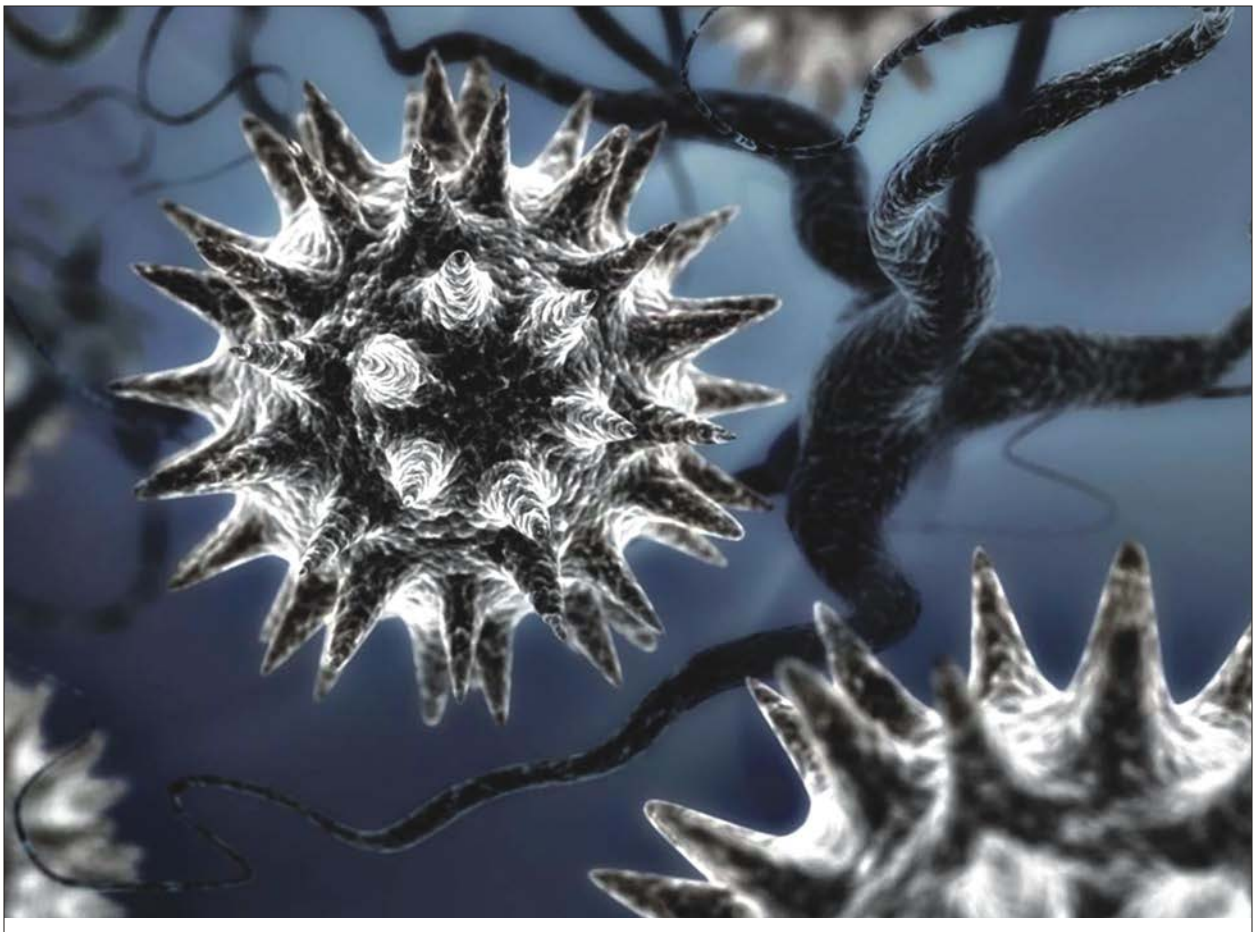

International Journal of Virology

ISSN 1816-4900

Academic Journals Inc.

