The effects of Soilborne Sugar Beet Viruses and Their Vector *Polymyxa betae* Keskin on Symptom Expression and Root Weight of Sugar Beet

Nazlı Dide KUTLUK YILMAZ Miray ARLI SOKMEN

Ondokuz Mayis University, Faculty of Agriculture, Department of Plant Protection 55139, Samsun, Turkey.

ABSTRACT

Sugar beet bait plants grown in soil samples collected in central and northern parts of Turkey were previously found to be infected with *Beet necrotic yellow vein virus* (BNYVV) and *Beet soilborne virus* (BSBV) through enzyme-linked immunosorbent assay (ELISA), and with *Polymyxa betae* using direct coloration method of bait plant root tissues. The effects of BNYVV, BSBV and *P. betae*, alone and in combination on the growth of sugar beet seedlings were investigated by considering root weight and the mean ELISA values. The result from the statistical analysis showed that total fresh root weight was negatively correlated with BNYVV, BSBV and BNYVV+BSBV infections, but not with aviruliferous *P. betae* infection. Additionally, the most common symptom after six weeks growing periods was chlorosis for single BNYVV (46%), BSBV (28.1%) and double BNYVV and BSBV (58.5%) infections. Also, the number of plants with no visible symptoms in single BSBV infections (60.3%) was higher than that obtained in single BNYVV infections (39.5%). Most of plants (65.3%) infected with aviruliferous *P. betae* populations had no visible symptom, however, 24.7% of the plants showed chlorosis. This study indicated that various populations of both sugar beet viruses and *P. betae* could be present in the region, and BNYVV seems to provoke chlorosis in both single and double infections.

Key words: Bait plant, BNYVV, BSBV, sugar beet

INTRODUCTION

Among all the sugar beet (*Beta vulgaris* L.) virus diseases, rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV) and transmitted by the plasmodiophorid vector *Polymyxa betae* Keskin (Fujisawa and Sugimoto, 1976), is one of the most devastating agents (Whitney and Duffus, 1998). This virus disease causes large economic losses by reducing yields up to 100% (Whitney and Duffus, 1998) and decrease the sugar content from 16-18% to less than 7% (Bongiovanni and Lanzoni, 1964). Typical symptoms are characterized by a massive proliferation of lateral roots beneath an enlarged crown, constricted growth of tap root and severe stunting of whole plant (Rush and Heidel, 1995). Resting spore form of the vector can retain the virus in soil for more than 15 years (Abe and Tamada, 1986). Other soilborne virus, BSBV has a similar vector and host ranges that infect *Chenopodiaceae* family like BNYVV (Asher and Thompson, 1987; Prillwitz and Schlösser, 1992). BSBV is morphologically similar to BNYVV but serologically different. Unlike BNYVV, different serological types of BSBV exist (Mouhanna et al., 2002). 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; Kutluk Yilmaz and Arli Sokmen, 2010). These viruses have been reported frequently in Turkey since the 1990s (Vardar and Erkan, 1992; Erdiller and Özgür, 1994; Kutluk Yilmaz and Yanar, 2001; Meunier et al., 2003; Kutluk Yilmaz et al., 2004; Özer and Ertunç, 2005; Kutluk Yilmaz et al., 2007; Kutluk Yilmaz and Arli Sokmen, 2010).

The aim of this investigation was to determine the effects of BNYVV, BSBV and their vector *P. betae* on symptom expression and root weight of sugar beet plants.

