DETECTION OF BEET NECROTIC YELLOW VEIN VIRUS IN LITHUANIA

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Summary

A survey of sugar beet crops for the presence of \textit{Beet necrotic yellow vein virus} (BNYVV) in Lithuania was undertaken from 1998 using DAS-ELISA as a detection method. In 2004 in one area of the South-west region of Lithuania, sugar beet roots with branched tips and enlarged quantities of small rootlets were collected and samples of rootlets reacted positively for BNYVV using the kits from BIOREBA AG and LOEWE. When sugar beet seeds were sown in soil collected from the rhizomania infected field, the germinated plants rootlets appeared thicker and had brown discoloration. The samples of rootlets gave a positive reaction with BNYVV in DAS-ELISA tests. These samples in immunosorbent electron microscopy with trapped antibodies from the BIOREBA AG kit revealed the presence of straight, very short and longer particles, about 20 nm in diameter. Mechanical inoculations to \textit{Chenopodium amaranticolor} resulted in development of local chlorotic lesions. These sugar beet rootlets were used for RT-PCR detection BNYVV with primer pairs as recommended by V.Harju from Central Science Laboratory (UK) in Protocol for the diagnosis of the quarantine organism \textit{Beet necrotic yellow vein virus} (2003) and a slightly modified one-step protocol. Using both RT-PCR protocols, amplified products of the expected size were obtained. Thus, detection of BNYVV in Lithuania was confirmed by ELISA in naturally infected sugar beet roots, in rootlets of plants grown in soil from rhizomania infected field, by mechanical transmission to plant-indicator, by morphology of detected virus particles in ISEM, and according to RT-PCR amplification products.

Introduction

Rhizomania is one of the most economically significant sugar beet diseases and can cause reduction in sugar yield of up to 70\% (Richard-Molard, 1985). The agent of the disease is \textit{Beet necrotic yellow vein virus} (BNYVV). The virus is transmitted by the plasmodiophorid, obligate parasite of sugar beet roots \textit{Polymyxa betae} Keskin. Initially detected and described in Italy (Canova, 1959), rhizomania is widely distributed in most sugar beet growing areas in Europe and world-wide (Rush and Heidel, 1995; Suarez et al., 1999; Ratti et al., 2005). In Europe, BNYVV has spread from Southern to Northern Europe reaching northern sugar beet growing regions. Recently the first detection of BNYVV was recognized in Sweden (Lennefors et al., 2000) and Denmark (Nielsen et al., 2001). Molecular studies based on reverse transcription-polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP) analysis have revealed that two major strain groups of the virus (A and B types) containing only four RNA species are prevalent in
Europe (Kruse et al., 1994). The A type is widespread in Southern Europe, the type B predominates in Germany and France, whereas in the United Kingdom both the A and the B types have been found (Koenig et al., 1995). Type P of BNYV contains RNA 5, has limited distribution in France and UK and is known to be responsible for severe rhizomania symptoms (Ratti et al., 2005).

Sugar beet crops in Lithuania cover more than 20,000 Ha. Surveys of sugar beet crops for the presence of Beet necrotic yellow vein virus have been regularly contacted out since 1998, using DAS-ELISA kits from BIOREBA AG and LOEWE for detection. Here we report evidence of the first detection BNYVV in sugar beet crops in Lithuania.

Materials and Methods

During surveys for the presence of BNYVV, usually about 1/10 of fields are checked. In 2004 in one area of Southwest region of Lithuania, sugar beet plants with branched root tips and enlarged quantities of small rootlets were detected and subjected to standard DAS-ELISA using BIOREBA AG and LOEWE immunological kits (Clark and Adams, 1977). Soil samples from fields with sugar beet plants positive for BNYVV were used for growing sugar beet bait plants of the susceptible variety 'Belmonte', as recommended in Annex 5 of Protocol for the diagnosis of the quarantine organism Beet necrotic yellow vein virus, version Jan 2003, drafted by Val Harju and approved by European and Mediterranean PPO as Standard (EPPO, 2004). Roots of these bait plants were used for DAS-ELISA tests, for mechanical inoculation of Chenopodium amaranticolor plants, for electron microscopic (EM) investigation of virus particles and for PCR detection of BNYVV.

For observation of presence of virus particles, EM carbon coated grids were sensibilized with BIOREBA antibodies to BNYVV diluted 1:50 in FBS buffer. The grids were placed on drops of root extracts (1:20 tissue/buffer), washed, stained with 2% of UA, and observed with JEOL JEM-100S EM at x 25 000.

For PCR tests, RNA extraction from sugar beet bait plant root tissue was carried out according to the instruction of “QuickPrep™ Total RNA Extraction Kit” for the direct isolation of total RNA (Amersham Biosciences, UK). For this investigation, primer pairs were synthesized and reaction conditions were adopted according to the Protocol for the diagnosis of BNYVV, with some details indicated in a previous publication (Staniulis et al., 2004). For nested PCR, RNA extraction and reaction conditions were according to Annex 2 of the above indicated Protocol.

Results and Discussion

In one region of southwest part of Lithuania BNYVV was detected using two different sources of BNYVV antibodies. Soil samples from BNYVV positive fields were used for growing sugar beet bait plants, ‘Belmonte’. Bait plants had roots with dark brown discoloration and slightly bearded appearance (Fig. 1). Tissue extracts from these rootlets gave positive DAS-ELISA results. Leaves from mechanically inoculated Chenopodium amaranticolor developed local lesions (Fig. 2). EM investigation using BIOREBA antibodies to BNYVV for increased trapping of virus particles revealed presence of characteristic BNYVV particles about 20 nm in diameter and of several
different lengths, the majority of them being slightly longer than 260 nm (Fig. 3). Agarose gel electrophoresis of RT-PCR products revealed expected BNYVV products of 500 bp size (Fig. 4.) and for nested PCR – specific products of expected size – 326 bp (Fig. 5.).

Fig. 1. Rootlets of sugar beet bait plants grown in soil from a field of rhizomania infected plants.

Fig. 2. Local lesions on Chenopodium amaranticolor after inoculation with extract from bait plant rootlets.

Fig. 3. Characteristic BNYVV particles trapped on an EM grid coated by antibodies against BNYVV from extracts of sugar beet bait plant rootlets. Virus particles in the middle of the picture are a bit longer than 260 nm.
Results of this investigation indicate that sugar beet seedlings, grown in soil collected from rhizomania infected fields bore diagnostic root symptoms and contained BNYVV. Extracts of these rootlets gave positive results in DAS-ELISA tests, revealed characteristic BNYVV particles, caused LL on inoculated leaves of C. amaranticolor, and produced BNYVV specific products in RT-PCR and nested PCR. These findings verify the presence of BNYVV in at least one location in Lithuania. Our findings were confirmed by Dr. C. Ratti at DiSTA, Bologna, who also identified the isolate of BNYVV as the to B type. Limited observation of BNYVV infected plant symptoms on leaves and roots indicate that they are very mild in comparison with described classical symptoms. Positive ELISA results were obtained from sugar beet roots showing a mild degree of degeneration.

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References


