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BEET NECROTIC YELLOW VEIN VIRUS: PURIFICATION AND DETECTION BY ELECTRON MICROSCOPY AND WESTERN BLOT

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The best results for Beet necrotic yellow vein virus (BNYVV) purification were obtained from Chenopodium quinoa leaf tissue mechanically inoculated with the virus using slightly modified method described by Bouzoubaa [1]. The presence of BNYVV was confirmed by immunosorbent electron microscopy (IEM) and Western blot analysis. IEM with antiserum to BNYVV coat protein (CP) revealed virus particles morphology typical for rhizomania agent [8]. Purified virus suspension was used for CP evaluation and detection by Western blot.

Introduction. BNYVV (genus *Benyvirus*) causes rhizomania – destructive disease of sugar beet (*Beta vulgaris* L. ssp. vulgaris). BNYVV is rigid, rod-shaped virus, composed of particles 390, 265, 100 and 85 nm long and 20 nm wide [8]. The multipartite single-stranded RNA genome consists of four RNA species, and the 5th species is being found in some isolates (6.8, 4.7, 1.8, 1.5 and 1.45 kb) [10]. The virus is transmitted by vector, the soilborne plasmodiophorid *Polymyxa betae* Keskin, which survives in infested soil for many years [11]. The disease now has a worldwide distribution (Europe, Asia and North America) [9] and is characterized by reduced taproot size with extensive proliferation of lateral rootlets that leads to decline in root yields and sugar content [6].

Surveys to detect the presence of BNYVV have been regularly carried out in Lithuania since 1998 using DAS-ELISA test. In recent years this virus was detected in two areas of Lithuania [3, 13]. *C. quinoa* plant tissues mechanically inoculated with crude extract of infected sugar beet rootlets were used for investigation of rhizomania agent. Here we present identification of BNYVV using EM (i.e., the particle morphology) and Western blot assay (i.e., CP molecular weight).

Materials and methods. Isolation of BNYVV was performed by mechanical inoculation of *C. quinoa* using infected sap of sugar beet bearded roots. Symptomatic leaf tissue was tested for BNYVV by DAS-ELISA [2] using DSMZ immunological kit, for virus particles morphology by EM and further it was used for virus purification.

BNYVV was purified as described by Bouzoubaa with some modification [1]. Healthy *C. quinoa* extract was used as negative control. The virus was concentrated twice by PEG, NaCl. Afterwards the virus was purified by high speed ultracentrifugation through 20 % sucrose cushion and in 5-45 % sucrose density gradient centrifugation. Purified virus suspension was used for EM examination and Western blot analysis.

In attempts to examine virus particles, carbon coated grids were placed on drops of purified virus suspension, stained with 2% uranyl acetate (UA), and were viewed using JEOL JEM-100S electron microscope at x 20 000 magnification.

For BNYVV protein analysis purified virions were denatured and CP was separated by 12% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) in duplicate [5]. One gel was stained with PageBlue™ Protein Staining Solution. Capsid molecular mass was estimated using PageRuler™ Prestained Protein Ladder. For Western blot analysis the other gel with BNYVV CP was transferred to nitrocellulose membrane [12]. Membrane was probed with IgG to BNYVV (diluted 1:250) and the bound IgG was detected with secondary antibody conjugated to alkaline phosphatase (diluted 1:1000). Antibodies used for Western blot analysis were the same as supplied for DAS-ELISA kit. Blot was visualized with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) substrate.

Results and Conclusions. IEM investigation of infected sugar beet rootlets revealed the presence of characteristic different lengths BNYVV particles about 20 nm in diameter (Fig. 1) [8, 10]. Tissue extracts from these rootlets was used for mechanical inoculation of indicator plant - *C. quinoa*, which leaves developed local chlorotic lesions after 5-7 days (Fig. 2).

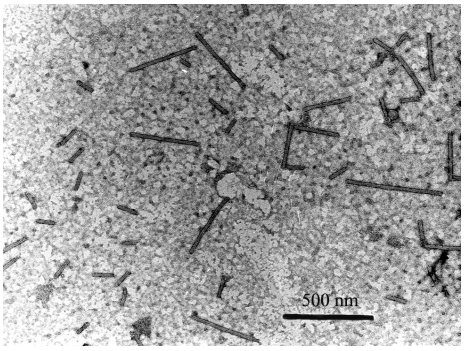

Fig. 1. IEM (instrumental magnification 20 000 x): BNYVV characteristic virus particles about 20 nm in diameter from extracts of sugar beet rootlets.

