

SHORT COMMUNICATION

DETECTION OF *BET SOIL-BORNE VIRUS* AND *BET VIRUS Q*
IN SUGARBEET IN GREECEO.I. Pavli^{1,2}, M. Prins³ and G.N. Skaracis¹¹*Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Breeding and Biometry, Iera Odos 75, 11855 Athens, Greece*²*University of Wageningen, Department of Plant Sciences, Laboratory of Virology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*³*Keygene N.V., Agro Business Park 90, 6708PW Wageningen, The Netherlands*

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 BSBV was restricted to rhizomania-infected fields from only two sugarbeet 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 and BVQ in Greece. The future assessment of the impact of each of these viruses on sugarbeet 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, sugarbeet, 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 sugarbeet. 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, sugarbeet 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 sugarbeet 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. be-*

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

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

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 (Weiland *et al.*, 2007).

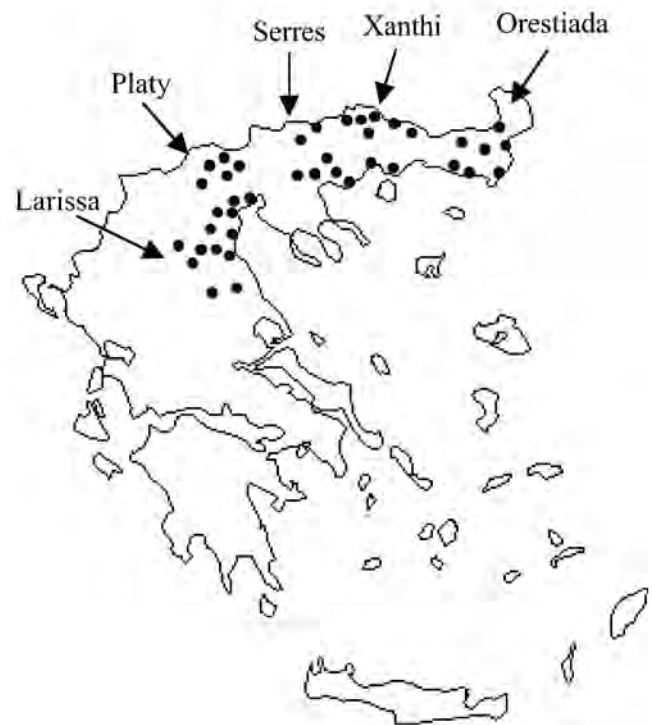
In the present study, the five major sugarbeet-growing regions of Greece were surveyed for the presence of the soil-borne viruses BSBV and BVQ, which are frequently associated with rhizomania disease in Europe. BSBMV and BBSV were not included in this survey, as the former occurs only in the USA (Lee *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 elonga-

tion 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-

**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.

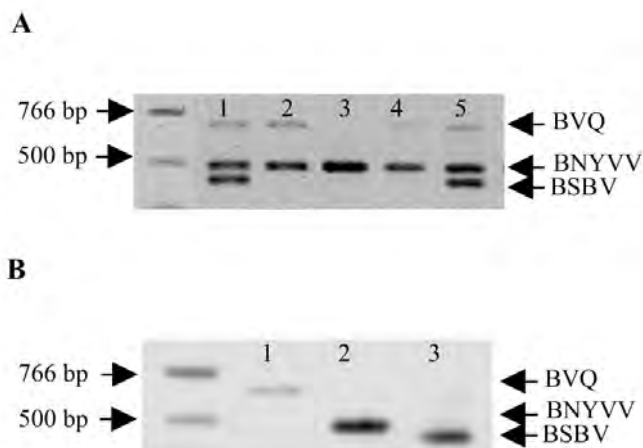


Fig. 2. Amplification products, corresponding to BNYVV, BSBV and BVQ, obtained by (A) multiplex RT-PCR. Lanes 1, 2, 3, 4 and 5 correspond to samples collected from Xanthi, Larissa, Platy, Orestiada and Serres, respectively; (B) individual RT-PCR assay. Ladder: PCR Marker (New England Biolabs).

BV RNA-1 and BVQ RNA-3, respectively (Fig. 2). Both BSBV and BVQ were found in rhizomania-infested fields. To our knowledge, this is the first report of BSBV and BVQ in Greece.

The most frequently detected virus was BNYVV (95%), followed by BVQ (57.5%), whereas BSBV was much less represented (22.5%). More specifically, of the 38 BNYVV-infected samples, 23 contained also BVQ and 9 contained BSBV. In all cases, BSBV occurred in triple infections with BNYVV and BVQ, while 15 BNYVV-infected samples tested negative for the presence of BSBV or BVQ.

The 459 bp amplicon sequence corresponding to BNYVV (accession No. FN812746, FN812747, FN812748) had a 99% nucleotide and amino acid sequence identity with the Japanese BNYVV isolate S (accession No. D84410, BAA12339). The 400 bp fragment of BSBV (accession No. FN812749, FN812750) had a 98% nucleotide sequence identity with the Polish BSBV isolate (accession No. FJ971717), whereas the BVQ-derived amplicon of 690 bp (accession No. FN812751, FN812752) had a sequence identity of 97% with a BVQ isolate from Germany (accession No. AJ223598).

Our data point to the systematic co-existence of BNYVV and BVQ in the Greek sugarbeet cultivation zones, in contrast to previous studies where BNYVV was more often associated with BSBV (Meunier *et al.*, 2003). The presence of BVQ prevailed in rhizomania-infested fields throughout the country, whereas BSBV was only found in Xanthi and Serres. In surveys conducted in countries with climatic conditions similar to those of Greece, single infections prevailed over mixed infections and the combination of BNYVV with BSBV was prevalent (Kutluk Yilmaz *et al.*, 2004; Farzadfar *et al.*, 2007).

Since all samples included in this survey originated from rhizomania-infested 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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