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FIRST FINDING OF BEET NECROTIC YELLOW VEIN FUROVIRUS IN CROATIA

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In fields of sugar beet (***Beta vulgaris L. var. saccharifera***) near Osijek and Županja (eastern Croatia) virus symptoms similar to those of beet necrotic yellow vein furovirus (BNYVV) were observed on plant specimens in 1997. The main symptoms were pale greenish yellow foliage, upright leaves, reduced root size and root proliferation characteristic of rhizomania. Samples of sugar beet collected in the fields during June, July and August 1997 were checked for BNYVV. In these samples BNYVV was revealed. This was established on the basis of test plant reactions, serology, electron microscope analysis of virus particles and soil transmission experiments. That is the first certain finding of rhizomania caused by BNYVV in sugar beet in Croatia. Attempts to isolate BNYVV from sugar beet growing in fields near Čakovec and Virovitica (northwestern and central Croatia, respectively), which also showed rhizomania symptoms, were unsuccessful.

Introduction

In Croatia, sugar beet (*Beta vulgaris* L. var. *saccharifera*) is cultivated in three distinct geographic regions. The most intensive production is in Slavonia and Baranja, to a lesser extent in Virovitica and sporadically in Medjimurje. Among several factors which have contributed to a decline in sugar beet

production in Croatia in recent years in part is rhizomania, a devastating disease of sugar beet caused by beet necrotic yellow vein furovirus (BNYVV). According to Horvát h (1994) the economic loss caused by the virus may amount to 60-80% of beet root weight, 20-30% in sugar content and 70-90% in sugar output. Rhizomania caused by BNYVV is one of the most demaging of all beet diseases in many regions of the world (Rush and Heidel 1995). This disease was first described in Italy (Canova 1959) and the causative virus was identified in Japan (Tamada and Baba 1973). Afterwards BNYVV was found in more than 20 European countries. This virus has been revealed in China (Gao et al. 1983) and in the United States (Duffus et al. 1984) as well.

It seems rhizomania has spread to most sugar beet production areas of the world (Gerik 1989). However, so far, this plant disease caused by BNYVV was not identified in Croatia. It is true that Šutic and Milanovic (1978) described rhizomania symptoms in sugar beet in eastern Croatia but they did not prove that these symptoms were really caused by BNYVV. As is known, rhizomania symptoms in sugar beet can be caused by agents other than BNYVV (Rush and Heidel 1995).

BNYVV belongs to furoviruses, a taxonomic group of fungal-transmitted, rod-shaped and single-stranded RNA viruses (Brunt and Richards 1989). Unlike most furoviruses, which possess bipartite genomes, BNYVV usually contains four single-stranded RNA species (Richard and Tamada 1992). The rod-shaped virus particles of BNYVV have four lengths, i. e. 85, 100, 265 and 390 nm and they are 20 nm wide with RNA species of 1.5, 1.8, 4.7 and 6.8 kb, respectively (Rush and Heidel 1995). The vector of BNYVV is the soilborne fungus *Polymyxa betae* Keskin, one of the *Plasmodiophoromycetes* (Abe and Tamada 1986). The fungus *Polymyxa betae* is an obligate parasite and has a limited host range, primarily within *Chenopodiaceae*, *Amaranthaceae* and *Portulacaceae* (Abe and Ui 1986). This fungus only infects the primary root tissue of young roots. BNYVV has so far been isolated not only from sugar beet but also from spinach (*Spinacia oleracea* L.) and Swiss chard (*Beta vulgaris* var. *cycla*) as well (Russo et al. 1981; Fujisawa et al. 1982).

In 1997 several fields planted with sugar beet in the western, central and eastern part of Croatia were inspected in order to find out whether BNYVV infects sugar beet in Croatia. The results of the preliminary findings are presented herein.

