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INTERACTIONS BETWEEN *BEET NECROTIC YELLOW VEIN VIRUS* AND *BEET SOILBORNE VIRUS* IN DIFFERENT SUGAR BEET CULTIVARS

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ABSTRACT: In the present study, non-infested soil, naturally infested soils of aviruliferous *Polymyxa betae* and viruliferous *P. betae* carrying *Beet necrotic yellow vein virus* (BNYVV) and *Beet soilborne virus* (BSBV) were obtained from sugar beet fields during surveys in central and northern parts of Turkey in 2005. These soils, alone and in combination were compared to non-infested soil for their effects on plant fresh weight and virus content by using partially resistant (cv. Leila) and susceptible (cv. Arosa) varieties to the rhizomania disease (caused by BNYVV). Soils infested with *P. betae*, carrying one and both viruses, showed significantly reduced fresh weight of seedlings, and aviruliferous *P. betae* significantly decreased sugar beet growth. Partially resistant cultivar to rhizomania did not show resistance to BSBV in single infection under controlled room conditions. However, ELISA result for BSBV in mixed infection was negative in the partially resistant cultivar to rhizomania. Also, the ELISA absorbance value of BNYVV in the susceptible cultivar to rhizomania was found to be lower in mixed infection than in single infection.

Key Words: Sugar beet, rhizomania, *Beet soilborne virus*, *Polymyxa betae*, ELISA, bait plant.

FARKLI ŞEKER PANCARI ÇEŞİTLERİNDE *BEET NECROTIC YELLOW VEIN* VIRUS VE *BEET SOILBORNE VIRUS* ARASINDAKİ İLİŞKİLER

ÖZET: Bu çalışmada, Türkiye'nin iç ve kuzey bölgelerinde şeker pancarı tarlalarında 2005 yılında yapılan sürvey çalışmaları ile aviruliferous (virüs taşımayan) *Polymyxa betae*, *Beet necrotic yellow vein virus* (BNYVV) ve *Beet soilborne virus* (BSBV)'ü içeren *P. betae* ile doğal olarak bulaşık ve bu etmenler ile bulaşık olmayan topraklar elde edilmiştir. Rhizomania hastalığına (BNYVV tarafından sebep olunan) kısmi dayanıklı (cv. Leila) ve hassas (cv. Arosa) şeker pancarı çeşitleri kullanılarak, tek ya da karışık enfeksiyona sahip bu topraklar, bulaşık olmayanlar ile virüs içerikleri ve yaş bitki ağırlıkları yönünden karşılaştırılmıştır. Bir ya da her iki virüsü birden taşıyan *P. betae* ile enfekteli topraklardaki fidelerin yaş bitki ağırlıklarının önemli derecede sınırlandığı ve virüs içermeyen *P. betae* ile bulaşık topraklarda ise şeker pancarı gelişiminin önemli ölçüde azaldığı görülmüştür. Rhizomania'ya kısmi dayanıklı olan çeşit, kontrollü şartlar altında tek enfeksiyonda BSBV'ye dayanıklılık göstermemiştir. Bununla birlikte, bu çeşitte karışık enfeksiyonlarda BSBV'nin ELISA değeri negatif olmuştur. Rhizomania'ya hassas çeşitte ise tek BNYVV enfeksiyonu ile karışık enfeksiyon kıyaslandığında, BNYVV'nin ELISA absorbans değerinin karışık enfeksiyonda BNYVV'nin tekli enfeksiyonuna göre daha düşük olduğu belirlenmiştir.

Anahtar Sözcükler: Şeker pancarı, rhizomania, *Beet soilborne virus*, *Polymyxa betae*, ELISA, tuzak bitki.

1. INTRODUCTION

Beet necrotic yellow vein virus (BNYVV) and *Beet soilborne virus* (BSBV) are important soilborne viruses in the production areas of sugar beet (*Beta vulgaris* L.) in Turkey. BNYVV is member of the genus *Benyvirus* (Tamada, 1999) while BSBV is classified in the genus *Pomovirus* (Koenig and Lesemann, 2005). Both viruses are transmitted by the plasmodiophorid vector *Polymyxa betae* Keskin (Ivanovic et al., 1983; Asher and Thompson, 1987; Prillwitz and Schlösser, 1992).