MATERIALS and METHODS

Soil material: Soil samples (n= 510) were collected mainly from fields in the sugar beet-growing areas of Samsun, Amasya, Tokat, Corum, Yozgat, Cankırı and Kastamonu provinces in central and northern parts of Turkey in 2004-2005. In each field, composite soil sampling was obtained by randomly taking five to twelve samples from the upper surface of 20 cm of the soil. All soil samples were dried at room temperature, ground to dust and diluted to 1: 1 vol/vol with autoclaved sand. A total of 510 mixed soil samples were filled in 300 mL pots, and there were two replicates for each soil sample. Then, 10 sugar beet seeds of the rhizomania-susceptible cultivar (cv. Arosa) were sown as bait plants in each pot. The plants were grown under controlled conditions with a 12-h photoperiods at 20°C (night) and 23°C (day). All plants were watered generously every other day with Hoagland’s solution. After six weeks of growth, leaf symptoms on bait plants were recorded. These conditions were chosen on the basis of data from similar, previous glasshouse-based experiments with *P. betae* transmitted benyviruses of sugar beet (Asher and Blunt, 1987; Rush, 2003). In the harvest, the number of plants per pot was determined, their roots were carefully washed in running top water, cut and weighted. After that the combined roots of each pot were divided into two parts. One was used to test for the presence of viruses by ELISA and the other for *P. betae*. Plant material was stored at -20°C until used.

Some physical and chemical characteristics of the soil samples used in this study were previously determined. Out of 510, 144 soil samples were randomly selected from each provinces without considering disease status. The same soil samples were also used in the concurrent parallel study which has already been published (Kutluk Yilmaz et al., 2010). 77% of the samples were in fine textural class (clay and clay loam), 23% in coarse textural class (loam, sandy loam or sandy clay). Of the soils, 44.4% were low, 41.7% in moderate, 11.8% in high and 2.7% in very high organic matter. According to the soil reaction (pH), 3.4% were slightly acid, 2.8% neutral, 41.7% slightly alkaline and 50.7% moderately alkaline. Of the soils, 16.7% had low lime contents, 69.4% were in moderate, 12.5% in high and 2.1% in very high lime contents (Soil Surv. Manual, 1993).

ELISA tests: The double-antibody sandwich (DAS)-ELISA tests for BNYVV and a triple-antibody sandwich (TAS)-ELISA tests for BSBV were performed using Sediag Biochemica (France) and Adgen (Scotland) kits following the manufacturer's instructions, respectively. The optical density was measured at 405 nm using the Tecan Spectra II (Grödig, Salzburg, Austria) from 30 to 120 min, after the addition of the *p*-nitrophenyl phosphate.

A sample was considered as positive or infected when absorbance value at 405 nm was more than two times the mean of the negative control (Meunier et al., 2003).

Detection of *P. betae*: The root samples were stained with lactophenol containing 0.1% acid fuchsin and examined under a light microscope (Leica, Switzerland) to detect the presence of *P. betae* cyctosori (Abe and Tamada, 1986).

Statistical analysis: Data obtained from each individual pot were used in statistical analyses. Pearson correlation analysis was performed to determinate the relationship between root weight and ELISA absorbance values of BNYVV and BSBV, either alone or in combination. Also, Kendall's tau rank correlation analysis was used to determine the relationship between *P. betae* and either ELISA absorbance values or root weight since the data of *P. betae* was nonparametric. In order to reveal how the soilborne diseases affect root weight, regression analysis was applied. The regression equation was calculated based on the following formula;

$$\hat{y} = a + b_1 x_1 + b_2 x_2 + b_3 x_3$$

which is $\hat{y}$: dependent variable (root weight of i^{th} plant); x_i is independent variables (BNYVV, BSBV and *P. betae*); a is an intercept, and b_i is regression coefficients.

In addition to these analysis, one-way ANOVA was performed in a completely randomized design: $\hat{Y}_{ij} = \mu + \alpha_i + e_{ij}$ where $\hat{Y}_{ij}$ is observation values (absorbance values of BNYVV and BSBV and root weight), μ is the overall mean, α_i is the effect of the it parameter (symptom) and e_{ij} = residual error. Then, Duncan Multiple Range Test was used to reveal if difference is present. All the computational work was performed by MINITAB software (Minitab V. 13.20, 2000).