Material and Methods

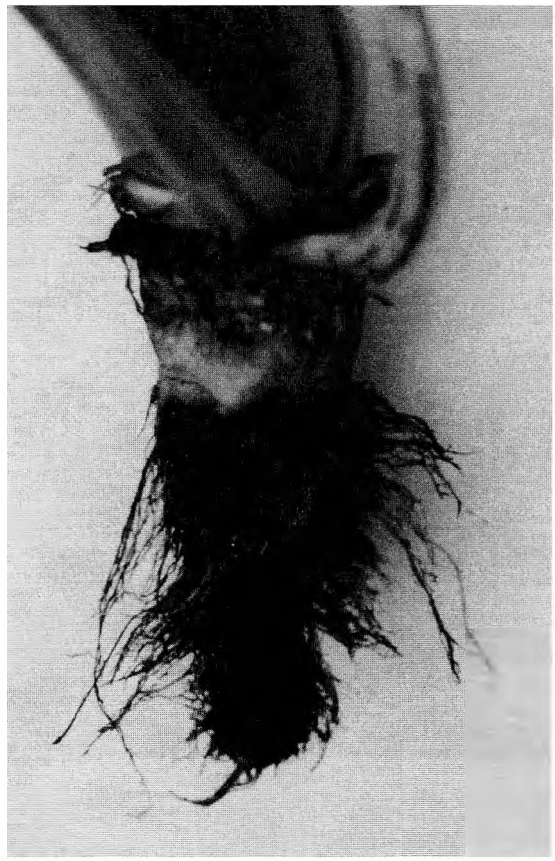
Infected sugar beet plants with typical rhizomania virus symptoms were collected in June, July and August 1997 in four sugar beet growing localities situated in three geographical regions in Croatia: in fields near Čakovec (north-

Fig. 1. Foliar symptoms of rhizomania caused by beet necrotic yellow vein furovirus on field-grown sugar beet (B) in comparison with healthy sugar beet (A): symptoms are stunting, mild chlorosis and a more upright leaf position.

↑

Fig. 2. Root bearding of sugar beet caused by beet necrotic yellow vein furovirus in fields of eastern part of Croatia: the root on right belongs to a healthy sugar beet.

Fig. 3. A sugar beet root with typical symptoms of rhizomania previously shown in Fig. 2 (the second root from the left), somewhat enlarged. →



western region, locality I), Virovitica (central region, locality II), Osijek (eastern region, locality III) and Županja (eastern region, locality IV).

Trials of virus transmission on test plants were performed by mechanical inoculation of plant sap and by field soil in contact with test plant root system. Inocula (plant saps) for mechanical inoculations were prepared separately from leaves and rootlets of the plants which showed typical rhizomania symptoms. The tissue sap was diluted with 0.1 M phosphate buffer, pH 7.0 at a ratio of 1:1. Carborundum was used as abrasive. Transmission of virus isolates from soil to plants was done according to T a r a k u and J u r e t i c (1990) but in a somewhat modified way. Soil samples were collected in beet fields in which rhizomania symptoms were present. Each sample consisted of approximately 0.5 litre of soil taken from the upper 20 cm of the soil profile. The soil samples were put separately in pots, and sugar beet seeds were sown in them.

Leaf sap of naturally infected sugar beet was examined electron microscopically by the dipping method, using 2% uranyl acetate. Serological experiments were carried out by means of the agar gel double-diffusion test. The antiserum to BNYVV (titre 1/64) was kindly supplied by Dr. J. R i c h t e r (Aschersleben, Germany).

R e s u l t s a n d D i s c u s s i o n

1. Disease symptoms

In all four (I-IV) localities, many beets exhibited excessive crown growth, and the leaves had a more upright position than normal. The plants were more or less stunted (Fig. 1). The tip of the taproot of such plants usually was killed and the proliferation of lateral rootlets resulted in typical rhizomania symptoms (Figs. 2 and 3). Necrotic yellow vein symptoms, which are characteristic for a BNYVV, were not detected. Only beets in localities III and IV showed foliar symptoms in the shape of light green or yellow blotches. Infected plants were in circular groups. The number of sugar beets with rhizomania symptoms varied from about 10% in localities I and II to about 30% in localities III and IV.

2. Transmission experiments

Twenty samples of beets with rhizomania symptoms collected in the four localities (five samples per locality) were tested on several different hosts using inocula prepared from the leaf and root sap for mechanical inoculation. Several plants such as *Beta vulgaris* L. var. *saccharifera*, *Chenopodium amaranticolor* Coste et Reyn., *Ch. quinoa* Wild. and *Spinacia oleracea* L. served as test plants. However, the results of the inoculations were negative.

Consequently, we tried to transmit the suspected causative virus isolates by bringing the root systems of the test (bait) plants into contact with field soil. In these experiments sugar beet seeds were sown on soil taken from the fields of the four localities in which the originally symptom bearing sugar beet were grown. Of the 50 sugar beet seeds sown on soil collected from locality III, 19 developed plants showing alterations on leaves in the form of mild chlorotic symptoms and, rarely, yellow vein symptoms. Similar results were obtained in experiments with soil taken from locality IV, i. e. out of 60 seeds sown, 22 gave plants with the symptoms. However, we could not discover any virus infection in sugar beet from localities I and II. Successful transmission of virus isolates throught the soil and their symptoms showed that virus isolates L3 (found in locality III) and L4 (found in locality IV) could belong to BNYVV.