www.academicjournals.com

International Journal of Virology 2 (1): 50-54, 2006
ISSN 1816-4900
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Alternative Weed Hosts of *Beet Necrotic Yellow Vein Virus* and *Beet Soil Borne Virus* in North East of Turkey

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Abstract: Purpose of this study was to determine the alternative weed hosts of two important viral diseases of sugar beet *Beet necrotic yellow vein virus* (BNYVV) and *Beet soil borne virus* (BSBV). In addition to the existing hosts of BNYVV and BSBV, different weed species were collected at production areas of sugar beet in North East of Turkey and both viruses were detected by ELISA. For BNYVV and its vector *Polymyxa betae* six weed species were determined as an alternative host of BNYVV. The highest absorbance values of ELISA were obtained from *Solanum nigrum* (0.725). This is the first report of *Cichorium intybus, Heliotropium eurapaeum* and *Plantago major* as an alternative host of BNYVV. None of the weed species collected from infested sugar beet fields was infected by BSBV.

Key words: *Beet necrotic yellow vein virus, beet soilborne virus, rhizomania*, weed, sugar beet

Introduction

Sugar beet (*Beta vulgaris* var. *saccharifera* L.) is extensively cultivated in North East of Turkey, over 19.049 ha (Anonymous, 2003). Rhizomania, a soilborne virus disease of sugar beet is caused by *Beet necrotic yellow vein virus* (BNYVV). BNYVV is a member of genus *Benyvirus* (Koening and Lesemann, 2000). BNYVV is vectored by soil Protist *Polymyxa betae*, Keskin (Abe and Tamada, 1986). Rhizomania is a severe disease of sugar beet world wide and affecting sugar beet yield and consequently the amount of extractable sucrose (Rush and Heidel, 1995). Since the first case reported in Turkey in 1976, the disease has been widely distributed in the majority of the sugar beet growing regions of Turkey, causing severe yield losses ranging from 30 to 80% (Kutluk and Yanar, 2001; Kutluk *et al*., 2004; Ozgur, 1995).

Beet soil borne virus (BSBV) another soil borne virus was first reported in England in 1982 (Ivanovic and Mcfarlane, 1982). Later, it was reported from many other researchers that BSBV is present in many other countries around the world (Lindsten, 1991; Prillwitz and Schlosser, 1992; Turina and Rubies-Autonell, 1996). Prillwitz and Schlosser (1992) reported the occurrence of BSBV in soils in which BNYVV was also present. BSBV and BNYVV are morphologically similar to each other; however, they are serologically different from each other. Unlike BNYVV, different serological types of BSBV exist. BSBV belongs to *Pomovirus* genus and *P. betae* is also its vector as well as

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BNYVV (Prillwitz and Schlosser, 1992). According to, Prillwitz and Schlosser (1992) BSBV causes rhizomania-like symptoms and results in yield loses up to 70% in sugar beets.

Polymyxa betae has a host range mainly limited to the *Chenopodiaceae*. Abe and Tamada (1986) reported that the ability of *P. betae* to transmit BNYVV differs among isolates or *Formae speciales* of the fungus. The virus was maintained only in cystosori extracted from sugar beet, spinach (*Spinacia oleracea*), *Chenopodium murale* and *Chenopodium capitatum*. However, a similar fungus has been reported from the family of Amarantheceae and thought to be a forme specialis of *P. betae* (Barr, 1979). Ivanovic (1988) determined that *Claytonia perfoliata* and *Stelleria media* were the hosts of *P. betae*. Barr and Asher (1992) reported that three biotypes of *P. betae* were identified in Britain and divided into 3 groups: The biotype infecting Chenopodiaceous species, the biotype with a narrower host range (including *B. vulgaris*) and the biotype infecting only *Silena alba*.

The objective of this study was to determine the alternative weed hosts of BNYVV and BSBV in sugar beet growing areas of North East Turkey.

