SHORT COMMUNICATION

SURVEYS OF *BEET NECROTIC YELLOW VEIN VIRUS*, *BEET SOIL-BORNE VIRUS*, *BEET VIRUS Q* AND *POLYMYXA BETAE* IN SUGAR BEET FIELDS IN IRAN

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SUMMARY

The main Iranian sugar beet (*Beta vulgaris*) growing areas were surveyed for the occurrence and incidence of *Beet necrotic yellow vein virus* (BNYVV), *Beet soil-borne virus* (BSBV) and *Beet virus Q* (BVQ). Root samples from 3,486 plants were collected from 184 commercial sugar beet fields of 14 provinces from randomly selected and symptomatic plants and analysed by tissue-blot immunoassay (TBIA). Serological diagnoses were confirmed by reverse transcription-polymerase chain reaction tests (RT-PCR) using specific primers. Based on TBIA data, BNYVV was prevalent (overall incidence of 52.4%), followed by BSBV (9.8%) and BVQ (1.4%). High levels of virus infections (more than 70%) were found in Ardabil, Esfahan, Fars, Khorasan and Zanjan provinces, whereas Khuzestan was apparently "virus-free". The putative vector of the soil-borne viruses, *Polymyxa betae*, was also detected by RT-PCR in 179 fields surveyed.

Key words: Surveys, sugar beet, BNYVV, BSBV, BVQ, TBIA, RT-PCR.

Among all the sugar beet diseases having viral, bacterial or fungal origins, rhizomania is one of the most devastating (Whitney and Duffus, 1998). This virus disease causes large economic losses by reducing yields up to 100% (Whitney and Duffus, 1998) and decreasing the sugar content from 16-18% to less than 7% (Bongiovanni and Lanzoni, 1964). Rhizomania has a worldwide distribution, apparently being present everywhere sugar beet is cultivated (Tamada, 2002). Its causal agent, *Beet necrotic yellow vein virus* (BNYVV), is transmitted and preserved in the soil by *Polymyxa betae* Keskin. Like BNYVV, the plasmodiophorid has a host range mainly restricted to the roots of Chenopodiaceae spp. (Barr and Asher, 1992; Tamada, 2002) and is present in most sugar beet growing countries (Payne and Asher, 1990). The virus can survive in the soil within resting spores of *P. betae* for several years (Rush and Heidel, 1995; Rush, 2003).

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Beet soil-borne virus (BSBV) and *Beet virus Q* (BVQ), the two tripartite genome viruses in the genus *Pomovirus* (Koenig and Lesemann, 2005), have also been reported in sugar beet fields throughout the world (Lindsten, 1989; Meunier *et al.*, 2003). In common with BNYVV, the two viruses have similar vectors (*P. betae*), host ranges and particle morphologies, but differ in serological properties, genome structure and sequence (Koenig *et al.*, 1998; Koenig and Lesemann, 2005). These viruses often occur together in the same field and, not rarely, in rhizomania-affected sugar beets (Meunier *et al.*, 2003), although their etiological role in the disease remains a matter of debate (Prillwitz and Schlosser, 1992).

Sugar beet is one of the main sources of sugar in Iran. In recent years, the average root yield of sugar beet has been considerably reduced, e.g. from 29.8 t/ha in 1999 to 26.6 t/ha in 2000 (FAO, 1999; 2000), and many farmers have replaced sugar beet crops, mainly with corn. Several virus diseases have been reported previously in sugar beet in Iran (Farzadfar *et al.*, 2006), but there is little information on the incidence and distribution of soil-borne viruses of beet and *P. betae* in the country. Rhizomania disease symptoms were first observed in sugar beet fields in Fars province in 1996 (Izadpanah *et al.*, 1996), where subsequent soil bioassays indicated the widespread occurrence of *P. betae* (Kamran *et al.*, 2000). In the present work, the main Iranian sugar beet cultivation areas were surveyed for the presence of BNYVV, BSBV, BVQ and their soil-borne vector, *P. betae*.

