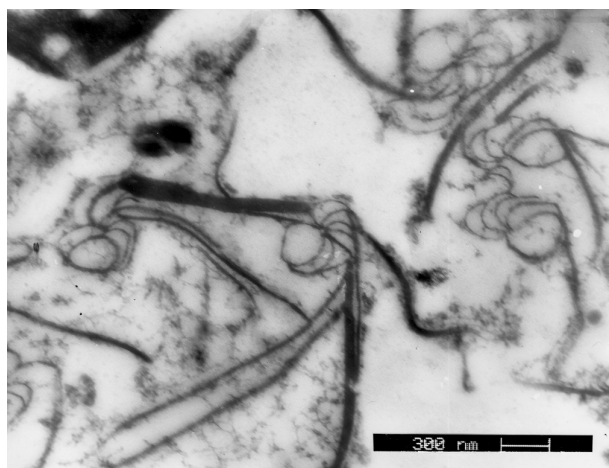


Figure 1. Pinwheels and laminated aggregates in cytoplasm of PPV infected leaf cell of plum

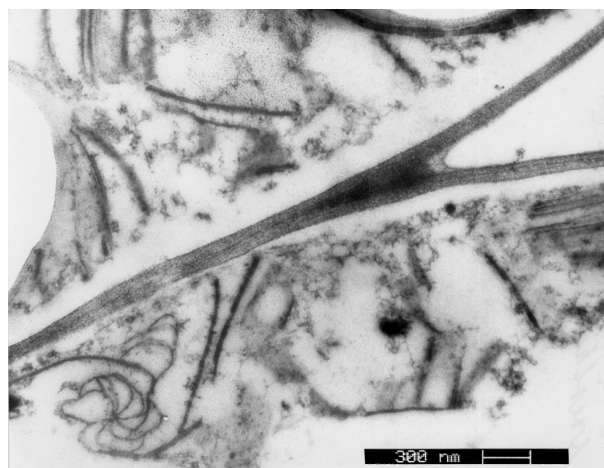


Figure 2. Pinwheels and laminated aggregates in cytoplasm of PPV infected leaf cell of apricot

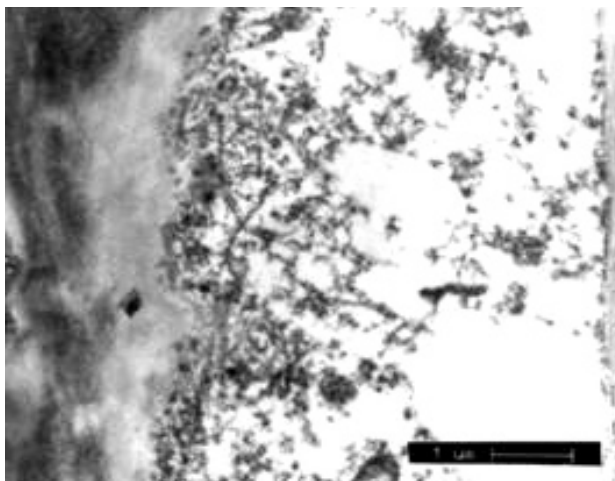


Figure 3. PPV particles in cytoplasm of meristematic cell of plum cv. Švestka domácí

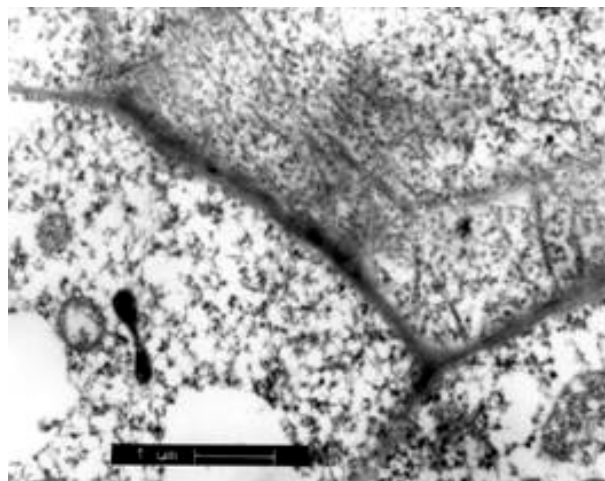


Figure 4. PPV particles in cytoplasm of meristematic cell of apricot cv. Karola

rose (6.5 g/l), saccharose (20 g/l) and myo-inositol (0.1 g/l). On this medium the *in vitro* cultures were grown for 3 weeks. Three to five leaves appeared in this time; they were used as samples for detection of PPV by RT-PCR.