RESULTS

Symptoms expression on bait plants: After six weeks growth period, sugar beet plants revealed different kinds of virus symptoms on leaves of rhizomania susceptible cultivar (cv. Arosa) in the bait plant tests (Table 1). Chlorosis was the most common symptom (32.7%) (Figure 1B) followed by leaf rolling and curling (6.3%) (Figure 1D), chlorosis+leaf distortion (2.4%) (Figure not shown), green vein banding and distortion (1.6%) (Figure 1A and 1E), chlorotic lesions (1.2%), necrotic spots (0.2%) and chlorotic+necrotic spot (0.2%) and root rot (0.6%) (Figures not shown). On the other hand, 54.9% of the samples which were found to be infected gave no visible symptom in bait plant tests (Figure 1C) (Table 1).

Table 1. Infection phenotypes were observed on leaves of rhizomania susceptible cultivar (cv. Arosa) in the bait plant tests after six weeks growth period for BNYVV, BSBV, BNYVV and BSBV and aviruliferous *P. betae*.

Infection phenotypes	BNYVV	BSBV	BNYVV+ BSBV	Aviruliferous *P. betae*	Healthy	Total
No visible symptom	15 (39.5)	73 (60.3)	21 (17.8)	106 (65.3)	65 (89.0)	280 (54.9)
Chlorosis	17 (46.0)	34 (28.1)	69 (58.5)	39 (24.7)	8 (11.0)	167 (32.7)
Leaf rolling+Curling	4 (10.8)	9 (7.4)	7 (6.0)	12 (7.6)	0 (0.0)	32 (6.3)
Chlorosis+Leaf distortion	2 (5.4)	1 (0.8)	9 (7.8)	0 (0.0)	0 (0.0)	12 (2.4)
Green vein banding+distortion	0 (0.0)	3 (2.5)	3 (2.6)	2 (1.3)	0 (0.0)	8 (1.6)
Chlorotic lesions	0 (0.0)	0 (0.0)	5 (4.3)	1 (0.6)	0 (0.0)	6 (1.2)
Necrotic lesions	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
Chlorotic+Necrotic lesions	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.2)
Root rot	0 (0.0)	0 (0.0)	3 (2.6)	0 (0.0)	0 (0.0)	3 (0.6)
Total	38 (7.5)	121 (23.7)	118 (23.1)	160 (31.4)	73 (14.3)	510

* Percentage infections are given in brackets.

As shown in Table 1, three distinct types of symptoms were observed for single BNYVV infection. Chlorosis was the most prevailing symptom (46%), followed by leaf rolling+curling (10.8%) and chlorosis+ leaf distortion (5.4%), and 39.5% of the plants gave no visible symptom. The results showed that there was no statistically difference in BNYVV titres (Figure 2A) and root weights (data not shown) according to phenotypes ($P<0.05$).

