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Effects of Soil Properties on the Occurrence of Beet Necrotic Yellow Vein Virus and Beet Soilborne Virus on Sugar Beet in Tokat, Turkey

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Abstract: Beet necrotic yellow vein virus (BNYVV) and Beet soilborne virus (BSBV) are the important soilborne virus diseases in the production areas of sugar beet. A survey was carried out to detect these viruses in soil samples using bait plant test and double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) and triple antibody sandwich-ELISA (TAS-ELISA) in sugar beet production areas in Tokat, Turkey in 2001. Of the 26 soil samples taken from 8 districts in Tokat province, it was found that 23 samples (85.5%) were infected with BNYVV and 24 samples (92.3%) with BSBV. In 2002, total 49 root samples were tested for the presence of these viruses by ELISA. The incidence of BNYVV (67%) was greater than that of BSBV (8%). Furthermore, in this study it was determined the relationships between physical and chemical properties of soils and the appearance of BNYVV and BSBV. But, it could not determine any connection between infectious potential of viruses and soil properties.

Key words: Beet necrotic yellow vein virus, beet soilborne virus, *Polymyxa betae,* rhizomania, soil properties, sugar beet

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is cultivated extensively in Tokat, Turkey, over the areas of 19.049 ha in 2001[1]. Rhizomania, a soilborne virus disease, is one of the most important sugar beet disease in that district. In severely infected fields the yield loss can be 70% or more[2] and sugar content can be reduced from 16-18 to 7% due to the virus infection[3]. The disease was first reported in Italy in the early 1950's[4] and has been distributed in many sugar beet growing countries in the world since then[5,6]. The agent of rhizomania is Beet necrotic yellow vein virus (BNYVV) and member of the genus *Benyvirus*[6]. The virus is transmitted by soilborne vector protozoa *Polymyxa betae* Keskin[7]. Virus can survive within thick-walled resting spores (cystosori) of *P. betae* for at least 15 years in soil[8,9].

Another soil borne virus, beet soilborne virus (BSBV) was first reported from England in 1982[10]. Later, it was indicated that BSBV can be found to occur in many other contries[11-14]. Prillwitz and Schlösser[15] reported its occurrence in soils in which BNYVV was present. BSBV is morphologically similar to BNYVV but serologically different from it. Unlike BNYVV, different serological types of BSBV exist. BSBV belong to *Pomovirus* genus and also *P. betae* is its vector[15]. According to Prillwitz and Schlösser[15] BSBV, taken up infested soil, may cause rhizomania-like symptoms and yield loses up to 70% in sugar beets.

Rhizomania is common in poor drained soils such as kepir and vertisol which have high clay contents as well as in soils with high ground water levels[16]. Soil is main factor in spreading of the diseases. Main factors that affect the BNYVV infection are inoculum level of *P. betae*, soil temperature, soil moisture[15] and pH of soil[17].

In the present study, sugar beet plants with rhizomania-like symptoms from Tokat, Turkey were tested for BNYVV and BSBV with ELISA. On the other hand, the relationships between the occurrence of BNYVV and BSBV and the physical and chemical properties of soils were investigated.

MATERIALS AND METHODS

Soil material: A total of 26 soil samples were collected from the fields in Central, Pazar, Turhal, Zile, Erbaa, Artova, Niksar and Almus districts of Tokat province of Turkey during August, 2001. All samples were taken

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considering the visual indications for the presence of rhizomania in field grown plants, such as pistachio green coloration of leaves and beard-like appearance of the roots. At least one kilogram of soil was obtained from 0-20 cm depth[18,19] and brought to laboratory in labelled polyethylene bags. In these specimens total salt[20], pH[21], lime[21] and soil texture properties[22] were determined.

Virus infected plant material: Sugar beet growing areas in the villages belonging to Tokat Center, Pazar and Turhal were surveyed in the period of August- September of 2002. Considering virus infection symptoms in the plants, the root samples were taken from the plants in 49 fields.