BNYVV is responsible for rhizomania disease of sugar beet, was first reported in Turkey in 1988 (Vardar and Erkan, 1992), and then it has spread throughout most provinces where sugar beet is grown (Özgör, 2003).

The 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). Also, BSBVcan cause a yield loss of up to 70% (Prillwitz and Schlösser, 1992; Prillwitz, 1993).

BNYVV and BSBV are closely related pathogens and these viruses often occur together in the same field (Prillwitz and Schlösser, 1992; Turina et al., 1996; Mouhanna et al., 2002; Meunier et al., 2003; Kutluk Yilmaz et al., 2005). Both viruses can survive within thick-walled resting spores of *P. betae* for several years in soil (Abe and Tamada, 1986, Prillwitz and Schlösser, 1992). Therefore, partially resistant cultivars have been the only economical way of controlling rhizomania disease. A number of cultivars with varying degrees of resistance or tolerance to BNYVV have been developed and presently grown in rhizomania infested regions. Resistance to rhizomania in most sugar beet cultivars is controlled by the dominant gene *Rz* (Wisler et al., 1999). The resistance of such cultivars has been reported to be caused by a restriction of virus multiplication and/or translocation in the roots (Scholten et al., 1994). Some of these cultivars have shown a variable response in yield when grown in different countries and/or under different conditions (Heijbroek et al., 1999). Besides

this, wild beet accessions like *Beta vulgaris* spp. *maritima* WB41 and WB42 served as sources for additional resistance genes *Rz2* and *Rz3*, respectively (Lewellen et al., 1987; Whitney, 1989).

The aim of this experiment was to determine the effects of *P. betae*, BNYVV, BSBV, alone and in combination, on growth and the virus titers of partially resistant and susceptible sugar beet cultivars to the rhizomania disease in naturally infested soils.

2. MATERIALS AND METHODS

2. 1. Soil Samples

Aviruliferous *P. betae*, non-infested, BNYVV and BSBV-infested soils were collected from sugar beet fields during surveys in central and northern parts of Turkey in August and September 2005 (Table 1). After soil samples were dried at room temperature in a laboratory and sieved through 2 mm screens, some physical and chemical soil properties were determined as follows; particle size distribution by hydrometer method (Day, 1965), soil reaction, pH, 1:1 (w:v) soil:water suspension by pH meter, electrical conductivity (EC_{25^oC}) in the same suspension by EC meter, organic matter (OM) content by Walkley-Black method, exchangeable cations by ammonia acetate extraction (Kacar, 1994), and according to Bower (US Salinity Lab. Staff, 1954). Locations, physical and chemical properties of the soil samples were given in Table 1.

Before this experiment, all virus-infested and virus free soils were tested prior to this study to confirm the presence or absence of the desired viruses. For this reason, roots from sugar beet plants grown in these soil samples containing cystosori of *P. betae* isolates were stained with lactophenol containing 0.1 % acid fuchsin and were detected by light microscope (Leica, Sweden) (Abe and Tamada, 1986). Then, seedlings were tested for BNYVV and BSBV by ELISA. Non-infested soil was autoclaved prior to use.

2. 2. Bait Plant Technique

A rhizomania-susceptible cultivar (cv. Arosa) and a rhizomania-partially resistant cultivar (cv. Leila) were used in this experiment. This study consisted of following treatments: (i) non-infested soil, (ii) aviruliferous *P. betae* infested soil, (iii) BNYVV-infested soil, (iv) BSBV-infested soil, (v) BNYVV- and BSBV-infested soil. In this trial, pots were arranged on controlled room benches in a randomized complete block desing with three replications for each treatment.

Each of the soil samples were mixed in equal parts with autoclaved sand to facilitate ease of root removal of bait plants at harvest. Then, approximately 10 sugar beet seeds were sown in 300 mL each plastic pots containing mixed soil. The plants were grown under controlled conditions with a 16-h photoperiod at 20°C (night) and 23°C (day). The pots were watered directly as needed. The bait plants were harvested weekly for 6 weeks starting 2 weeks post emergence of seedlings. In the harvest, the number of plants per pot was determined, their roots were carefully washed in running top water, cut and weighted. After the fresh weight was obtained, the combined roots of each pot were tested for BNYVV and BSBV by enzyme linked immunosorbent assay (ELISA).