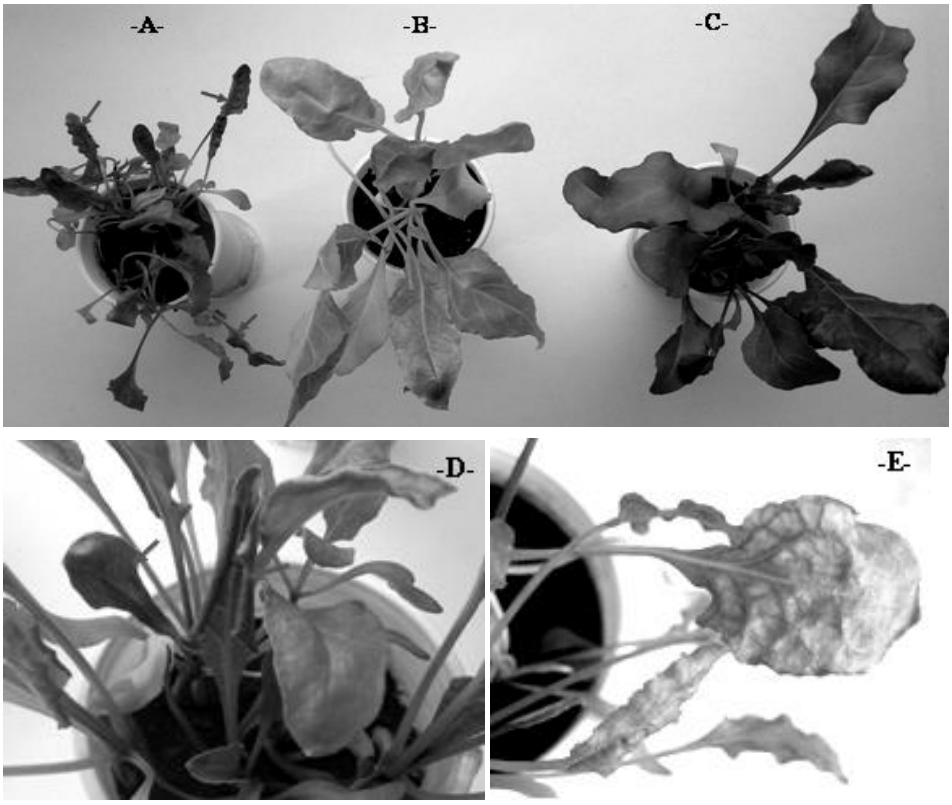

Figure 1. Infection phenotypes on leaves of the rhizomania susceptible cultivar (cv. Arosa) in the bait plant tests after six weeks growth period. These phenotypes were green vein banding and distortion (A and E), chlorosis (B), no obvious symptom (C), leaf rolling and curling (D).

In single infected samples with BSBV, asymptomatic phenotype is largely observed (60.3%) on sugar beet seedlings. In addition to that, some samples showed chlorosis (28.1%), leaf rolling+curling (7.4%), green vein banding+distortion (2.5%), necrotic lesions (0.8%) and chlorosis+leaf distortion (0.8%). ELISA value of BSBV infected sample with necrotic lesion was significantly lower (0.650) than those of leaf distortion (2.550) ($P<0.05$) but there was no any statistically difference found in ELISA values of other symptomatic plants (Table 1, Figure 2B). In addition to that, the mean root weights were not statistically different among samples expressing different symptoms (data not shown).

In BNVYY and BSBV mixed infection, chlorosis was the most common type of symptom. Of the 118 double infected plants, 69 had chlorosis (58.5%). The other phenotypes of mixed infection were no visible symptoms (17.8%), chlorosis+leaf distortion (7.8%), leaf rolling+curling (6%), chlorotic lesion (4.3%), green vein banding+distortion (2.6%), root rot (2.6%) and, chlorotic+necrotic lesion (0.9%)

(Figure not shown) (Table 1). The highest BSBV titres in mixed infection were obtained with curling+rolling type of symptom (2.66) and chlorotic+necrotic lesions (2.69) while the highest BNYVV titre was recorded with cholorotic+necrotic lesions (2.50). Interestingly, the mean absorbance values of BSBV (2.69) and BNYVV (2.50) in mixed infection for chlorotic and necrotic lesion type of symptom were statistically different than those of BSBV (0.87) and BNYVV (0.33) mixed infection for root rot symptom ($P<0.05$) (Figure 2C), but there was no difference at significant level among symptom types in terms of root weights.

