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Short communication. Relationships between soil properties and soilborne viruses transmitted by *Polymyxa betae* Keskin in sugar beet fields

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Abstract

Sugar beet plants (cv. Arosa), susceptible to rhizomania, were grown in 144 soil samples taken from sugar beet fields in north and central part of Turkey in 2004. Incidences of *Beet necrotic yellow vein virus* (BNYVV) and *Beet soilborne virus* (BSBV) by ELISA and of their vector *Polymyxa betae* by root staining method were determined in bait plants. Some soil properties such as, texture, pH, organic matter, cation exchange capacity, electrical conductivity, lime ($CaCO_3$) and exchangeable Ca, Mg, K and Na contents were determined. Sand content and pH values of the soils gave significant positive correlations with *P. betae* (0.177* and 0.164*, respectively). Lime ($CaCO_3$) and exchangeable Mg contents had also significant positive correlations with soil pH. Increasing $CaCO_3$ (0.189*) and exc. Mg (0.235**) content of soils induced BNYVV and BSBV infections respectively, transmitted by *P. betae*, due to their increasing effects on soil pH.

Additional key words: bait plant test, BNYVV, BSBV, lime ($CaCO_3$), soil pH.

Resumen

Comunicación corta. Relaciones entre las propiedades del suelo y virus del suelo transmitidos por *Polymyxa betae* Keskin en campos de remolacha azucarera

Se cultivaron plantas de remolacha azucarera (cv. Arosa), susceptibles a rhizomania, en 144 muestras de suelo recogidas de campos del norte y centro de Turquía en 2004. Se determinó la incidencia de *Beet necrotic yellow vein virus* (BNYVV) y *Beet soilborne virus* (BSBV) por ELISA y de su vector *Polymyxa betae* por el método de tinción de raíz en plantas cebo. Se determinaron algunas propiedades del suelo tales como textura, pH, materia orgánica, capacidad de intercambio catiónico, conductividad eléctrica, y contenidos en cal ($CaCO_3$), y Ca, Mg, K y Na intercambiables. El contenido en arena y los valores de pH de los suelos presentaron correlaciones positivas con *P. betae* (0,177* y 0,164*, respectivamente). La cal ($CaCO_3$) y el contenido en Mg intercambiable estuvieron también positivamente correlacionados con el pH del suelo. Contenidos crecientes en $CaCO_3$ (0,189*) y en Mg intercambiable (0,235**) de los suelos indujeron infecciones de BNYVV y BSBV respectivamente, transmitidas por el vector *P. betae,* debido a su creciente efecto en el pH del suelo.

Palabras clave adicionales: cal ($CaCO_3$), BNYVV, BSBV, pH del suelo, plantas cebo.

Rhizomania is one of the most economically significant and widespread virus diseases in most sugar beet (*Beta vulgaris* L.) growing areas in the world. The disease is caused by *Beet necrotic yellow vein virus* (BNYVV). Characteristics of rhizomania include proliferation of lateral roots and loss in both yield and sugar production (Canova, 1967; Brunt and Richards, 1989). In most cases, BNYVV and *Beet soilborne virus* (BSBV) occurred together (Meunier *et al.*, 2003). BSBV and BNYVV have a similar vector *Polymyxa betae* Keskin and host ranges that infect *Chenopodiaceae* family (Keskin, 1964; Asher and Thompson, 1987;

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Abbreviations used: BNYVV (*Beet necrotic yellow vein virus*), BSBV (*Beet soilborne virus*), CEC (cation exchange capacity), DAS-ELISA (double antibody sandwich-enzyme linked immunosorbent assay), EC (electrical conductivity), OM (organic matter), TAS-ELISA (triple antibody sandwich-ELISA).

Prillwitz and Schlösser, 1992; Koenig and Lesemann, 2005). BSBV may cause symptoms resembling rhizomania on sugar beet, but it also frequently occurs in symptomless plants (Prillwitz and Schlösser, 1992; Koenig *et al.*, 2000).

The sugar beet is a main source of sugar in Turkey and grown extensively in northern and central part of Anatolia (Anonymous, 2005). In this study, relationships among the occurrence of soilborne viruses, their vector *P. betae* and the physico-chemical soil properties were investigated with direct coloration of bait plant root tissues for *P. betae* cystosori and ELISA tests for viruses.

Surface soil samples (0 to 20 cm depth) were randomly collected from 144 different sugar beet fields from Samsun, Amasya, Tokat, Corum, Cankiri and Yozgat provinces located in northern and central part of Turkey in 2004. Air dried and sieved soil samples were used to prepare 1:1 soil:sand mixture with autoclaved sand. After filling 144 soil:sand mixtures in pots with two replications, 10 sugar beet seeds of the rhizomania-susceptible cultivar (cv. Arosa) were sown in each 300 mL pot. All plants were harvested after six weeks of controlled growth with a 12 h photoperiod at 20°C (night) and 23°C (day). The presence of viruses by ELISA and the *P. betae* were tested in the plant roots.