Fig. 2. Mechanical inoculations with infected plants sap: local chlorotic lesions on indicator *C. quinoa* plant.

The lesions differ in size and appearance, depending on the viral isolate. Isolates carrying RNA3 produce intence yellow lessions, which have a tendency to extend along veins [4]. Isolates carrying only RNA1 and RNA2 induce mild chlorotic local lesions and give a lower purification yield. Systemic infection hosts (e.g. spinach) did not give superior yields of BNYVV [1].

Symptomatic *C. quinoa* leaf tissue with local lesions was collected and used for BNYVV purification. An OD_{260}/OD_{280} ratio was estimated 1.11-1.17 for a pure *benyvirus* suspension. The yield of purified BNYVV preparations was 0.237-0.515 mg from 100 g infected leaves material, assuming that extinction coefficient was 3.2 [1].

EM of purified BNYVV suspension showed rod-shaped virus particles of lengths typical for rhizomania agent (Fig. 3) [8].

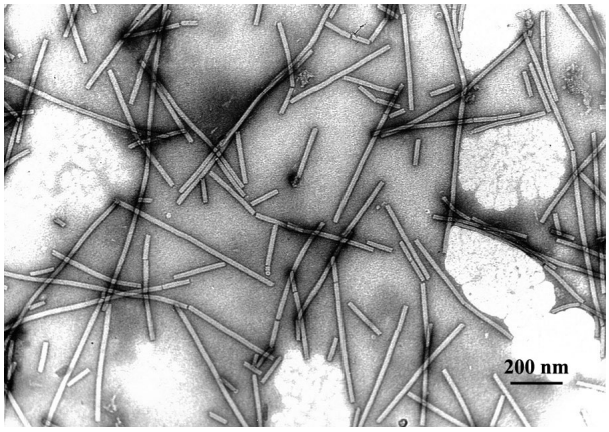

Fig. 3. EM (instrumental magnification 20 000 x): purified BNYVV particles.

The virus so obtained was stored for further examination and antisera production.

Electrophoresis of purified plant viruses can be a helpful and simple tool for characterization of their particles. For this purpose usually SDS-PAGE is used. Viruses can be separated by electrophoresis, and then they can be effectively transferred by electroblotting onto nitrocellulose membranes for further analysis. A great advantage of this technique is that it identifies the virus by two idependent properties of its coat protein – molecular weight and serological specifity [7]. The molecular mass of BNYVV coat protein was estimated by SDS-PAGE to be 21 kDa (Fig. 4). In Western blot BNYVV antiserum reacted only with the CP of purified BNYVV virions and did not reacted with any proteins from healthy *C. quinoa* extracts (Fig. 5).

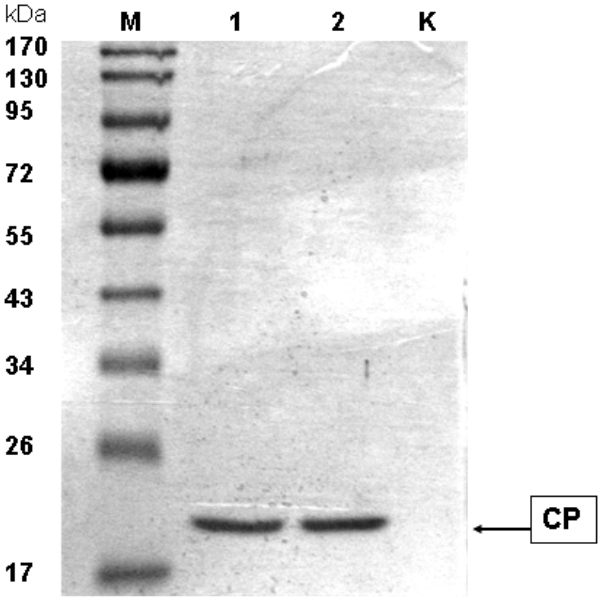

Fig. 4. Analysis of BNYVV CP in 12% SDS-PAGE stained with PageBlue™. An extract from healthy *C. quinoa* was included as control.

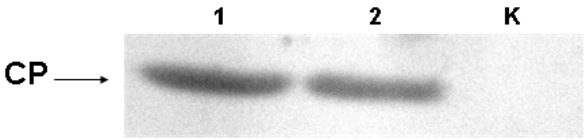

Fig. 5. Western blot analysis of BNYVV CP. An extract from healthy *C. quinoa* was included as control.

These results confirm the identification of BNYVV and so purified virus suspension could be used for antisera preparation.

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