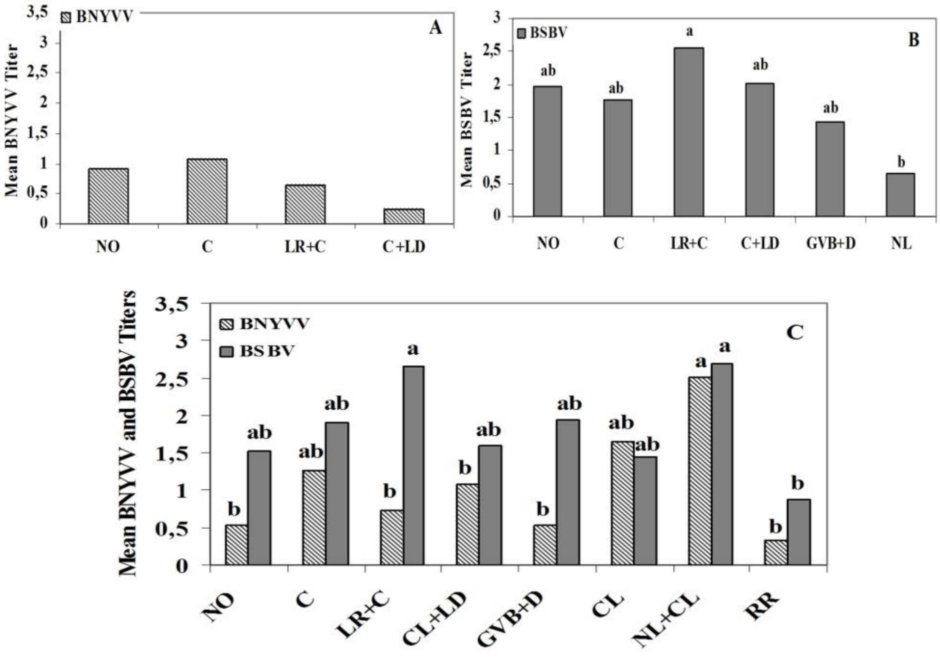

Figure 2. The mean titres of BNYVV **(A)**, BSBV **(B)** and BNYVV+BSBV **(C)** detected by ELISA according to phenotypes of bait plants. These phenotypes are no visible symptom (NO), chlorosis (C), leaf rolling and curling (LR+C), chlorosis and leaf distortion (C+LD), green vein banding and distortion (GVB+D), chlorotic lesions (CL), necrotic lesions (NL), chlorotic and necrotic lesions (NL+CL) and, root rot (RR).

Besides these virus infected samples, aviruliferous *P. betae* infected samples also showed different kinds of symptoms in bait plat tests. The most common symptom was chlorosis (24.7%), followed by leaf rolling+curling (7.6%), green vein banding+distortion (1.3%) and chlorotic lesion (0.6%) (Figures not presented). There was no visible symptom observed at 65.3% of the beet plants. The roots considered healthy according to ELISA and direct coloration methods did not generally showed any symptom (89%), the only eight samples had chlorosis (11%) (Table 1).

Interactions among BNYVV, BSBV and *P. betae* in sugar beet: In order to reveal whether the soilborne virus diseases affect root weight, correlation analysis was applied. The results from the statistical analysis showed that the total fresh root weights were reduced significantly by single and mixed infections of BSBV and BNYVV after six weeks inoculation compared to healthy samples. At the same time, single infections of BSBV or BNYVV may have more negative impact on root weight ($P<0.01$) than mixed infection of BNYVV+BSBV ($P<0.05$) (Table 2).

Table 2. Correlations on the mean ELISA values and root weights of plants infected with BNYVV, BSBV, BNYVV+BSBV, aviruliferous *P. betae*.

	BNYVV	BSBV	BNYVV+BSBV	Avr *P. betae*
BSBV	0.262**			
BNYVV+BSBV	0.542**	0.409**		
Avr P. betae	0.121**	0.237**	0.226**	
Root weight (g)	-0.274**	-0.173**	-0.092*	0.038

** Correlation is significant at 0.01 level, * correlation is significant at 0.05 level.

The formula below can be used to calculate the root weight (RW) of infected beet plants.

RW= 0.249 - 0.061 (BNYVV) - 0.020 (BSBV) + 0.053 (Avr *P. betae*).

Based on this equation, BNYVV and BSBV have negative but Avr *P. betae* has positive effect on root weight (RW).

