DETECTION OF BEET SOIL-BORNE VIRUS AND BEET VIRUS Q IN SUGARBEET IN GREECE

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SUMMARY

Sugar beet plants with typical rhizomania symptoms were collected from the five major cultivation zones of Greece. The presence of Beet necrotic yellow vein virus (BNYVV), the primary causal agent of the disease, was ascertained by DAS-ELISA in 38 out of 40 fields surveyed and the positive samples were subsequently examined for the presence of other soil-borne viruses which are frequently associated with rhizomania, using a multiplex RT-PCR assay targeting BNYVV, Beet soil-borne virus (BSBV) and Beet virus Q (BVQ). The occurrence of BSBV and BVQ was confirmed in 9 and 23 rhizomania-infected fields, respectively. In contrast to surveys conducted in other countries, the presence of BVQ prevailed throughout Greece in dual infections with BNYVV, whereas BSVB was restricted to rhizomania-infected fields from only two sugar beet cultivation areas. Nine of the samples tested were infected with all three viruses. BSBV was always found in triple infections. To our knowledge, this is the first report of BSBV in Greece. The future assessment of the impact of each of these viruses on sugar beet could prove significant for breeding objectives in terms of achieving a more durable resistance to the rhizomania syndrome.

Key words: Beet soil-borne virus, Beet virus Q, rhizomania, sugar beet, survey, diagnosis

Rhizomania, initially described in Italy in the mid 50’s, is now distributed worldwide (Tamada, 1999; Lennefors et al., 2000) and is considered as the most destructive disease of sugar beet. The disease causes serious or even complete loss of potential sugar yield by reducing both root yield and sugar content. Typical disease symptoms consist of a mass of fine hairy secondary rootlets that give the taproot a beard-like appearance, accompanied by a constriction of the main taproot and stunting of the infected plant (Tamada, 1999). The disease agent is Beet necrotic yellow vein virus (BNYVV) (Tamada and Baba, 1973), the type species of the genus Benyvirus (Torrance and Mayo, 1997; Tamada, 1999), which is transmitted through the soil by the plasmodiophorid Polymyxa betae Keskin. In Greece, rhizomania was first identified in the mid 70’s and has since spread to all sugar beet cultivation areas of the country (Richard-Molard, 1985).

Apart from BNYVV, sugar beet is a host for several other soil-borne viruses such as Beet soil-borne mosaic virus (BSBMV), another member of the genus Benyvirus (Koenig and Lesemann, 2000; Lee et al., 2001; Wisler, 2001), Beet soil-borne virus (BSBV) and Beet virus Q (BVQ), both members of the genus Pomovirus (Koenig et al., 1998, 2000), and Beet black scorch virus (BBSV), a necrovirus belonging to the family Tombusviridae (Cai et al., 1999; Cao et al., 2002; Lommel et al., 2005; Yuan et al., 2006). BBSV is transmitted in a non-persistent manner by Olpidium brassicae (Jiang et al., 1999), whereas the other three viruses share the same vector with BNYVV and are frequently found in mixed infections in sugar beet roots (Prillwitz and Schlösser, 1993; Heidel and Rush, 1994; Meunier et al., 2003).

With the exception of BBSV, which has isometric particles and a genome organization typical of necroviruses (Cao et al., 2002; Yuan et al., 2006), the remaining four viruses have rod-shaped particles and multipartite positive-sense, single-stranded RNA genomes. BNYVV has four genomic RNAs, but some isolates possess a fifth RNA species (Tamada et al., 1989; Tamada, 1999). BSBMV has four RNAs and a genome organization similar to that of BNYVV, whereas the genome of BSBV and BVQ consists of three RNA segments (Hutchinson et al., 1992; Koenig et al., 1998).

Studies on the interactions between BSBMV and BNYVV have revealed that prior inoculation with one virus elicits protection against infection by the other, and that cross-protection is due to the inhibition of replication of the second virus (Mahmood and Rush, 1999). Later experiments using infections through P. betae.
tae have further provided evidence that in dual infections BNYVV may suppress BSBMV (Wisler et al., 2003). Similarly, Prillwitz and Schlösser (1993) showed that initial infection with BSBV results in a considerably reduced disease severity upon subsequent infection with BNYVV, in comparison with sugarbeets infected only with BNYVV. The possible involvement of BSBV and BVQ in the epidemiology of rhizomania disease is not yet well elucidated (Heidel and Rush, 1997; Meunier et al., 2003). Likewise, although the impact of BBSV infections to sugarbeet is poorly characterized, this virus can cause rhizomania-like symptoms in the absence of BNYVV (Welldon et al., 2007).

In the present study, the five major sugarbeet-growing regions of Greece were surveyed for the presence of the soil-borne viruses BSBV and BVQ, which are frequently associated with rhizomania disease in Europe. BSBMV and BBSV were not included in this survey, as the former occurs only in the USA (Lee et al., 2001; Rush, 2003) and the latter has a limited distribution in Europe (González-Vázquez et al., 2009).

Sugarbeet roots with rhizomania symptoms were collected from 40 fields covering the entire crop growing area of Greece (Fig. 1) and the occurrence of the disease was verified by DAS-ELISA, using an antiserum specific to BNYVV (Bio-Rad, France). Total RNA was isolated from taproots and rootlets with an RNeasy plant mini-kit (Qiagen, USA). First-strand cDNA synthesis was performed using approximately 0.5 µg of total RNA, isolated from ELISA-positive roots, and 0.5 µM of each of the viral specific reverse primers (Table 1) with the Im-Prom II Reverse Transcriptase System (Promega, USA).