2. 3. Serological Tests

The roots of sugar beet plants were tested for the presences of BNYVV and BSBV by ELISA. The double-antibody sandwich (DAS)-ELISA was used to determine BNYVV infection by using antiserum supplied by Sediag Biochemica (France). DAS-ELISA was performed according to Clark and Adams (1977), except that extraction buffer included 0.1% nonfat dry milk instead of bovine serum albumin (Arif et al., 1994).

A triple-antibody sandwich (TAS)-ELISA were used to test for BSBV. TAS-ELISA was performed according to instructions of the antiserum producer (Adgen, England). The plates were measured using a microplate reader (Tecan Spectra II, Grödig/ Salzburg, Austria). All reported ELISA values were taken after 2 hr substrate incubation and samples were considered positive when the absorbance at A_{405} nm values exceeded the mean of the healthy controls by at least factor of three (Wisler et al., 2003).

2. 4. Statistical Analyses

Data obtained from each individual pot were used in statistical analyses. Analyses of variance were run using SPSS 11.0 statistical software programme (SPSS Inc., Cary, NC, USA). Then, LSD Multiple Range Test was used to reveal if difference is present. Significance was evaluated at $P<0.01$ or $P<0.05$ for all tests.

3. RESULTS

All soil samples in this experiment showed similar properties each other according to the results that can be summarized as follows; textural classes of the soils are clay, slightly alkaline in pH, moderate in organic matter, non saline according to EC value (Soil Survey Staff., 1993) (Table 1).

F values and significant levels of the variance analyses for ELISA absorbance values and total plant fresh weight are shown in Table 2. There were significant differences among cultivars, soil treatments, harvest date, and their interactions for the BNYVV ELISA values. Significant differences among the BSBV ELISA absorbance values occurred for soil treatments, interactions of cultivar x soil treatments and cultivar x harvest date under controlled room conditions. There were also significant differences for the total plant fresh weight among cultivars, soil treatments, harvest date, interaction of cultivar x soil treatment and soil treatments x harvest date (Table 2).

The total plant fresh weight was significantly decreased by single and mixed infections of BSBV, BNYVV and *P. betae* when compared with the non-infested treatment (Table 3). Additionally, infection by aviruliferous *P. betae* caused a significant reduction in plant weight in both partially resistant and susceptible cultivars (Figure 1). The *P. betae* cystosori in the roots of BNYVV-infected sample is shown in Figure 2.

Table 1. Origines and physicochemical properties of aviruliferous *P. betae*, non-infested, BNYVV and BSBV-infested soil used in this study.

	Non-infested soil	Aviruliferous *P. betae* infested soil	BNVYY infested soil	BSBV infested soil	BNYVV+ BSBV infested soil
Soil Locations	Kocakavak/ Carsamba/ Samsun	Durakbasi / Carsamba / Samsun	Kiyikavurgali / Kizilirmak / Cankiri	Derekoy / Havza / Samsun	A. Narli / Vezirkopru / Samsun
Clay, %	58.14	47.66	48.07	42.83	48.06
Silt, %	28.70	36.15	30.09	25.34	25.39
Sand, %	13.16	16.88	21.83	31.83	26.54
Texture class	Clay	Clay	Clay	Clay	Clay
pH (1:1)	7.75	7.75	7.77	7.73	7.88
EC, dSm^{r}	0.598	0.583	0.850	0.367	0.615
CEC, cmol kg^{-1}	21.25	35.49	37.12	27.15	26.43
Organic matter, %	3.27	3.17	4.19	2.66	3.81
$CaCO_3$, %	5.22	8.52	7.00	2.62	18.75
Na, cmol kg^{-1}	0.281	0.512	0.540	0.081	0.435
K, cmol kg^{-1}	0.410	0.641	0.973	0.492	0.949
Ca, cmol kg^{-1}	35.31	31.84	29.40	28.5	29.66
Mg, cmol kg^{-1}	6.46	11.77	11.66	5.66	7.83

Table 2. *F* values from variance analyses for ELISA absorbances values and total fresh weight.