During the growing seasons of 2001, 2004 and 2005, a total of 2,870 sugar beet samples taken at random and 616 samples that showed rhizomania-like symptoms were collected from 184 fields of 14 Iranian provinces (Table 1 and 2, Fig. 1). Virus incidence was determined on the basis of the serological results obtained by testing exclusively the samples collected at random from each province. Disease incidence was also estimated based on the virus-like symptoms observed on plants randomly collected from each surveyed field.

Sugar beet root tips were tested by tissue blot immunoassay (TBIA) (Lin *et al.*, 1990) as described by Farzadfar *et al.* (2006), using BNYVV (As-0799.1/CG6-

Table 1. Results of TBIA laboratory tests on sugar beet root samples taken at random from 122 fields in different Iranian provinces (in 2001).

Province	No. of fields surveyed	No. of root samples	Samples infected with:			Single infections with:			Mixed infections with:				Overall [f]
			BNYVV	BSBV	BVQ	BNYVV	BSBV	BVQ	BNYVV, BSBV	BNYVV, BVQ	BSBV, BVQ	BNYVV, BSBV, BVQ	
Ardabil	3	81	48 [a] (59.3) [b] 44.4-66.7 [c]	21 (25.9) 0.0-44.4	0 (0.0)	36 (44.4)	9 (11.1) 0.0-22.2	0 (0.0)	12 (14.8) 0.0-22.2	0 (0.0)	0 (0.0)	0 (0.0)	57 (70.4) 44.4-88.9
Azarbayejan-e-gharbi	5	96	20 (20.8) 0.0-65.0	16 (16.7) 0.0-60.0	29 (30.2) 0.0-70.0	5 (5.2) 0.0-8.8	0 (0.0)	10 (10.4) 0.0-42.1	0 (0.0)	3 (3.1) 0.0-10.0	4 (4.2) 0.0-10.0	12 (12.5) 0.0-50.0	34 (35.4) 0.0-75.0
Esfahan	16	473	332 (70.2) 40.9-95.5	78 (16.5) 0.0-91.4	8 (1.7) 0.0-25.0	270 (57.1) 8.6-95.2	22 (4.7) 0.0-20.0	2 (0.4) 0.0-6.3	56 (11.8) 0.0-71.4	6 (1.3) 0.0-18.8	0 (0.0)	0 (0.0)	356 (75.3) 54.5-100
Fars	8	236	170 (72.0) 33.3-100	9 (3.8) 0.0-21.4	1 (0.4) 0.0-3.6	161 (68.2) 33.3-89.3	1 (0.4) 0.0-3.4	0 (0.0)	8 (3.4) 0.0-21.4	1 (0.4) 0.0-3.6	0 (0.0)	0 (0.0)	171 (72.5) 33.3-100
Kermanshah	22	473	134 (28.3) 5.3-72.7	21 (4.4) 0.0-31.3	1 (0.2) 0.0-5.6	123 (26.0) 0.0-68.2	11 (2.3) 0.0-20.0	0 (0.0)	10 (2.1) 0.0-12.5	1 (0.2) 0.0-5.6	0 (0.0)	0 (0.0)	145 (30.7) 5.3-72.7
Khorasan	18	444	314 (70.7) 43.8-96.4	20 (4.5) 0.0-13.3	1 (0.2) 0.0-3.3	297 (66.9) 43.8-90.0	4 (0.9) 0.0-10.0	0 (0.0)	16 (3.6) 0.0-11.8	1 (0.2) 0.0-3.3	0 (0.0)	0 (0.0)	318 (71.6) 43.8-96.4
Khuzestan	9	144	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Qazvin	16	329	166 (50.5) 0.0-100	18 (5.5) 0.0-33.3	0 (0.0)	152 (46.2) 0.0-89.5	4 (1.2) 0.0-13.3	0 (0.0)	14 (4.3) 0.0-20.0	0 (0.0)	0 (0.0)	0 (0.0)	170 (51.7) 0.0-100
Semnan	18	432	221 (51.2) 5.9-88.9	23 (5.3) 0.0-44.8	1 (0.2) 0.0-3.7	205 (47.5) 0.0-88.9	7 (1.6) 0.0-10.3	1 (0.2) 0.0-3.7	16 (3.7) 0.0-34.5	0 (0.0)	0 (0.0)	0 (0.0)	229 (53.0) 5.9-88.9
Zanjan	7	162	100 (61.7) 41.7-72.0	75 (46.3) 0.0-76.0	0 (0.0)	49 (30.2) 12.0-44.0	24 (14.8) 0.0-36.0	0 (0.0)	51 (31.5) 0.0-52.0	0 (0.0)	0 (0.0)	0 (0.0)	124 (76.5) 41.7-88.0
Average [e]	122	2870	1505 (52.4) 0.0-100 (110) [d]	281 (9.8) 0.0-91.4 (56)	41 (1.4) 0.0-70.0 (9)	1298 (45.2) 0.0-95.2 (108)	82 (2.9) 0.0-36.0 (30)	13 (0.5) 0.0-36.8 (4)	183 (6.4) 0.0-71.4 (47)	12 (0.4) 0.0-18.8 (6)	4 (0.1) 0.0-10.0 (2)	12 (0.4) 0.0-50.0 (3)	1604 (55.9) 0.0-100 (111)