Primers (1 and 2, 3 and 4, 5 and 6) (Table 1) targeting BNYVV RNA-1, BSBV RNA-1 and BVQ RNA-3 were used either individually or combined in a multiplex-PCR assay. For individual reactions, PCR mixture contained 1 µl of the RT reaction, 0.5 µM of each primer, 250 µM dNTPs, 1.25 mM MgCl₂, 1x Taq buffer and 1.25 U Taq polymerase (GoTaq Flexi DNA polymerase, Promega, USA) in a final volume of 20 µl. Amplification cycles included a first denaturation cycle of 3 min at 94°C, then 35 cycles composed of 30 sec at 94°C, 1 min at 62°C, 1 min at 72°C with a final elongation cycle of 7 min at 72°C. Multiplex PCR reactions were performed by using 2 µl of the RT reaction and 0.25 µM of each primer using the reaction conditions described above. Amplification products were visualized in 2% TAE agarose gels stained with ethidium bromide and examined under ultraviolet light. Following elution from agarose gels, PCR products corresponding to the three viruses were cloned into TOPO TA vector and sequenced using M13 forward and reverse primers.

Having established that BNYVV is widespread in the major sugarbeet production areas of Greece (Pavli, 2010), next it was investigated whether the distribution of this virus would coincide with that of BSBV and/or BVQ. Primers for both individual and multiplex RT-PCR assays were selected, specifically amplifying fragments of 459, 400 and 690 bp for BNYVV RNA-1, BS-

**Table 1.** Primers used for the specific amplification of *Beet necrotic yellow vein virus*, *Beet soil-borne virus* and *Beet virus Q* RNA segments.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5' to 3')</th>
<th>5'-nt position</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. BNYVV-F</td>
<td>CGA AGA TAG CAG ACG ACA GGT TC</td>
<td>RNA 1: 6116</td>
<td>Suárez et al., 1999</td>
</tr>
<tr>
<td>2. BNYVV-R</td>
<td>TCA AGA TAG GAG GCC TGT GCC AT</td>
<td>RNA 1: 6574</td>
<td>Suárez et al., 1999</td>
</tr>
<tr>
<td>3. BSBV-F</td>
<td>CTT ACG CTT CTT ACT TTT ATG CC</td>
<td>RNA 1: 2828</td>
<td>Meunier et al., 2003</td>
</tr>
<tr>
<td>4. BSBV-R</td>
<td>GTC CGC ACT CTT TGC AAC TGT TC</td>
<td>RNA 1: 3227</td>
<td>Meunier et al., 2003</td>
</tr>
<tr>
<td>5. BVQ-F</td>
<td>GTT TTC AAA CTT GTC ACG CT</td>
<td>RNA 3: 921</td>
<td>Rubies et al., 2006</td>
</tr>
<tr>
<td>6. BVQ-R</td>
<td>CCA CAA TGG GCC AAT AGA</td>
<td>RNA 3: 1610</td>
<td>Rubies et al., 2006</td>
</tr>
</tbody>
</table>

Fig. 1. The five major sugarbeet-growing areas of Greece (Orestiada, Xanthi, Serres, Larissa, Platy). The spots correspond to the areas where samples were collected. Distances between adjacent locations range between 70 to 100 Km.
was prevalent (Kutluk Yilmaz et al., 2007). In surveys conducted in countries with climatic conditions similar to those of Greece, single infections prevailed over mixed infections only with BNYVV and BVQ. In this respect, it should be noticed that by testing only BNYVV-infected samples, a bias was possibly induced. As a consequence, it would be interesting to investigate the possible occurrence and pathogenic effect of BSBV and BVQ in single infections in rhizomania-free areas or in symptomless plants collected from fields where this disease is unevenly distributed. Although apparently there are no reports of single infections by BVQ in the literature, it cannot be excluded that this virus occurs in the absence of BNYVV, but in mixed infection with other beet viruses. At the same time, it would be worthwhile investigating the sequence variability of BSBV and BVQ isolates present in the country. To this aim, the choice of RNA-1, customarily used for obtaining a reliable RT-PCR amplification based on the highly conserved polymerase sequences, would not be appropriate. Instead, the less conserved nature of the genes encoding the coat protein or the triple gene block proteins renders them better targets for monitoring virus variants. Gathering all relevant information, would definitely assist in designing and implementing a more rational breeding program for the production of commercial varieties with better resistance against the rhizomania syndrome.

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REFERENCES


Since all samples included in this survey originated from rhizomania-infected fields, both pomoviruses (BSBV and BVQ) were found only in co-infection with BNYVV. At the same time, BSBV was found in triple infections only with BNYVV and BVQ. In this respect, it should be noticed that by testing only BNYVV-infected samples, a bias was possibly induced. As a consequence, it would be interesting to investigate the possible occurrence and pathogenic effect of BSBV and BVQ in single infections in rhizomania-free areas or in symptomless plants collected from fields where this disease is unevenly distributed. Although apparently there are no reports of single infections by BVQ in the literature, it cannot be excluded that this virus occurs in the absence of BNYVV, but in mixed infection with other beet viruses. At the same time, it would be worthwhile investigating the sequence variability of BSBV and BVQ isolates present in the country. To this aim, the choice of RNA-1, customarily used for obtaining a reliable RT-PCR amplification based on the highly conserved polymerase sequences, would not be appropriate. Instead, the less conserved nature of the genes encoding the coat protein or the triple gene block proteins renders them better targets for monitoring virus variants. Gathering all relevant information, would definitely assist in designing and implementing a more rational breeding program for the production of commercial varieties with better resistance against the rhizomania syndrome.

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