Viral RNA isolation and RT-PCR detection of PPV. The total RNA was isolated from samples of 0.1 g of plant tissue, using an RNeasy Plant Mini Kit (Qiagen) according to manufacturer's instructions.

Primers P1-P2 (WETZEL *et al.* 1991) were used for the RT-PCR detection of PPV. RT-PCR was carried-out using a Qiagen OneStep RT-PCR Kit in a PTC200 thermocycler (MJ Research). Reverse transcription was done at 45°C for 60 min, followed by a denaturation step at 95°C for 15 minutes. Subsequently, PCR was done at 40 cycles. Each cycle consisted of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 45 seconds. The reaction was completed by elongation at 72°C for 10 minutes.

Aliquots of PCR products were run on 1.5% agarose gels. A product of 243 bp appeared in samples infected with PPV.

RESULTS AND DISCUSSION

Typical pinwheels were found in ultrathin sections of leaves of PPV infected plums, apricots and peaches. Pinwheels and laminated aggregates were observed in the cytoplasm of infected cells (Figures 1 and 2). Pinwheels or aggregates were not found in ultrathin sections prepared from leaf tissue of healthy control trees. Neither pinwheels

nor laminated aggregates were present in embryonic cells of plants infected with PPV.

Filamentous virus particles or their aggregates approximately 750 nm long were found in ultrathin sections of growth tips with a diameter of 0.5 mm of shoots of plum, apricot and peach. The presence of PPV in growth tips 0.5 mm in diameter was confirmed by RT-PCR. Virus particles were observable in the cytoplasm, close to the cell walls. PPV particles approximately 750 nm long or their aggregates present in the cytoplasm close to the walls of meristematic cells were found in ultrathin sections of plum, cv. Švestka domácí (Figure 3), apricot, cv. Karola (Figure 4), and peach, cv. Earliglo (Figure 5).

Virus particles were never observed in ultrathin sections of growth tips of 0.2 mm in diameter from

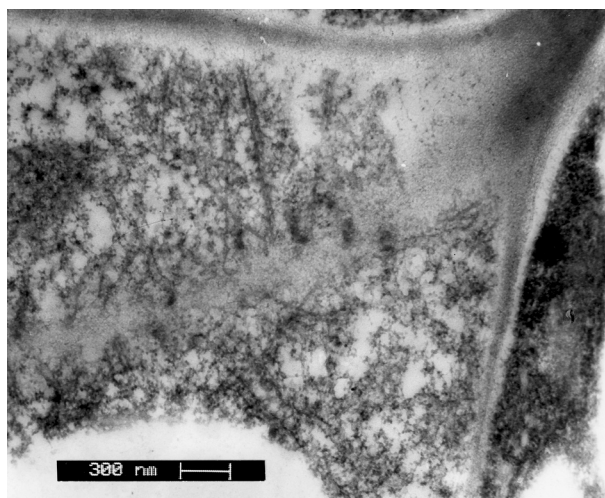


Figure 5. PPV particles in cytoplasm of meristematic cell of peach cv. Earliglo

PPV infected plum, apricot, and peach shoots. PPV was usually not detected by RT-PCR in growth tips 0.2 mm in diameter from PPV infected plum, apricot, and peach shoots, or the reactions were weak. The results of this electronmicroscopical study indicate that embryonic cells of growth tips 0.2 mm or less in diameter can be used in meristem cultures to obtain virus free plants without application of thermotherapy or chemotherapy. Thus, virus free plants of plum, apricot, and peach were obtained from meristematic cells of plants infected with *Plum pox virus*. This is the first report on the presence of PPV particles in cells 0.2–0.5 mm distant from the top of growth tips in stone fruit trees infected with *Plum pox virus*.

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Received for publication August 22, 2008

Accepted after corrections September 19, 2008

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