[a] Number of infected sugar beet samples; [b] Average of virus incidence (%); [c] Range of virus incidence (%); [d] Number of fields with virus infection; [e] Incidence of each virus in the surveyed provinces; [f] Total virus incidence in each province calculated on the basis of single or mixed infections.

F4), BSBV (As-0576.1) and BSBV/BVQ (As-0576.2) antisera kindly provided by S. Winter (DSMZ, Braunschweig, Germany). Infected root beard extracts from sugar beet cvs. IC1 and Opus grown in infested soils (kindly supplied by A. Meunier, Unité de Phytopathologie-UCL-AGRO-BAPA, Louvain-la-Neuve, Belgium) were used as positive controls.

According to TBIA results, BNYVV was the prevailing virus (52.4%), followed by BSBV (9.8%) and BVQ (1.4%) (Table 1). Similarly, serological results from samples with rhizomania-like symptoms indicated the highest incidence of BNYVV (61.2%); low levels of infections with BSBV and BVQ also were found in association with rhizomania disease, 2.4% and 1.6%, respectively. Moreover, BNYVV was the predominant virus in symptomatic samples from all provinces surveyed, including Azarbayejan-e-gharbi and Zanjan where high levels of BSBV and BVQ infections were found in the randomly collected samples (Table 1, 2). The virus incidence ranged from 0% to 76.5%, with an average rate of 55.9% in randomly collected sugar beet samples, but reached peaks of up to 100% infection in symptomatic samples (average: 61.7%). Of 122 fields surveyed, 111 contained plants infected by at least one virus. Mixed infections were detected in 7.3% of samples compared to 48.6% of samples with single infections; the combination BNYVV + BSBV was the most frequent (6.4%) (Table 1). A map of the distributions of BNYVV, BSBV and BVQ in the different provinces of Iran is shown in Figure 1.

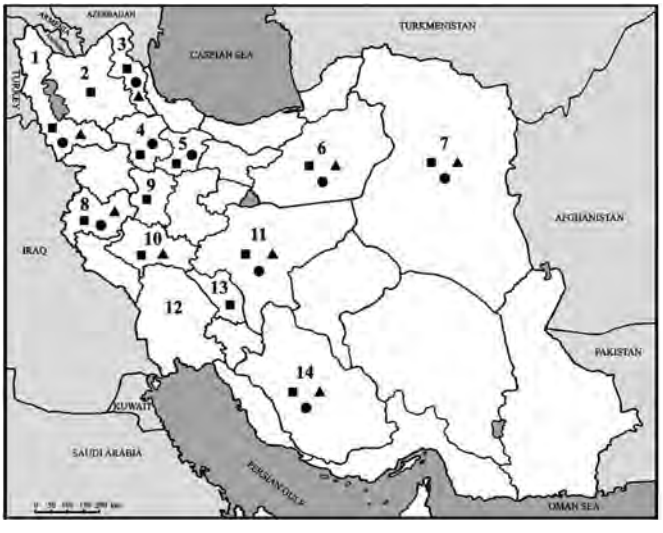

Fig. 1. Map of Iran showing the location of provinces surveyed (1 to 14) and the distribution of soil-borne viruses infecting sugar beets. 1: Azarbayejan-e-gharbi, 2: Azarbayejan-e-sharqi, 3: Ardabil, 4: Zanjan, 5: Qazvin, 6: Semnan, 7: Khorasan, 8: Kermanshah, 9: Hamedan, 10: Lorestan, 11: Esfahan, 12: Khuzestan, 13: Chaharmahal-va-bakhtyiari, 14: Fars, ■: *Beet necrotic yellow vein virus* (BNYVV), ●: *Beet soil-borne virus* (BSBV), and ▲: *Beet virus Q* (BVQ). Courtesy of Farzadfar et al. (2002).