Bait plant tests: Soil samples taken from 26 areas were used in bait plant tests. Twenty sugar beet seeds, one of which was the rhizomania-susceptible cultivar (Fiona), were sown in 500 mL of pots containing saturated soil. Each treatment was replicated three times. After the growing period of six weeks under room temperature, each pot was harvested separately. The root samples were divided into two parts: one of them was used to test for the presence of viruses by ELISA tests and the other for *P. betae*.

Serological tests: The double antibody sandwich ELISA (DAS-ELISA) was used the test for BNYVV. DAS-ELISA method was performed according to Clark and Adams[23] and instructions of the antiserum producer (Loewe Biochemica, Sauerlach, Germany), except that extraction buffer included 0.1% nonfat dry milk instead of bovine serum albumin[24]. Root samples of sugar beet were ground in mortar with extraction buffer (PBS: 0.13 M NaCl, 0.014 M KH_2PO_4, 0.002 M KCL, pH 7.4) containing 0.05% Tween 20 and 0.1% nonfat dry milk and put to wells, which were precoated with BNYVV-specific antiserum diluted in coating buffer (pH: 9.6). Later, the plates were incubated at 4°C overnight and after the incubation period washed three times with PBST-Tween 20 buffer. Then, the plates were coated with alkaline phosphatase conjugated antibody diluted in extraction buffer and incubated for 4 h at 37°C. After washing, p-nitrophenyl phosphate in diethanolamine substrate buffer (1 mg mL^{-1}, pH: 9.8) was added to each well and maintained at room temperature for 30 to 120 min. Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra II, Grödig/Salzburg, Austria).

A triple antibody sandwich (TAS)-ELISA was used to test for BSBV. Antiserum against BSBV was obtained from the Loewe Biochemica, Sauerlach, Germany. The plates were coated with BSBV polyclonal antiserum diluted to 1: 500. The root extracts were diluted in coating buffer (1: 5). Monoclonal antibody (Mab) were incubated at 37°C for 2 h and rat-anti-mouse conjugate at 1: 1000 was incubated at the same temperature for a further 2 h. After addition of the substrate (1 mg mL^{-1}), the plates were incubated at room temperature for 30 to 120 min and absorbance values at 405 nm were than measured using a microplate reader (Tecan Spectra II, Grödig/Salzburg, Austria). The positive-negative threshold was taken as the mean absorbance value of healthy plants plus five times the SD of the absorbances of six buffer control wells[25].

Detection of *P. betae*: Rootlet samples were taken from bait plant test and they washed to remove soil debrits, and were stained with lactophenol containing 0.1% acid fuchsin. *P. betae* was checked by observing the amount of resting spore clusters in 10 pieces of the rootlets (about 1 cm long) of each plant under a light microscope[8].

Statistical analysis: In order to detect the various properties of soils the present work was used that can affect disease occurrence. Logistic Regression analysis method was applied using SPSS for Windows 10.0 software[26].

RESULTS

The appearance of rhizomania-affected sugar beet leaves became pistachio green, wilted and with reduced grew more straight than those of healthy plant. Its lateral roots profilerated severely and its vascular bundles became dark and fibrous. In addition, it was observed that infected plants were smaller than healthy plants. These changes occurred in all locations in which BNYVV and BSBV were detected.

Detection of BNYVV and BSBV in the soils using the bait plant test and soil properties: Soils were collected from 26 different fields from 8 districts in Tokat province, based on the symptoms caused by rhizomania in order to reduce the risk of missing BNYVV-BSBV infected fields. However it is sometimes difficult to detect infected sugar beet using such criteria. For this reason, soil samples were taken from these fields in 2001. On the other hand, the relationships between the occurrence of BNYVV and BSBV and the physical and chemical properties of soils were investigated. These soils were used to detect the presence of BNYVV, BSBV and *P. betae* using the bait plant test. ELISA tests were performed six weeks later on the young sugar beet roots. Of the 26 soil samples taken from 8 districts in Tokat province, 23 samples (85.5%)

