Electronmicroscopic Study of *Beet Soilborne Pomovirus*

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Abstract

Beet soilborne pomovirus (BSBV) was observed both in the sap and in tissues from local lesions on *Chenopodium quinoa* leaves after their embedding into acrylic resin LR White. Immunocapturing with polyclonal antibodies was used to enhance number of particles on grids and immunolabelling by colloidal gold was used for better visibility of virus particles in tissues. BSBV has rod-like particles of various length and it forms inclusions of several particles adhering side to side each to another.

Keywords: *Beet soilborne virus*; *Chenopodium quinoa*; polyclonal antibodies; immunolabelling; electron microscopy

INTRODUCTION

BSBV was first found by IVANOVIC and MCFARLANE (1982) in England and better described by HENRY *et al*. (1986). Particles of BSBV have several modal lengths (65, 150 and 300 nm) and about 20 nm in diameter. The virus is transmitted by soil protist *Polymyxa betae* (IVANOVIC *et al*. 1983) and fulfils criteria to be included into genus *Pomovirus* (HULL 2002). It is very widespread in sugar beet growing areas all around the world (LINDSTEN 1991; PRILLWITZ & SCHLOSSER 1992; TURINA *et al*. 1996) and is present also in the Czech Republic (RYŠÁNEK & KUDLÁČKOVÁ 2000). It may cause symptoms resembling rhizomania caused by *Beet necrotic yellow vein benyvirus* (BNYVV) on sugar beet but frequently it also occurs in symptomless plants (PRILLWITZ & SCHLOSSER 1992). In pot trials the weight of plants after inoculation by viruliferrous zoospores of *P. betae* was decreased even by 40% (PRILLWITZ & SCHLOSSER 1992). The losses of infected plants under field conditions but after mechanical infection of roots caused 20% decreasing of root weight of young plants (KAUFMANN *et al*. 1993). Morphology of BSBV particles has already been studied by HENRY *et al*. (1986) and by LESEMANN *et al*. (1989) but little is still known about BSBV appearance in tissues of infected plants.

MATERIALS AND METHODS

BSBV was mechanically inoculated onto *Chenopodium quinoa* leaves from roots of sugar beet baiting plants growing in contaminated soil from Rostoklaty (Central Bohemia). Local lesions (5 to 8 days post inoculation) or roots of baiting plants were cut into small pieces 2 × 1 mm which were fixed 2 h in 2% glutaraldehyde at 4°C, washed in phosphate buffer, postfixed 2 h in 1% osmium tetraoxide and again washed in redestilled water. Then the tissue was dehydrated in a graded ethanol series (30, 50, 70, 90%, 30 min each and 100% overnight), infiltrated by LR White resin (1:3, 1:1, 3:1 mixture with ethanol, 1 h each and 100% resin 2 × 1 h) and embedded in resin in gelatine capsules with polymerization at 60°C for 2 days. Ultrathin sections were cut with LKB Ultratome III and put onto nickel grids covered with pioloform membrane. Immunolabelling was done placing the grids onto drops of chemicals: water 5 min, saturated solution of $NaIO_4$ 15 min, water 3 × 2 min, 0.1 M HCl 10 min, water 2 × 2 min, PBS with 1% BSA and 0.1% Tween 15 min, antibodies to BSBV (prof. Lindsten, Sweden) in PBS – BSA 1:50 1 h at 37°C, washing in PBS 10 min and 5 × 2 min, antirabbit IGg coupled to colloidal gold 15 nm (Biocell) 1:20 in PBS-BSA 1 h at 37°C, washing in PBS as above,

washing in water 2 × 5 min, 2% uranylacetate 5 min, washing in water 10 × 1 min, lead citrate 5 min, washing in water 10 × 1 min. For ISEM grids were incubated in antibodies in PBS (1:800) 1 h at 37°C, briefly washed with PBS, leaf homogenate (1:10 in PBS-BSA, spinned for 10 min at 10 000 g) 1 h at 37°C, washing with PBS and water, 2% uranylacetate 5 min, brief washing in water. Observation of grids was done with Tesla BS 500 electron microscope.

RESULTS

BSBV was observed both in sap and in tissues from local lesions on *Chenopodium quinoa* leaves. In sap both single particles and aggregates of several particles were seen. The particles were decorated to some extent with antibodies (Figure 1). In tissues from local lesions inclusions of virus particles were also seen with gold labelling. The inclusion consisted usually from only small number of particles adherring side to side each to another (Figure 2). They occured either isolated or groups of them were scattered in the cytoplasm of parenchyma cells. Because of small concentration of virus in tissues it was rather difficult to find it even using immunolabelling. Till now the virus has not been observed in roots of sugar beet.

DISCUSSION

It is the first observing of BSBV directly in tissues of infected plants. The inclusions of BSBV are quite different from those of BNYVV in the shape of fish skeleton (PUTZ & VUITTENEZ 1980) with which BSBV frequently occurs in mixed infections. Comparing to BNYVV, the concentration of BSBV in tissues was very small as already mentioned by HENRY *et al*. (1986) and confirmed by our work testing PCR (ZOUHAR & RYŠÁNEK 2000). That is why immunolabelling was very useful during the work as it enabled to find virus inclusions in the cytoplasm of infected cells but the antibodies had to be rather concentrated (1:50) for this purpose. In this case danger of backgroud exists but with some exceptions we did not have this problem. BSBV particles from sap were of different length as already described by IVANOVIC *et al*. (1983) and LESEMANN *et al*. (1989) but we have not mesured them. The particles were slightly decorated by antibodies. Without ISEM it was almost impossible to find the virus.

Figure 1. BSBV from sap from local lesions on *Chenopodium quinoa* leaves adsorbed onto grids coated with BSBV antiserum dilluted 1:800 (magnification 500 000×)

Figure 2. BSBV in cytoplasm in local lesions from infected leaf of *Chenopodium quinoa* labelled with colloidal gold (15 nm). Bar represents 100 nm

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