Rhizomania symptoms of yellows (31.8%), upright leaf position (17.7%), root madness (37.5%), lateral root proliferation (29.2%), xylem necrosis (26.6%) and malformation (19.2%) were observed in the surveyed fields. Leaf symptoms of necrotic yellow vein were observed only in Semnan. Also, the rate of root constriction resulting in a "wine glass" appearance of roots was particularly high in Esfahan. Both the incidences of diseases determined visually in the field and of virus infections detected by serological assays differed markedly between the different areas surveyed. In some provinces (Azarbayejan-e-gharbi, Fars, Kermanshah and Khorasan) the incidence of virus assessed by serological tests closely matched that of plants with symptoms, especially yellowing, lateral root proliferation and root madness. In contrast, this correlation was very weak in Arbadil, Esfahan and Qazvin. Similar results were obtained by analysing the rhizomania-symptomatic samples (data not shown).

The presence of viruses was also confirmed by reverse transcription-polymerase chain reaction (RT-PCR) in 122, 28 and 19 root samples that were positive in TBIA to BNYVV, BSBV and BVQ, respectively (data not shown). These samples, as well as 396 samples mainly from fields where these viruses had not been detected by TBIA (5-20 samples per field), were tested for the presence of *P. betae*. RT-PCR was carried out in a two-step process using specific primers designed to amplify regions on BNYVV RNA 1 to 5, BSBV RNA-2, BVQ RNA-1 and the mRNA for repetitive *Eco*RI-like fragments of *P. betae* (Koenig *et al.*, 1995; Kiguchi *et al.*, 1996; Meunier *et al.*, 2003). Total RNA was extracted from sugar beat root beards using RNA extraction solution (RNX^{TM}-plus, CinnaGen Inc., Iran) according to the manufacturer's instructions. The same sources of positive and negative controls used for TBIA tests were also used for PCR reactions. PCR products were analysed by electrophoresis through 1% agarose gel, using 1 Kb DNA ladder (Life Technologies, Gibco BRL, Rockville, MD, USA) for size comparison.

About 200 µg of RNA per gram of sugar beet rootlets could be extracted by using RNX^{TM}-plus. A PCR product of the expected size was obtained for each of the viral RNAs investigated (Koenig *et al.*, 1995; Meunier *et al.*, 2003). The expected 515 bp DNA fragment corresponding to BNYVV RNA-5 was not observed in the isolates under study. Moreover, *P. betae* was detected by RT-PCR in 421 out of 565 samples tested (Meunier *et al.*, 2003).

Three to five samples from the upper 15 cm of soil were collected randomly in each field surveyed and bulked. For the fields that were free of BNYVV, BSBV and BVQ in TBIA assays, the soil from each bulked sample was dried, mixed 1:5 with sterile sand, placed in pots (10 cm in diameter) and used to grow plants of Opus and IC1 cultivars. Infested soil samples kindly

Table 2. Results of TBIA laboratory tests on symptomatic sugar beet root samples taken from 62 fields in 13 provinces of Iran.