		F values		
Source	df	BNYVV	BSBV	Weight (g)
Cultivar	1	210.62**	1.01 ns	17.58**
Soil treatment	4	152.66**	48.33**	259.34**
Cultivar x Soil treatment	4	84.41**	5.47**	10.30**
Harvest date	5	4.26**	1.91 ns	28.83**
Cultivar x Harvest date	5	3.88**	3.06*	1.94 ns
Soil treatment x Harvest date	20	11.20**	2.00 ns	21.83**
Cultivar x Soil treatment x Harvest date	20	7.83**	2.38 ns	1.98 ns
Error	118			

ns: not significant; * and ** indicate significance at the $P \leq 0.05$ and 0.01 levels, respectively, according to *F* test.

Table 3. Main effect treatment means for ELISA values for BNYVV, BSBV and total fresh weight evaluated for two cultivars over five soil treatments and six weekly harvest dates*

Treatments	BNYVV	BSBV	Weight (g)
Grand mean	0.567	0.444	0.520
Cultivar			
Susceptible (cv. Arosa)	0.809^{a}	0.464	0.572^{a}
Partially Resistant (cv. Leila)	0.325^{b}	0.423	0.467^{b}
Soil treatment			
Noninfested	0.235^{c}	0.223^{c}	1.319^{a}
Polymyxa betae	0.235^{c}	0.223^{c}	0.416^{b}
BSBV	0.235^{c}	0.887^{a}	0.254^{c}
BNYVV	1.166^{a}	0.223^{c}	0.337^{bc}
Mixed infection	0.965^{b}	0.662^{b}	0.271^{c}
Harvest date			
Week 1	0.528^{ab}	0.361	0.264^{d}
Week 2	0.572^{ab}	0.409	0.383^{c}
Week 3	0.641^{a}	0.483	0.530^{b}
Week 4	0.586^{a}	0.398	0.646^{a}
Week 5	0.654^{a}	0.461	0.690^{a}
Week 6	0.423^{b}	0.549	0.604^{ab}

- Means within the colums followed by a different letter are significant at $P \leq 0.01$ according to LSD multiple range test.

Table 4. ELISA values for BNYVV and BSBV in single and mixed infections rhizomania-partially resistant and -susceptible sugar beet cultivars*

	Susceptible		Partially Resistant	
Soil treatment	**BNYVV**	**BSBV**	**BNYVV**	**BSBV**
Noninfested	0.235^{ef} (-)	0.223^{f} (-)	0.235^{ef} (-)	0.223^{f} (-)
Polymyxa betae	0.235^{ef} (-)	0.223^{f} (-)	0.235^{ef} (-)	0.223^{f} (-)
BNYVV	1.885^{a} (+)	0.223^{f} (-)	0.436^{de} (-)	0.223^{f} (-)
BSBV	0.235^{ef} (-)	0.794^{c} (+)	0.235^{ef} (-)	0.980^{c} (+)
Mixed infection	1.447^{b} (+)	0.860^{c} (+)	0.484^{d} (-)	0.469^{d} (-)
		LSD= 0.2095		

* Means within columns followed by a different letters are significant at *P≤0.01* according to LSD multiple range test. Ratios of ≥ 3 times the healthy ELISA absorbance mean are considered positive (+).

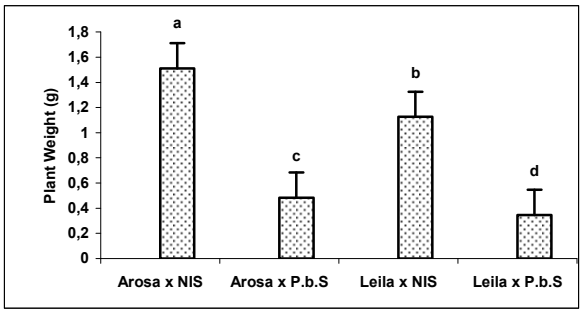

Figure 1. Effects of aviruliferous *P. betae*-infected soil on plant weight in rhizomania- susceptible (cv. Arosa) and rhizomania-partially resistant (cv. Leila) sugar beet cultivars. Means followed by a different letters are significant at *P≤0.05* according to LSD multiple range test (NIS: non-infested soil; P.b.S: *P. betae* infested soil).