BNYVV and BSBV were tested by DAS-ELISA using the antiserum from Sediag Biochemica (France) (Clark and Adams, 1977) and TAS-ELISA using antiserum from Adgen (Scotland), respectively. Samples were considered positive when the absorbance values at 405 nm were more than two times the mean of the negative control (Meunier *et al.*, 2003). Also, the root samples were stained with lactophenol containing 0.1% acid fuchsin to examine *P. betae* cyctosori under a light microscope (Leica, Switzerland) (Abe and Tamada, 1986).

Table 1. Descriptive statistics of some properties of soil samples[1]

	Minimum	Maximum	Mean	Std. deviation
Clay, %	4.13	68.66	38.36	11.77
Silt, %	13.67	72.98	30.16	9.22
Sand, %	4.27	65.93	31.48	11.92
EC[2], dS m^{-1}	0.20	2.95	0.76	0.49
pH (sat. ex.)	5.59	8.33	7.75	0.38
OM[3], %	0.84	4.34	2.21	0.68
$CaCO_3$, %	0.81	27.99	10.10	5.65
CEC[4], cmol kg^{-1}	9.27	48.76	27.65	8.92
Extractable cations				
Ca, cmol kg^{-1}	9.89	66.83	27.21	6.92
Mg, cmol kg^{-1}	3.15	28.72	9.41	4.01
K, cmol kg^{-1}	0.12	1.79	0.68	0.32
Na, cmol kg^{-1}	0.08	5.64	0.70	0.86

[1] The number of soil samples is 144. [2] EC: electrical conductivity. [3] OM: organic matter. [4] CEC: cation exchange capacity.

Some basic soil properties were determined according to the standard methods and given in Table 1. According to the results, 77% of the soil samples were in fine (clay and clay loam), 23% in coarse (loam, sandy loam and sandy clay) textural class, 3.4% of the samples were in slightly acid, 2.8% in neutral, 41.7% in slightly alkaline and 50.7% in moderately alkaline as soil pH (Soil Survey Staff, 1993) (Table 1).

ELISA test results showed that BNYVV and BSBV were detected in 54 (37.5%) and 53 (36.8%) fields, respectively (Table 2). Single infections with BNYVV

Table 2. Infection of *Beet necrotic yellow vein virus, Beet soilborne virus* and *Polymyxa betae* in different textural soils

Textural class	Number of samples[1]	Viruliferous *P. betae*			Aviruliferous *P. betae*	Total *P. betae*
		BNYVV	BSBV	BNYVV + BSBV		
Clay	64 (44.4)	10 (15.8)	10 (15.8)	15 (23.4)	20 (30.3)	55 (85.9)
Clay loam	47 (32.6)	7 (14.9)	11 (23.4)	8 (17.0)	18 (38.3)	44 (93.6)
Loam	12 (8.3)	3 (25.0)	1 (8.3)	2 (16.7)	5 (41.7)	11 (91.7)
Sandy clay	10 (6.9)	2 (20.0)	1 (10.0)	3 (30.0)	3 (30.0)	9 (90.0)
Sandy loam	6 (4.2)	2 (33.3)	0 (0.0)	1 (16.7)	3 (50.0)	6 (100.0)
Silty clay	2 (1.4)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	2 (100.0)
Silty clay loam	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)
Silt loam	1 (0.7)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
Total	144	25 (17.4)	24 (16.7)	29 (20.1)	51 (35.4)	129 (89.6)

[1] % distributions are given in brackets.

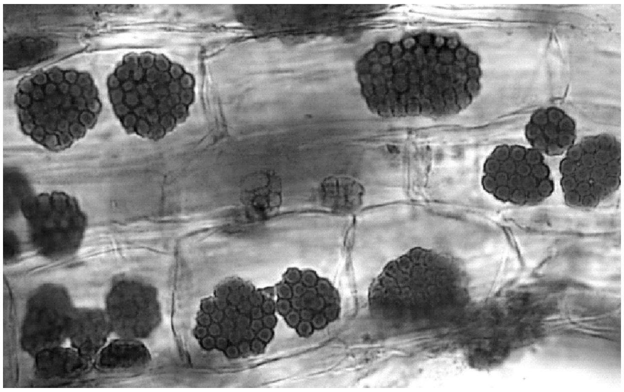

Figure 1. *P. betae* cystosori in sugar beet root tissues.