Province	Year	No. of fields surveyed	No. of root samples	Samples infected with			Overall [c]
				BNYVV	BSBV	BVQ	
Ardabil	2005	4	17	17 [a] (100) [b]	0 (0.0)	2 (11.8)	17 (100)
Azarbayejan-e-gharbi	2005	5	49	24 (49.0)	6 (12.2)	4 (8.2)	26 (53.1)
Azarbayejan-e-sharqi	2005	1	16	11 (68.8)	0 (0.0)	0 (0.0)	11 (68.8)
Chahrmahal-va-bakhtiyari	2001	3	40	12 (30.0)	0 (0.0)	0 (0.0)	12 (30.0)
Esfahan	2005	3	86	86 (100)	0 (0.0)	0 (0.0)	86 (100)
Fars	2005	2	60	51 (85.0)	0 (0.0)	0 (0.0)	51 (85.0)
Hamedan	2001	3	20	12 (60.0)	0 (0.0)	0 (0.0)	12 (60.0)
Kermanshah	2005	11	109	48 (44.0)	2 (1.8)	0 (0.0)	48 (44.0)
Khorasan	2005	5	67	38 (56.7)	5 (7.5)	2 (3.0)	39 (58.2)
Khuzestan	2005	4	30	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Lorestan	2001	5	16	9 (56.3)	0 (0.0)	2 (12.5)	9 (56.3)
Lorestan	2004	4	15	13 (86.7)	0 (0.0)	0 (0.0)	13 (86.7)
Lorestan	2005	5	49	23 (46.9)	0 (0.0)	0 (0.0)	23 (46.9)
Qazvin	2004	3	13	13 (100)	2 (15.4)	0 (0.0)	13 (100)
Zanjan	2004	4	29	20 (69.0)	0 (0.0)	0 (0.0)	20 (69.0)

[a] Number of infected sugar beet samples; [b] Average of virus incidence (%); [c] Total virus incidence in each province calculated on the basis of single or mixed infections.

provided by A. Meunier were used as positive controls. After growing plants for 9-12 weeks in a greenhouse (Gerik, 1992), roots were harvested, washed and tested by RT-PCR for viruses and *P. betae*.

P. betae was also detected by RT-PCR in root beards of susceptible sugar beet plants grown in soil samples from 10 out of 15 fields where soil-borne viruses had not been detected by serological or RT-PCR tests. The results indicated that all fields analysed, except 5 in Khuzestan, were infested with *P. betae*.

Some plants infected with BSBV or BVQ showed rhizomania-like symptoms, but this association was not always observed in all the fields surveyed, as observed by others (Prillwitz and Schlosser, 1992; Meunier *et al.*, 2003). Our findings suggest that soil-borne viruses, especially BNYVV, are responsible for sugar beet disorders in Iran. The failure to detect virus in some plants with rhizomania-like symptoms was perhaps because similar symptoms can be induced by several soil-borne fungi, adverse soil conditions, such as hardpans (Rush and Heidel, 1995), or other sugar beet viruses (Whitney and Duffus, 1998).

In contrast to the presence of *P. betae*, BNYVV and other soil-borne viruses were not found in root or soil samples collected in Khuzestan province (Fig. 1, area 12), where sugar beet is sown in early autumn (October) and harvested in early spring. The average daily temperatures in November-April period from 1987 to 2003 in Safi-abad (I. R. of Iran Meteorological Organization), the main sugar beet cultivation area in Khuzestan, were low enough (11.6-22.7°C) to prevent infection of plants by *P. betae* (Legreve *et al.*, 1998). Also, the alkalinity of soils in Khuzestan (pH 7.0-8.0) (Farmanara and Veenenbos, 1959) is not optimal for infection by *P. betae* (Uchino and Kanzawa, 1995).

The wide distribution of rhizomania in Iran could be due to many factors, i.e. the use of highly contaminated tools in cultural practices, the long distance transportation of infested soil on the surface of sugar beet roots or of other crops, and the use of sewage and waste resulting from sugar beet factories (Heijbroek, 1988; Scott and Cooke, 1993). In this study, *P. betae* was found in soils of all provinces surveyed, indicating the potential for BNYVV spread in regions where rhizomania has not yet been detected. An additional problem is the possible role that aviruliferous *P. betae* can play in decreasing sugar beet growth (Wisler *et al.*, 2003).

In 2000, many sugar beet cultivars with a partial tolerance to rhizomania were tested in Iran. In these cultivars the incidence of BNYVV was similar to that for susceptible varieties (data not shown), thus suggesting that the multiplication and spread of the virus still occurs in their roots (Asher *et al.*, 2002). As a consequence, the spread of the virus and the increase the inoculum in the soil is not stopped but continues undisturbed.

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