Table 1: The results of soil analysis, ELISA and light microscopic observation of selected fields in the sugar beet production areas of Tokat province/Turkey

Districts, villages	pH	Salt (%)	Lime (%)	Texture component			Texture class*	ELISA* *		Plants
				Sand	Clay	Silt		BNYVV	BSBV	*P. betae*
Center										
Kiliçli	8.12	0.021	3.9	30	21	49	L	+	+	+
Çamagzi	7.79	0.049	1.2	36	48	17	C	-	+	+
Bakisli	7.85	0.045	4.6	45	45	10	SC	-	+	+
Pinarli	7.95	0.027	3.5	30	38	32	CL	+	+	+
Döllük	7.87	0.027	4.1	40	48	12	SC	+	+	+
Bula	7.70	0.018	1.2	35	49	17	C	+	-	+
Kizilhasan	8.06	0.019	2.9	82	10	8	LS	+	-	+
Pazar										
Çerçi	7.97	0.032	15.1	47	35	18	SCl	+	+	+
Dereköy	8.35	0.029	7.7	25	35	40	CL	+	+	+
Söngüt	8.04	0.041	18.5	10	48	42	SC	+	+	+
Turhal										
Tatlicak	8.16	0.033	10.9	50	30	16	SCl	+	+	+
Çayköy	8.00	0.030	13.2	50	32	18	SCl	+	+	+
Zile										
Beylikçayiri	7.92	0.048	6.3	12	48	40	SiC	+	+	+
Koças	7.96	0.038	36.2	35	37	28	SiCl	+	+	+
Akyazi	8.00	0.035	3.3	3	52	45	SC	+	+	+
Emirören	8.17	0.031	14.7	50	32	18	SCl	+	+	+
Kireçli	7.98	0.032	20.4	48	25	27	SCl	+	+	+
Erbaa										
Çalkara	8.02	0.034	9.6	11	47	42	SC	+	+	+
Çalkara	8.09	0.019	11.7	19	43	38	-	+	+	+
Çalkara	7.87	0.039	11.4	4	55	41	SiC	-	+	+
Çalkara	7.90	0.035	14.3	17	38	45	SiCL	+	+	+
Çalkara	8.01	0.021	12.2	25	38	27	CL	+	+	+
Çalkara	8.09	0.015	13	25	38	37	CL	+	+	+
Niksar										
Buzköy	7.92	0.019	10.9	34	50	17	C	+	+	+
Artova										
Kunduz	7.93	0.037	9.3	18	35	47	SC	+	+	+
Almus										
Çevreli	7.94	0.022	8.4	50	32	8	SCI	+	+	+0
Total	-	-	-	-	-	-	26	23	24	26
Infection %	-	-	-	-	-	-	-	85.5	92.3	100

* L: Loam, S: Sand, Si: Silt, C: Clay, ** Beet necrotic yellow vein virus (BNYVV) and Beet soilborne virus (BSBV)

Table 2: BNYVV and BSBV in sugar beet grown in Tokat, Turkey[a]

District	No. of samples tested	BNYVV	BSBV
Center	28.00	20.00	2
Pazar	14.00	10.00	2
Turhal	7.00	3.00	-
Total	49.00	33.00	4
Percent Infected	67.35	8.20	

[a] Beet necrotic yellow vein virus (BNYVV) and beet soilborne virus (BSBV)

were infected with BNYVV and 24 samples (92.3%) with BSBV. In all roots were found characteristic structures of *P. betae* (plasmodia and cystosori) when examined under the light microscope (Table 1).

Seven soil samples were taken from in sugar beet fields in Center, 5 (71%) of the samples reacted with antisera to BNYVV and BSBV (Table 1). The incidence of BNYVV and BSBV in Pazar, Turhal, Zile, Niksar, Artova and Almus districts was 100%. Six soil samples were taken from different location in Zile district and it was found that 83% of them were infected with BNYVV and 100% with BSBV.

The results from these soil analysis showed that the texture of the soils were generally differed fine to heavy and soil reactions were slightly alkaline (>7.3) to moderately alkaline (>7.8). Lime contents were low (>2%) to high (>15%) in the soils mentioned (Table 1).