DISCUSSION

According to the results of our study, BNYVV and BSBV can be considered to have impact on root weight. Reduction in taproot weight indicates great BNYVV and BSBV damages, but this weight decline could also be related to differences in nutrition contents of various soils or other soilborne pathogens. Under natural conditions, the root yield in rhizomania infected sugar beet can be reduced by 90% (Johansson, 1985). Besides this, Wisler et al. (2003) found that the plant weights measured for BNYVV and for mixed infections with BNYVV and *Beet soilborne mosaic virus* (BSBMV), another soilborne virus by transmitted by *P. betae*, were lower than those of the seedlings in the healthy treatments in greenhouse experiments. Similarly, Kutluk Yilmaz (2010) demonstrated that 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 soil treatment. Kaufmann et al. (1993) stated that BSBV reduced tap root weight of sugar beet cv. Rizor and Carla of about 20% in pot experiments. There is considerable variability among different BSBV isolates in the same soil sample. It might explain why different estimations of potential yield reduction between 0 and 70% have been found with BSBV in previous study (Koenig et al., 2000). However, in our study, aviruliferous *P. betae* did not have negative impact on root weight of beet seedlings. Similarly, 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. In contrast, 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. Wisler et al. (2003) have emphasized that aviruliferous *P. betae* infection caused a significant reduction in seedling weight for both BNYVV-susceptible and resistant cultivars, compared to sugar beet plants grown in non-infested soil in greenhouse studies. Also, Blunt et al. (1991) reported that *P. betae*, which was assumed to be virus-free, reduced dry weight of roots of young plants. Conflicting studies on root weight could be due to fact that isolates of *P. betae* might be differ in aggressiveness.

After six weeks growth, the certain types of symptoms on bait plants were dominant for different viruses. The most common symptom was chlorosis with single BNYVV (46%), BSBV (28.1%) and BNYVV+BSBV (58.5%) infections. Indeed, foliar chlorosis is typically associated with rhizomania (Rush et al., 2006). On the other hand, the number of plants with no visible symptoms in singly BSBV infected samples (60.3%) was higher than that in BNYVV-single infected samples (39.5%) (Table 1). Some researchers stated that 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). Rush et al. (2006) indicated that some BNYVV-infected plants never exhibit foliar symptoms. Besides this, soil samples containing aviruliferous *P. betae* also had symptom on 34.7% of bait plants. The reason for that could be the presence of different *P. betae* populations or the presence of other soilborne viruses simultaneously infecting bait plants. Unexpectedly, eight of uninfected plants also showed chlorosis type of symptom (11%) which may be caused by other biotic or abiotic disease factors. According to our results on soil characteristics, 92.4% of soils were slightly alkaline and moderately alkaline (pH 7.4-8.4). High soil pH and $CaCO_3$ contents may induce chlorosis by affecting soil nutrient sufficiency. Different plant nutrient deficiency such as nitrogen, potassium, calcium, magnesium, sulfur and iron etc. may cause chlorosis in different plant species (Aydemir and İnce, 1988).

There are very few published studies about different infection phenotypes of BNYVV populations on sugar beet. Pale green leaves and abnormal growth of lateral root after fungal inoculation on sugar beet (*Beta vulgaris* spp. *vulgaris*) were obtained by Tamada and Maruyama (2005). Tamada (2007) and Chiba et al. (2008) demonstrated that the resistant plants after rub inoculation displayed a range of symptoms from no visible lesions to necrotic lesions with very low levels of virus accumulation, whereas the susceptible plants showed bright yellow lesions and developed high levels of virus accumulation.

