Warsaw Agricultural University, Warsaw, Poland

THE FAILURE OF BEET NECROTIC YELLOW VEIN VIRUS (BNYVV) DETECTION BY DAS-ELISA TEST

A. Kozłowska and S. Kryczyński

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Beet necrotic yellow vein virus (BNYVV) is responsible for rhizomania, an economically important disease of sugar beet (Beta vulgaris L. ssp. vulgaris convar. crassa Alef provar. altissima Döll). At present, rhizomania is considered a serious problem in almost all sugar beet growing regions of the world (Richard-Molard 1985, Hill 1989, Smith et al. 1994, Ascher 1999). The key role in its spread plays the root-invasive vector Polymyxa betae Keskin. Severe infection leads to reduction in taproot weight even by 80% and the sugar content can be reduced by 16–18% (Ascher 1999). In Poland BNYVV infection reduced root yield by 20% whereas decrease in sugar value was about 2.5% (Piszczek 2003). Nevertheless, in both cases occurrence of rhizomania can make sugar beet production uneconomic.

The original aim of our experiments was evaluation of 11 sugar beet cultivars reaction to infection by Beet necrotic yellow vein virus. Cultivar ‘Evita’ was used as a known susceptible standard while 10 other cultivars were issued by breeders during last 15 years. 20 plants of each cultivar were inoculated with BNYVV using the technique described by Tuitert (1990). The seeds were sown into pots filled with a mixture of Sphagnum peat with the soil collected from fields infested with BNYVV and Polymyxa betae (4:1) and they were additionally inoculated by gentle root rubbing with sap obtained from sugar beet leaves infected with BNYVV (Geyl et al. 1995) when the seedlings were transplanted to the field. Almost four months after transplanting these plants to the field, leaf samples were collected and tested with DAS-ELISA for BNYVV. DAS-ELISA kits originated from Loewe Biochemica GmbH. Surprisingly, the virus was not detected in any of the plants tested. E405 value for positive standard sample was over 1500. Testing was repeated three weeks later with negative results again. This time the samples were collected only from 12 plants showing some symptoms suggesting BNYVV infection (leaf distortion, narrowing, elongation, yellowing and wilting) and they were tested by Dr. M. Jeżewska at the Institute of Plant Protection in Poznań. Leaf and root samples (20 plants) were tested again at harvest time but again the extinction values were not higher than the values for the control plants. Microscopic examination of some minor roots (Phot. 1) showed P. betae presence in the examined preparations. The de-
tails of the methods used as well as the detailed results are presented by Kozłowska (2004).

The inoculation experiments were repeated in the greenhouse and in the growth-chamber conditions using seedlings of susceptible cv. ‘Evita’ sugar beet plants (three replications with 20 each) grown in the virus and *P. betae* infested soil. No BNYVV was detected again by DAS-ELISA although more than 50% of plants displayed symptoms characteristic of rhizomania (growth reduction, leaf yellowing and distortion, Phot. 2) and *P. betae* was present in their roots, which was revealed by microscopic examination.

Similar difficulties have already been reported. Because of the extremely low BNYVV concentration in sugar beet plants even ELISA test used as the best method for the efficient di-
agnosis of BNYVV gave ambiguous results (Jeżewska et al. 1991). Even in the presence of rhizomania root symptoms samples of field-grown sugar beet were negative in ELISA as a result of conditions not conducive to *P. betae* infection (Wisler et al. 2003).

Several possible explanations may be offered for this failure of BNYVV detection. At first, it is known that to obtain BNYVV detectable concentration virus multiplication by means of several secondary infection cycles is necessary (Tuittert 1993, Obermeier et al. 1996). In general, the life cycle of *P. betae* consists of zoosporangia releasing zoospores and thick-walled resting spores (Dahm and Buchenauer 1993). The plasmodium produces zoosporangia only when water (which is essential to enable cyst to germinate and zoospores to swim to roots) is available (Tuittert 1993). It is important to note that, especially with low inoculum density, primary infection may take place too late in the season so the detection level may or may not be reached (Tuittert 1990).

The difficulty during BNYVV detection might be explained also by non-even distribution of the virus in sugar beet plants due to very poor virus translocation and its restriction in the rootlets (Kaufmann et al. 1993). One obvious explanation for negative results obtained with DAS-ELISA techniques can be improper choice of the time to analyze the samples from affected sugar beet plants.

The occurrence of several virus variants (strains? serotypes?) offers another explanation for the failure of BNYVV detection (Tamada 1975) whereas rhizomania symptoms are observed and vector presence is confirmed by microscope examination. BNYVV nucleic acid detection by electrophoresis after RNA amplification by RT-PCR might be useful in such instances. It has been proved (Meunier et al. 2000, Morris et al. 2001) that some samples which were positive by the RT-PCR techniques had been shown to have an extremely low optic density with ELISA techniques.

To summarize, taking into consideration the ease with which agent causing rhizomania is transmitted and a remarkable change in rhizomania symptom expression in Poland (Jeżewska and Piszczek 2001), it might be necessary to use in some cases a more sensitive diagnostic method than that given by ELISA. Nevertheless, RT-PCR assays are specific, but not yet suitable for routine test of large number of samples because of high cost.

**Literature**


Authors’ address: Anna Kozłowska, M.Sc.,
Prof. dr hab. Selim Kryczyński,
Department of Plant Pathology,
Warsaw Agricultural University,
ul. Nowoursynowska 159,
02-787 Warsaw,
Poland
e-mail: kryczynski@alpha.sggw.waw.pl

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