In order to prove that the soil properties may affect the disease occurrence logistic regression and correlation analysis were applied using SPSS for Windows 10.0 software. A total 156 observations (26 areas X two viruses X three replications) were used. Soil characteristics such as pH, salt, lime and soil texture (i.e. sand, silt and clay content) were taken as independent variables, and clean (ELISA<0.96) and infected (ELISA=0.96) for BNYVV; clean (ELISA<0.148) and infected (ELISA=0.148) for BSBV were taken as the possible causes of the dependent variable in logistic regression and correlation equations. Based on the results, relationship between soil properties and the occurrence of BNYVV and BSBV were not statistically significant.

Detection of BNYVV and BSBV in the plant material: Plant samples were collected from Center, Pazar and Turhal districts (a total of 49 fields) in 2002. In the assays the viruses were detected in more than 75% of the samples (Table 2). The incidence of BNYVV (67.35%) was greater than that of BSBV (8.20%) in our root samples. The percentage of samples in which both viruses detected in the same sample ranged 8.20%.

DISCUSSION

In Turkey, rhizomania was first reported in the North of Turkey (Erbaa, Tasova, Kesan and Uzunköprü) in 1987. BNYVV and BSBV are closely related pathogens and widely distributed in sugar beet growing regions. Both pathogens are vectored by the zoospores plasmodiophoromycete *P. betae*. There have been reports on incidence of BNYVV in Tokat province[16,27], but BSBV and *P. betae* have never been investigated in same location. In most cases, BSBV and BNYVV were tend to occur together. Similarly, Mouhanna *et al.*[25] and Meunier *et al.*[28] found that BNYVV occurred with BSBV. In this study, BSBV was found in more than 90% of the analyzed soil samples and its frequency was higher in comparison to BNYVV. The soil survey findings in 2001 were generally more than expectation on BSBV occurrence in Tokat. But, the reverse case was in fields sampling in 2002 that the occurrence of BSBV in infected roots sharply decreased to 8% and approximately 67% of the samples were infected with BNYVV. The reason of different results obtained from bait plant test and plant samples might be due to the cultivating tolerant varieties with rotation (4 years) and existing the viruses concentration in the research areas. Indeed, in the field, rhizomania tolerant sugar beet varieties such as Gina, Gabriela, Rizor and Roxane have been grown in all production areas with rhizomania problem in Turkey since 1994. Tolerant varieties do not replicate the virus as much as classical ones. An explanation also might be that those fields had been recently infected by BNYVV and/or BSBV and the amount of viral particles present in the plant did not allow a significant identification of the presence of the viruses by ELISA. *P. betae* also was common in regions of our sampling areas where sugar beet is grown.

Although the results of the earlier study conducted by Kutluk Yilmaz *et al.*[29] mentioned that the significance relationship between soil properties such as pH, phosphorus and potassium contents and the occurrence of rhizomania disease was present, this relationship is very weak, because the coefficients are very small relative to their standard error sourced by soil properties. Most of the researchers independently focused on soil as a spreading factor of rhizomania and its vector[13,15,30]. Kastir and Widera[31] reported that *P. betae* was most common in loamy soils while Grünewald *et al.*[18] and Hillmann[32] indicated that rhizomania can occur in any soil from heavy clay to a light sand. In addition, some researchers explained that the heavily infected soils had soil reactions of neutral to alkaline[17,33,34]. Due to these soil samples classified according to texture as i.e. loam, clay, sandy clay, loamy sand silty clay, it was not proved that the soil properties were a speading factor of BNYVV, BSBV and its vector. On the other hand, many researchers concluded that the optimum pH values suitable for *P. betae* ranged from 6 to 8[30,35]. The analysis of the our soils showed that all of them had a pH above 7.70 which is favorable to the development of *P. betae*. However this study could not make any relation between the infectious potential of the soil and the concentration of Ca as suggested by Goffart and Maraite[36].

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