(17.4%) and BSBV (16.7%) were determined in 49 (34.0%) fields whereas double virus infection was found in 29 (20.1%) fields. BNYVV often has been reported in association with one or two different pomoviruses including BSBV and *Beet virus Q* (BVQ) in the nature (Turina *et al*., 1996; Mouhanna *et al*., 2002; Meunier *et al*., 2003). *P. betae* in this study was common (89.6%) in sugar beet fields and 35.4% of the roots were infested with aviruliferous *P. betae* cystosori (Table 2) (Fig. 1).

Although 77% of the soil samples were in fine textural soil classes, Grünewald *et al*. (1983) and Hillmann (1984) indicated that rhizomania can occur in any soil from heavy clay to light sand. Kastir and Widera (1988) reported that *P. betae* was common in loamy soils. Kutluk Yilmaz *et al*. (2004) did not found relationships among BNYVV, BSBV, and some soil properties of 26 samples from Tokat province in Turkey. In the current study, *P. betae* showed significant positive correlations with BNYVV (0.258**), BSBV (0.254*), sand content (0.177*) and pH (0.164*) of soil samples (Table 3). Increases in pH and sand content or macro porosity in soil samples induced vector infection in plants. Abe (1987) reported that soil pH affecting vector infection in sugar beet roots varied from neutral to slightly alkaline while soil pH was usually acidic in non-infested fields' soils. Many researchers concluded that *P. betae* has been shown to infect most rapidly and most severely at pH 6-8 with little development below this (Ui, 1973; Abe, 1974). In this study, 92.4% of soils were slightly alkaline and moderately alkaline (pH 7.4-8.4). The significant correlation between pH and *P. betae* (0.164*) showed that soil pH above 7.4 which is favorable to the development of *P. betae* vector infection in sugar beet roots. Germination of cystosori is inhibited at pH 5.5 but not at 6.5 or 7.0 (Ui, 1973) and also the activity of zoospores virtually ceases at pH values below 5.0 (Ivanovic, 1984).

Table 3. Correlations among *Beet necrotic yellow vein virus, Beet soilborne virus, Polymyxa betae* and soil properties

	P. betae	BNYVV	BSBV	pH
P. betae	1	0.258**	0.254**	0.164*
BNYVV	0.258**	1	0.277**	0.095
BSBV	0.254**	0.277**	1	0.115
Clay	–0.104	–0.053	0.067	–0.041
Silt	–0.096	–0.021	–0.145	–0.055
Sand	0.177*	0.069	0.047	0.083
EC	0.048	–0.097	0.075	0.089
OM	–0.092	–0.021	0.027	–0.181*
CEC	–0.067	–0.089	–0.041	–0.145
Ca	–0.143	–0.076	–0.129	–0.007
Mg	0.149	–0.140	0.235**	0.164*
K	0.066	–0.088	0.108	0.008
Na	0.095	–0.079	0.107	0.156
$CaCO_3$	–0.015	0.189*	0.058	0.332**

**,*: Correlation is significant at 0.01 and 0.05 level, respectively.

Lime ($CaCO_3$) and exchangeable Mg content of the soil samples had significant positive correlations with BNYVV (0.189*) and BSBV (0.235**), respectively (Table 3). Increases in lime content in the soils induced rhizomania disease. Abe (1987) reported that Ca application in form of $CaCO_3$ instead of $CaSO_4$ in soils with pH 5.1 increased soil pH and the *P. betae* infection. Hydrolysis of strong bases in the soil solution, such as Na_2CO_3, K_2CO_3 and $MgCO_3$, develop alkalinity and increase soil pH (Brady, 1974). Factors causing soil pH fluctuations in fields were i) nitrate nitrogen content in rhizosphere soil, ii) saturation rate of calcium in soil or iii) exchangeable calcium amount in soil (Abe, 1987). In this study, exchangeable Mg content of soil samples other than $CaCO_3$ content had also a significant positive correlation with soil pH (0.164*). Magnesium can be found in the formation of dolomitic limestone [$CaMg(CO_3)_2$] with Ca and can cause increase in soil pH (Bohn *et al.,* 1985). It indicates that increasing exchangeable Mg content in the soils induced BSBV infection due to increasing soil pH. Kutluk Yilmaz *et al*. (2003) also found that rhizomania disease was induced as soil pH, phosphorus and potassium contents increased.

As a result of this study, increasing lime ($CaCO_3$) and exchangeable Mg content of soils caused increases in soil pH and induced BNYVV and BSBV infections transmitted by vector *P. betae*, respectively.

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References

ABE N., 1974. Factors affecting the rhizomania of sugar bet. Bull Hokkaido Prefect Agric Exp Stn 30, 95-102.

ABE N., 1987. Studies of the ecology and control of *Polymyxa betae* Keskin as a fungal vector of the causal virus (beet necrotic yellow vein virus) of rhizomania disease of sugar beet. Bull Hokkaido Prefectural Agric Exp St 60, 81.

ABE H., TAMADA T., 1986. Association of *Beet Necrotic Yellow Vein Virus* with isolates of *Polymyxa betae* Keskin. Ann Phytopath Soc Jap 52, 235-247.

