MOLECULAR CHARACTERIZATION OF THREE SOIL-BORNE SUGAR BEET-INFECTING VIRUSES BASED ON THE COAT PROTEIN GENE

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SUMMARY

Sugar beet (Beta vulgaris L. ssp. vulgaris) hosts the soil-borne viruses Beet necrotic yellow vein virus (BNYVV), Beet soil-borne virus (BSBV), Beet soil-borne mosaic virus (BSBMV) and Beet virus Q (BVQ). Only a few isolates of these viruses have been characterized at the coat protein (CP) gene level. In this work, CP gene sequences were determined of three isolates each of BNYVV, BSBV and BVQ from different parts of Poland (Wielkopolska, Mazowsze and Dolny Slask). Phylogenetic analysis showed that Polish BNYVV isolates belong to the A and B type groups. The CP gene of the three BSBV isolates had 98-100% identity with comparable sequences available in GenBank. CP genes of the three BVQ isolates were almost identical to one another (98-99%) and contained three additional nucleotides, resulting in a further amino acid residue (arginine) at position 86, as reported by Lennefors et al. (2005).

Key words: Sugar beet, Beet necrotic yellow vein virus, Beet soil-borne virus, Beet virus Q, sequencing, coat protein, phylogenetic analysis.

Many soil-borne viruses are known to infect sugar beet worldwide. Rhizomania, caused by Beet necrotic yellow vein virus (BNYVV) (Tamada and Baba, 1973), the type member of the genus Benyvirus (Koenig and Lesemann, 2005), is the most important disease of sugar beet, causing high yield losses in many parts of the world. Other soil-borne viruses include Beet soil-borne mosaic virus (BSBMV), the other member of the genus Benyvirus (Lee et al., 2001), Beet soil-borne virus (BSBV), and Beet virus Q (BVQ) both of which belong to the genus Pomovirus and have multipartite genomes consisting of three RNA species (Hutchinson et al., 1992; Koenig et al., 1998). BSBMV occurs in the USA, where is widespread (Rush, 2003; Heidel and Rush, 1994). The coat protein (CP) gene of all these viruses is located on RNA-2 (Bouzoubaa et al., 1986; Koenig et al., 1997; Koenig et al., 1998; Lee et al., 2001).

BSBV was identified for the first time in sugar beet roots from the United Kingdom in 1982 (Henry et al., 1986). Since then it was reported from many European countries, the United States (Meunier et al., 2003), Iran (Farzadfar et al., 2002) and Syria (Mouhanna et al., 2002). Lesemann et al. (1989) described two BSBV serotypes, Ahlum and Wierthe, the second of which (Wierthe) was later classified as a distinct virus species called Beet virus Q (BVQ) (Koenig et al., 1998). BVQ has been detected in Belgium, Bulgaria, France, Germany, Hungary, Italy, Sweden, The Netherlands (Meunier et al., 2003) and the Czech Republic (Rysanek et al., 2006).

In Poland, rhizomania was first reported in the 1980s (Osinska et al., 1989). In 2006 BSBV and BVQ were described in fields where BNYVV had previously been found (Borodynko, 2006; Borodynko et al., 2006), suggesting that soil-borne sugar beet-infecting viruses are widespread in the country (Borodynko and Pospieszny, 2007).

This prompted a survey during which samples from 15 sugar beet plants showing yellow patches, reduced taproot size and strong proliferation of lateral rootlets were collected in three different parts of Poland (Wielkopolska, Mazowsze and Dolny Slask). Roots were analyzed by DAS-ELISA using a Bio-Rad antiserum (USA) to identify BNYVV, and by TAS-ELISA using a DSMZ antiserum (Germany) to identify BSBV and BVQ. The presence of the three viruses was confirmed by a multiplex RT-PCR using specific primers designed to amplify a fragment of RNA-2 for BNYVV and of RNA-1 for BSBV and BVQ (Meunier et al., 2003) (Fig. 1 A and B). These viruses often occurred in mixed infection, as shown by the identification of ten plants infected by all three of them. No positive reaction for BSBMV was obtained.

As a further step, RT-PCR was carried out using primers designed by Lennefors et al. (2005) which amplify part of the coat protein (CP) gene, obtaining products of the size expected for each virus. Amplicons were purified with Qiaex II gel extraction kit (Qiagen, USA) and cloned into pGEM-T-Easy vector (Promega, USA). Ten independent recombinant plasmids of each virus were se-
sequenced with an ABI automatic sequencer at the Institute of Biochemistry and Biophysics of the University of Warsaw, using standard T7 forward and Sp6 reverse primers. Pairwise comparison of the sequences obtained revealed a very high levels of similarity irrespective of the virus analyzed. Only a few point mutations among sequences representing part of the CP gene of each virus were found. Based on the non-synonymous mutations observed, three groups of sequences could be distinguished for each virus. Thus, three sequences of each group for each virus were chosen for phylogenetic analysis. Multiple sequence alignments using ClustalW were made (Thompson et al., 1994) and phylogenetic analyses were carried out using the Neighbor-Joining algorithm implemented with Mega version 3 (Kumar et al., 2004). The robustness of phylogenetic trees was tested by 1,000 bootstrap replicates.

Sequences of the CP gene of BNYVV isolates (WBcp, JAcp, ZAcp, accession Nos EU785962-64) determined in this study were 567 nucleotides (189 amino acids) in size, like those from other isolates available in GenBank. The identity of these sequences at the nucleotide and amino acid level ranged from 96 to 100%. Two groups of isolates could be identified by phylogenetic analysis, one of which contained the previously described types A and P, and included isolates JAcp and ZAcp. These two isolates were identical to isolate UF79 from the USA (Lennefors et al., 2005). The other group consisted of type B isolates and included our isolate WBcp. The CP gene of WBcp was identical to that of isolates GT85 from Germany, Kas3 from Kazakhstan, and FC from France. In accordance with previous findings (Lennefors et al., 2005), A+P group could be distinguished from B group by the amino acids at positions 62 (threonine versus serine), 103 (serine versus asparagines) and 172 (leucine versus phenylalanine). No additional nucleotide changes were found in the BNYVV sequences analyzed.

CP sequences of three BSBV isolates (WBScp, JBScp, ZBScp, accession Nos: EU785965-67) presently investigated were 495 nucleotide (165 amino acid) in length, i.e. the same size as those of other BSBV isolates available in GenBank. A comparison of Polish BSBV sequences with comparable sequences (Koenig et al., 1997; Lennefors et al., 2005; Wang et al., 2008) revealed a high nucleotide identity (98-100%). Our isolates, like those described by Lennefors et al. (2005) and Wang et al. (2008), contained an asparagine residue at position 65, instead of the threonine residue in the previously described German BSBV isolate (Koenig et al., 1997).

The CP gene of the Polish BVQ isolates (WQcp, JQcp, ZQcp, accession Nos EU785968-70) was 501 nt in size, as reported by Lennefors et al. (2005). Our isolates had 97% identity at the nucleotide level with the only complete BVQ genome available (Koenig et al., 1998) and 98-99% identity with isolates GT16 and FP71 described by Lennefors et al. (2005). Furthermore, the Polish isolates contained three additional nucleotides resulting in an extra amino acid residue (arginine) at position 86, as previously reported by Lennefors et al. (2005) and a serine at position 115 instead of glycine (Lennefors et al., 2005).

The very low level of genetic diversity among the Polish isolates of the three viruses under study may be due to their rapid adaptation to different environmental conditions and limited host range. Interactions between
BNYVV and BSBV or BVQ, or among the three viruses in triple infections, require further study.

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