Materials and Methods

Weed Material

During 2002 sugar beet growing season, more than 100 fields located in North East of Turkey were investigated. Fields investigated were identified either using the data reported by Kutluk and Yanar (2001) or by visual identification of yellow leaves on sugar beet or root symptoms. A total 180 samples belong to 36 different weed species were collected from sugar beet fields from August through October, when patches of Rhizomania disease were visible in crops. Each weed root was placed into polyethylene bag after labeling and washing. The samples were kept at -27°C until ELISA tests were done.

Serological Tests

The double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) performed with a specific antiserum to detect BNYVV. DAS-ELISA method was performed according to Clark and Adams (1977) and instructions of the antiserum producer (Loewe Biochemica, Sauerlach, Germany). Nonfat dry milk (0.1%) was used in extraction buffer instead of bovine serum albumin (Arif and Reavy, 1994). Root samples of weed 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, samples were 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 kept 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 performed to detect 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) was incubated at 37°C for 2 h and rabbit-anti-mouse conjugate (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

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 (Mouhanna *et al*., 2002).

Results and Discussion

ELISA tests revealed that BNYVV infection occurred within dicotyledonous weed species belonging to Asteraceae, Boraginaceae, Plantoginaceae, Polygonaceae and Solanaceae (Table 1) *Cichorium intybus*, *Heliotropium eurapaeum*, *Plantago major*, *Polygonum aviculare*, *Datura stramonium* and *Solanum nigrum* were found infected by BNYVV. The highest absorbance value of ELISA was observed for *Solanum nigrum* (0.725). There was no evidence that any of the other weed species were involved in spreading the rhizomania inoculum in the field. Any of the weed species collected from infested sugar beet fields were not infected by BSBV (Table 1).

The virus had ability to survive in air dried soil as well as in the field conditions for more than 15 years in cystosori of *Polymyxa betae* (Abe and Tamada, 1986). The cell walls of cystosori of

Table 1: Weed species tested for the presence of BNYVV and BSBV

Family	Species	BNYVV[a]	BSBV
Amaranthaceae	*Amaranthus blitoides* S. Watson	0.125	0.092
	Amararanthus lividus L.	0.177	0.131
	Amararanthus retroflexus	0.145	0.119
Apiaceae	*Daucus carota* L.	0.112	0.112
	Anethum graveolens L.	0.218	0.111
Arictolochiaceae	*Aristolochia maurorum* L	0.175	0.106
Asteraceae	*Cichorium intybus* L.	0.396(3)[b]	0.142
	Circium arvense (L.) Scop.	0.119	0.127
	Matricaria chamomilla L.	0.146	0.123
	Sochus oleraceus L.	0.208	0.119
	Xanthium strumarium L.	0.116	0.170
	Arctium lapa L.	0.104	0.091
Boraginaceae	*Echium vulgare* L.	0.233	0.160
	Heliotropium eurapaeum L	0.475(3)	0.173
Brassicaceae	*Sisymbrium officinale* (L.) Scop.	0.127	0.109
Chenopodiaceae	*Chenopodium album* L.	0.170	0.122
Convolvulaceae	*Convolvulus arvensis* L.	0.171	0.117
Cuscutaceae	*Cuscuta* sp.	0.116	0.114
Lamiaceae	*Lamium* sp.	0.119	0.105
	Mentha aquatica L.	0.136	0.118
Leguminosae	*Melilotus officinalis* (L.) De	0.240	0.108
	Vicia sp.	0.125	0.112
Malvaceae	*Hibiscus trionum* L.	0.154	0.114
	Malva neglacta Wallr.	0.173	0.114
Orabanchaceae	*Orabanche* sp.	0.260	0.109
Plantoginaceae	*Plantago major* L.	0.599(2)	0.147
Polygonaceae	*Polygonum aviculare* L.	0.309(5)	0.127
	Polygonum convolvulus L.	0.122	0.101
	Polygonum persicaria L.	0.116	0.100
Portulacaceae	*Portulaca oleracea L.*	0.604	0.140
Primulaceae	*Anagallis arvensis* L.	0.104	0.095
Ranunculaceae	*Adonis* sp.	0.101	0.101
Scrophulariceae	*Kickxia spuria* (L.) Dumort.	0.130	0.118
Solanaceae	*Datura stramonium* L.	0.471(4)	0.136
	Solanum nigrum L.	0.725(3)	0.112
Verbenaceae	*Verbena officinalis* L.	0.109	0.108

[a]ELISA absorbance values smaller than 0.303 is clean otherwise it is infected (BNYVV); Absorbance values smaller than 633 is clean otherwise it is infected (BSBV); [b] Number of samples infected with BNYVV.