ANONYMOUS, 2005. Agricultural structure (production, price, value). State Institute of Statistics Printing Division, Ankara, Turkey. [In Turkish].

ASHER M.J.C., THOMPSON K., 1987. Rhizomania in Europe. British Sugar Beet Rev 55, 24-28.

BOHN H.L., McNEAL B.L., O'CONNOR G.A., 1985. Soil chemistry, 2nd ed. John Willey & Sons, Inc, NY, USA.

BRADY N.C., 1974. The nature and properties of soils, 8th ed. MacMillan Publishing Co, Inc, NY, USA.

BRUNT A.A., RICHARDS K.E., 1989. Biology and molecular biology of furoviruses. Adv Virus Res 36, 1-32.

CANOVA A., 1967. «Rhizomania», a complex disease of sugar beet in Italy. Proc Int Symp Sugar Beet Prot, Novi Sad. pp. 381-382.

CLARK M.F., ADAMS A.N., 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J Gen Virol 34, 475-483.

GRÜNEWALD I., HORAK I., SCHLÖSSER E., 1983. Rhizomania. III. Verbreitung im Hessischen und im Raum Worms sowie Beziehungen zum Boden-pH und zur Fruchtfolge. Zuckerind 108, 650-652. [In German].

HILLMANN U., 1984. Neue Erkenntnisse über die Rizomania an Zuckerrüben mit besonderer Berücksichtigung bayerischer Anbaugebiete. Dissertation. Universitat Giessen. [In German].

IVANOVIC M., 1984. *Polymyxa betae* Kesk., as a parasite of sugar beet and vector of BNYVV. PhD thesis. Fac Agr, University of Belgrade, Serbia. 134 pp.

KASTIR U., WIDERA A., 1988. First results on the occurrence of the vectors of barley yellow mosaic virus and beet necrotic yellow vein virus in the GRD and on their multiplication on their host plants. Arch Phytopathol Pflanzenschutz 24, 93-101.

KESKIN B., 1964. *Polymyxa betae* n. sp., ein Parasit in den Würzeln von *Beta vulgaris* Tournefort, besonders wahrend der Jugendentwicklung der Zuckerrübe. Arch Microbiol 49, 348-374. [In German].

KOENIG R., PLEIJ C.W.A., BÜTTNER G., 2000. Structure and variability of the 3' end of RNA 3 of Beet soil-borne pomovirus – a virus with uncertain pathogenic effects. Arch Virol 145, 1173-1181.

KOENIG R., LESEMANN D.E., 2005. Genus *Pomovirus*. In: Virus taxonomy: classification and nomenclature of viruses (Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U., Ball L.A., eds). 8th Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA, USA. pp. 1033-1038.

KUTLUK YILMAZ N.D., ERKAN S., IKIZ F., 2003. Effects of soil properties on the occurrence of rhizomania disease in sugar beet cultivars. J Turk Phytopath 32(1), 45-52.

KUTLUK YILMAZ N.D., YANAR Y., GÜNAL H., ERKAN S., 2004. Effects of soil properties on disease occurrence of *Beet Necrotic Yellow Vein Virus* and *Beet Soilborne Virus* on sugar beet in Tokat, Turkey. Plant Pathol J 3(2), 56-60.

MEUNIER A., SCHMIT J.F., STAS A., KUTLUK N., BRAGARD C., 2003. Multiplex reverse transcription-PCR for simultaneous detection of *Beet Necrotic Yellow Vein Virus*, *Beet Soilborne Virus*, and *Beet Virus Q* and their vector *Polymyxa betae* KESKIN on sugar beet. Appl Environ Microbiol 69(4), 2356-2360.

MOUHANNA A.M., NASRALLAH A., LANGEN G., SCLÖSSER E., 2002. Surveys for *Beet Necrotic Yellow Vein Virus* (the cause of rhizomania), other viruses, and soil-borne fungi infecting sugar beet in Syria. J Phytopathol 150, 657-662.

PRILLWITZ H., SCHLÖSSER E., 1992. Beet soil-borne virus: occurrence, symptoms and effect on plant development. Med Fac Landbouw Univ Gent 57/2a, 295-302.

SOIL SURVEY STAFF, 1993. Soil survey manual. USDA Handbook No. 18, Washington.

TURINA M., RESCA R., RUBIES-AUTONELL C., 1996. Surveys of soilborne virus diseases of sugar beet in Italy. Proc 3rd Symp Int Work Group Plant Viruses Fungal Vectors, Dundee, Scodland, August 6-7. pp.121-124.

UI T., 1973. A monographic study of rhizomania of sugar beet in Japan. Proc 13th Research Meeting of the Sugar Beet Technological Cooperative of Japan 1, 233-265.