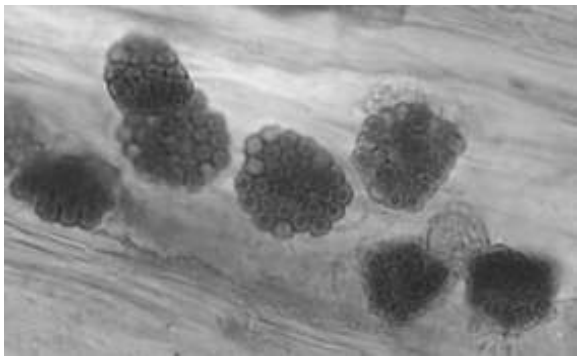

Figure 2. *P. betae* cystosori in the root tissues of BNYVV infected sample.

The absorbance values of BNYVV for single and mixed infection were 5 and 4.1 times higher than that of the healthy mean, respectively. Similarly, ELISA values of BSBV in single infection were almost 4 times greater than of healthy controls and 3 times greater in mixed infection (Table 3). There were slightly significant differences for the BNYVV ELISA values in six harvest dates, however there were not significant differences for the BSBV ELISA values (Table 3).

Rhizomania-partially resistant cultivar did not show resistance to BSBV in single infected plants under controlled room conditions (Table 4). In the partially resistant cv. Leila, titer of BSBV was significantly increased in single infected plants. However, the ELISA value in the mixed infection for BSBV was low in the partially resistant cultivar to rhizomania. But, the ELISA values for BSBV were not changed either in single or mixed infections in the rhizomania susceptible cultivar and their absorbance values were above the positive scoring threshold (Table 4).

In the absence of BSBV, BNYVV value had the highest titer in BNYVV-susceptible plants. For the cv. Leila, BNYVV ELISA values were negative for all treatments (Table 4). However, ELISA values of BSBV-infected plants never attained higher titers than the titers of BNYVV in mixed infection with either rhizomania susceptible or partially resistant cultivars. Additionally, the ELISA value of BNYVV in mixed infection in susceptible cultivar was lower than that of BNYVV in single infection (Table 4).

4. DISCUSSION

P. betae is not truly considered as pathogens but as vector of sugar beet viruses, and it plays crucial role in the epidemiology of viral diseases. Thus, little is known about the incidence and distribution of *P. betae* in the absence of BNYVV and BSBV. However, our previous study in sugar beet fields in central and northern parts of Turkey showed that percentage of soil samples with *P. betae* cystosori infestation was 91.25%. Besides this, 127 soil samples were infested with viruliferous *P. betae* cystosori (58%) (Kutluk Yilmaz et al., 2005). The previous study showed that aviruliferous *P. betae* is common in sugar beet fields in Turkey and a negative impact on sugar beet yield could be expected, despite many samples having neither BNYVV nor BSBV. Indeed, infection of aviruliferous *P. betae* caused at least 3 times more reduction in plant weight for both partially resistant and susceptible cultivars in this study comparing to non-infested soil (Figure 1). In contrast, Tamada et al. (1990) did not find any effect on root weight by virus-free *P. betae* when plants were grown for 40 days in a climate room followed by 3 months in a greenhouse. On the other hand, Blunt et al. (1991) reported that *P. betae*, which was assumed to be virus-free, reduced dry weight of roots of young plants. Gerik and Duffus (1988) found that three out of six isolates of *P. betae*

reduced lateral root weights compared to that in non-infested soil in a 2-months assay, whether or not the isolates were viruliferous. Similarly, Wisler et al. (2003) have emphasized that aviruliferous *P. betae* infection was caused a significant reduction in seedling weight for both BNYVV-susceptible and BNYVV-resistant cultivars, compared with sugar beet grown in non-infested soil in greenhouse studies. Conflincting studies on root weight could be due to fact that isolates of *P. betae* might be differ in aggressiveness.