P. betae are resistant to microbial degradation (Schlosser, 1988). It has been questioned for several decades whether weeds would serve as an alternative host without susceptible sugar beet cultivars in the field. Effects of the weeds on BNYVV survival in infested soils without Sugar beet cultivars are controversial. On the other hand, role of alternative weed hosts on survival of BSBV was relatively unexplored area in sugar beet growing areas of Turkey. To enhance the knowledge on alternative weed hosts of BSBV, it was also included in the present study. The highest absorbance values of ELISA were obtained for *Cichorium intybus*, *Heliotropium eurapaeum*, *Plantago major*, *Polygonum aviculare*, *Datura stramonium* and *Solanum nigrum*. The finding reported confirms the results of many earlier studies on alternative hosts of BNYVV (Al Musa and Mink, 1981; Hugo *et al*., 1996; Kutluk *et al*., 2000). Hess *et al*. (1982) reported that natural hosts allowing rapid development of virus and vector were *Beta vulgaris* and *Spinichia oleraceae*. Abe and Tamada (1986) reported that activity of virus protecting from cystosori was taken from only sugar beet, spinach, *C. album*, *Chenopodium capitatum* L. Al Musa and Mink (1981) determined *G. globosa* as the host of BNYVV in USA. Similarly, Hillmann (1984) confirmed the same finding in his study performed in Germany. Ivanovic (1988) stated that the hosts of vector (*P. betae*) were *Chenopodium perfoliata* and *Stelleria media* and Barr and Asher (1992) concluded that *Silena pratensis* was the host of the vector of BNYVV in England. Hugo *et al*. (1996) recorded *Chenopodium polyspermum* as host of BNYVV. In addition, Kutluk *et al*. (2000) determined *A. retroflexus*, *X. strumarium*, *C. album*, C. *vulvaria, C. arvense*, *Chamomilla recutita*, *Sonchus asper C. arvensis*, *P. aviculare*, *Polygonum persicaria*, *Portulaca oleracea*, *Veronica hederifolia*, *Datura stramonium*, *Solanum nigrum* and *Tribulus terrestris* as the alternative hosts of BNYVV in Turkey. In addition to the hosts reported from several researchers, the results obtained in this study revealed that *P. major*, *H. eurapaeum*, *C. intybus* are also weed hosts for BNYVV. This is the first report of these species as an alternative host of BNYVV.

Schlosser (1989) stated that weeds were not expected playing an important role, if any, in maintaining rhizomania inoculum in soils during the absence of susceptible crop plant. In contrary, Hugo *et al*. (1996) suggested that weed host has only a relatively small effect in building up rhizomania inoculum and spreading to sugar beet. The results confirmed that weeds were infected by BNYVV with in the sugar beet crops. Therefore, weed species infected by BNYVV should be responsible surviving and spreading of rhizomania in soil. In contrast, none of the weed species were infected by BSBV under natural conditions. Kudlackova and Rysanek (2003) conducted a similar study to determine the host range of BSBV using baiting plants of tested species into infested soil. They could not obtain BSBV from any of the plants studied except *Spinacia oleracea* and *Beta vulgaris* cultivars. The results suggest that the biotype of *P. betae* involved in BSBV transmission has a narrow host range as it was reported by Kudlackova and Rysanek (2003). However, no attempt was made in distinguishing vector isolates in this study. Further detailed studies are needed on *P. betae* isolates, before adequate conclusions can be drawn on characteristics of BSBV-transmitting vector isolates in Turkey.

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