After soil transmission of isolates L3 and L4 to sugar beet we tried to transmit them from the latter plants by mechanical inoculation to some BNYVV diagnostic plant species using leaf sap inocula. The results of these experiments are shown in the table. The host range and reactions in test plants

Table. Reactions of test plants mechanically inoculated with virus isolates L3 and L4 using leaf sap of beet artificially infected by field soil

Test plant	Symptom* Virus isolate L3	L4
Beta vulgaris L. var. *saccharifera*	N1, Sm St	N1, Sm St
Chenopodium amaranticolor Coste et Reyn.	N1	N1
Ch. quinoa Wild.	N1	N1
Cucumis sativus L. "Delicatess"	0	0
Datura stramonium L.	0	0
Gomphrena globosa L.	0	0
Nicotiana glutinosa L.	0	0
Petunia hybrida Vilm.	0	0
Phaseolus vulgaris L.	0	0
Spinacia oleracea L.	Sm	Sm

*N1 - necrotic local lesions; Sm - systemic mottling; St - stunting;
0 - symptomless

indicated that sugar beet in at least two localities in Croatia were infected with BNYVV (comp. T a m a d a 1975, H o r v á t h 1994).

3. Electron microscopy

In the leaf sap of sugar beet naturally infected with virus isolates L3 and L4, rod-shaped particles of different lengths were discovered; four particle lengths were predominant: 80, 95, 260 and 380 nm. On the basis of this

datum, the virus particles found correspond to those of BNYVV (T a m a d a 1975, P u t z 1977, R u s h and H e i d e l 1995).

4. Serological reactions

Virus isolates L3 and L4 reacted positively with undiluted antiserum to BNYVV giving precipitation lines characteristic of the virus antigen.

All the experiments performed proved that virus isolates L3 and L4 found in sugar beet in Croatia belong to BNYVV. This means that, this devastating virus has spread to Croatia as well as to the many European countries where it was known earlier.

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S A Ž E T A K

PRVI NALAZ FUROVIRUSA NEKROTIČNOG ŽUTILA ŽILA ŠEĆERNE REPE U HRVATSKOJ

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Više faktora utječe na urod šećerne repe u Hrvatskoj. Dosad nije bilo poznato je li među tim faktorima i štetna virusna bolest poznata pod imenom rizomanija koju uzrokuje virus nekrotičnog žutila žila šećerne repe (beet necrotic yellow vein virus; BNYVV). Taj virus pripada *furovirusima* (R u s h i H e i d e l 1995). Prema H o r v á t h u (1994) BNYVV može smanjiti urod šećerne repe i do 90%. Virus je rasprostranjen širom svijeta: otkriven je i u preko 20 europskih zemalja među kojima su i zemlje s kojima Hrvatska graniči.

Istražujući virusne bolesti šećerne repe u okolici Čakovca, Virovitice, Osijeka i Županje, opazili smo tijekom 1997. godine na listovima šećerne repe na sva četiri lokaliteta simptome slične onima koje uzrokuje BNYVV. Najupadljiviji simptomi bili su: usmjerenost listova prema gore a na njima su se tu i tamo zapažala zelenkastožuta područja (sl. 1). Nekrotično žućenje žila nije opaženo. Zapazili smo također da biljke s promjenama na listu imaju smanjen glavni korijen a na vršnom dijelu tog korijena redovito je nazočan abnormalno velik broj sitnih korjenčića; ti su korjenčići bili međusobno tijesno isprepleteni tako da su oblikovali gusti splet koji je nalikovao bradi (sl. 2, 3). Zbog toga se ta bolest šećerne repe naziva rizomanija ili bradatost korijena.

Na temelju simptoma opaženih na šećernoj repi u polju, reakcija na pokusnim biljkama, elektronskomikroskopskih istraživanja te serološke identifikacije ustanovili smo da opisanu bolest na šećernoj repi u okolici Osijeka i Županje uzrokuje BNYVV. To su potvrdili i pokusi prijenosa bolesti uzorcima tla u kojem su u polju rasli inficirani primjerci šećerne repe. Naime, naši su se virusni izolati, kao i svi dosad opisani izolati BNYVV-a, prenosili tlom. Iz literature je poznato da je prenosilac virusa u tlu gljivica *Polymyxa betae* (A b e i T a m a d a 1986). Naši pokusi pomoću kojih smo nastojali dokazati BNYVV u šećernoj repi te u pripadajućem tlu s područja Čakovca i Virovitice nisu dali pozitivan rezultat.

Izloženi rezultati nedvojbeno pokazuju da je i na području Hrvatske rasprostranjen BNYVV što je ujedno i prvi sigurni nalaz ovog virusa u našoj zemlji.

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