The factors affecting symptom expression for soilborne beet viruses are exactly unknown but might include sugar beet cultivar, virus strain and/or single nucleotide changes. Koenig et al. (2000) reported that there could be many mutants of BSBV which may differ in pathogenicity even in plantlets grown in the same soil. For BNYVV, three major pathotypes (A, B or P) have been reported (Kruse et al., 1994). Type A and B have 4 different RNAs while P-type also has a 5^{th} RNA. Type A is the most widespread (Kruse et al., 1994; Koenig et al., 1995), has been detected in Turkey (Kruse et al., 1994; Kutluk Yilmaz et al., 2007), however the B type seems to dominate in Germany and France, in some cases in Sweden (Koenig and Lennefors, 2000). From RNAs of BNYVV, the RNA-3-encoded p25 enables the systemic movement of the virus in sugar beet and is mainly responsible for symptom expression (Tamada et al., 1989; Jupin et al., 1992; Koenig et al., 1991) and root proliferation (Tamada et al., 1999). The amino acid tetrad in positions 67-70 of p25-coding region of RNA-3 has been found to be highly variable, especially in A type BNYVV sources collected at many different places in the world (Tamada et al., 2002; Schirmer et al., 2005; Kutluk Yilmaz, et al., 2007; Koenig et al., 2008). In our other study, partial nucleotide sequences of RNA-3 including p25 coding region of six different isolates from same regions, three BNYVV populations were determined as the amino acid tetrad in position 67-70 in terms of motifs of “ACHG”, “AHHG” and “VCHG”. The isolates with “ACHG” and “AHHG” gave chlorosis type of symptom on sugar beet seedlings in bait plant tests, whereas the isolate with ‘VCHG’ motif showed dark green vein banding and leaf distortion (unpublished data). Also, the severity of symptoms in sugar beet may be enhanced by the additional presence of RNA-5 and up to now, the fifth RNA was found frequently in Asia and in some specific locations in Europe (Tamada et al., 1996). In our previous study, RNA-5 was detected in samples having chlorosis and chlorotic lesions on leaves (Kutluk Yilmaz and Sokmen, 2007). This study indicates the presence of different BNYVV populations with various kinds of symptom appearance in the surveyed regions.

ÖZET

TOPRAK KÖKENLİ ŞEKER PANCARI VİRÜSLERİ VE VEKTÖR *POLYMYXA BETAE* KESKİN’İN ŞEKER PANCARINDA SİMPTOM OLUŞUMU VE KÖK AĞIRLIĞI ÜZERİNE ETKİLERİ

Daha önceki bir çalışmada, Kuzey ve İç Anadolu’dan alınan toprak örneklerinin şeker pancarı tuzak bitki testi, ELISA (enzyme linked immunosorbent assay) ve direkt tuzak bitki kök boyama yöntemleri sonucuna göre *Beet necrotic yellow vein virus* (BNYVV), *Beet soilborne virus* (BSBV) ve *Polymyxa betae* ile enfekteli olduğu belirlenmiştir. Bu çalışmada, ortalama ELISA değerleri ve kök ağırlıkları göz önüne alınarak, şeker pancarı fidelerinin gelişimi üzerine BNYVV, BSBV ve *P. betae*’nın tekli ya da karışık etkileri incelenmiştir. İstatistiki analizler, BNYVV, BSBV ve BNYVV+ BSBV enfeksiyonlarının bitkilerin yaş kök ağırlığını negatif olarak etkilediğini ancak aviruliferous *P. betae* enfeksiyonunun etkilemediğini göstermiştir. Altı haftalık yetiştirme peryodu sonrasında tuzak bitkilerde BNYVV, BSBV tekli ve BNYVV+ BSBV ikili enfeksiyonlarında en yaygın görülen simptomun sırasıyla % 46, % 28.1 ve % 58.5 oranlarıyla kloroz olduğu tespit edilmiştir. Ayrıca, tekli BSBV ile enfekteli bitkilerin % 60.3'ü, BNYVV ile enfekteli bitkilerin ise % 39.5'i herhangi bir simptom oluşturmamıştır. Kloroz (% 24.7), aviruliferous *P. betae* populasyonları ile enfekteli bitkilerde de en sık karşılaşılan simptom olurken, bitkilerin % 65.3'ü simptom sergilememiştir. Bu çalışma bölgede BNYVV, BSBV ve *P. betae*'nın farklı populasyonlarının mevcut olabileceğini ve BNYVV enfeksiyonunun kloroz oluşumunu artırdığını göstermiştir.

Anahtar Kelimeler: Tuzak bitki, BNYVV, BSBV, şeker pancarı.

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