This experiment also showed that there was no evidence of resistance to *P. betae* in partially BNYVV resistant cultivar. This confirms previous evidence that the resistance conferred by genes is expressed specifically against the BNYVV (Scholten et al., 1996). Also, evidence for the greater role of BNYVV than of *P. betae* in causing root yield reduction in the field is derivered from the results of a field trial with different initial inoculum levels of BNYVV (Tuitert and Hofmeester, 1992). Besides this, the total plant fresh weights were significantly decreased by single and mixed infections of BSBV and BNYVV (Table 3). In parts of the BSBV genome, there are considerable sequence variabilities among different isolates of the same soil sample. It might be explained why there have been different estimations of potential yield reduction between 0 and 70% (Koenig et al., 2000). Based on the these data, BSBV can be considered to have much impact on plant weight as well as BNYVV.

In BSBV alone treatment, the ELISA values for BSBV were high levels in both partially resistant and susceptible rhizomania varieties. Rhizomania partially resistant cultivar did not affect resistance to BSBV in sugar beet under controlled room conditions. In the absence of BSBV, BNYVV attained high titers in BNYVV-susceptible plants, but low titers in -partially resistant plants. Indeed, the effect of the partial resistance in Leila could be seen clearly, by considering the titer of ELISA for BNYVV in this study. In previous studies, the virus was also not often detectible or its concentration was very low in partially resistant genotypes (Bürcky and Büttner, 1988; Koenig and Stein, 1990; Sayama et al., 1991). Additionally, resistance in this original 'Holly' genotype has been shown to affect the multiplication of BNYVV in the lateral roots (Scholten et al., 1996) which are the site of initial infection, as well as reducing subsequent migration of the virus into the top root (Heijbroek et al., 1999).

When two viruses infected same plant simultaneously, disease symptoms may be increased, decreased, or unaffected. BNYVV and BSBV are often found in the same field, sometimes infecting same plant. Under these conditions, the potential for interaction between virus species is greatly increased (Rush, 2003). Prillwitz and Schlösser (1993) demonstrated that pre-infection with BSBV could reduce virus titer and attenuate symptom development in sugar beets subsequently challenged with BNYVV. So, damage from rhizomania was 50% less in protected beets than in non-protected control plants. Because, BSBV needs lower temperature than BNYVV, it was suggested that early infection by BSBV in the field might reduce incidence and severity of rhizomania. Similar results were obtained in the studies investigating interactions between BNYVV and another soilborne virus, *Beet soilborne mosaic virus* (BSBMV) (Mahmood and Rush, 1999). Using field soils naturally infested with BNYVV or BSBMV as inoculum, Wisler et al. (2003) found that in the absence of BNYVV, BSBMV always attained high titers in plants susceptible or resistant to BNYVV. In the absence BSBMV, BNYVV attained high titers in BNYVV susceptible plants, but low titers in resistant plants. However, when the soils with either BNYVV or BSBMV were mixed, BSBMV never attained high titers in either BNYVV susceptible or resistant plants. It was concluded that BNYVV was able to out-compete BSBMV or suppress BSBMV in mixed infections.

In this study, ELISA absorbance value in mixed infection for BSBV was significantly reduced in the rhizomania partially resistant variety in which BNYVV level was low. In this case, the ELISA values for BNYVV were not significantly changed either single or mixed infections. In the susceptible cultivar to rhizomania, the BNYVV ELISA absorbance value was lower in mixed infection compared with BNYVV alone. However, the titer of BSBV in the susceptible plants was not significantly changed in mixed infection compared with BSBV alone. There may be several reasons for low BSBV content in the roots of rhizomania-partially resistant plant in mixed infection. For example, BNYVV-infected zoospores of *P. betae* might be more aggressive than BSBV-infected zoospores of *P. betae*. On the other hand, there may be compatition for infection sites by viruliferous *P. betae*. Because, there are only fixed number of possible infection sites on the root system (Rush, 2003; Wisler et al., 2003). Whether BNYVV or BSBV predominates largely depens on environmental conditions and inoculum densities of the two viruliferous populations of *P. betae*. In this study, the mean absorbance value of BNYVV was 1. 885 whereas that of BSBV was 0.794 in single infection for rhizomania susceptible cultivar. Same infested soil was used in this experiment. Therefore, inoculum density of BNYVV carrying populations of *P. betae* was probably higher. Similarly, it was reported that the virus with the highest inoculum density in naturally infected soil samples usually colonize more in the root system, and the virus that infects the root system first usually reaches high levels (Rush, 2003).

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