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Research Article

Determination of *Benyvirus necrobetae* (Beet Necrotic Yellow Vein Virus) Infection from Spinach Fields of South Marmara Region in Türkiye

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Abstract: Spinach (*Spinacia oleracea* L.) is a major winter vegetable, produced and consumed in large quantities both globally and in Türkiye. Its cultivation faces significant economic losses due to various fungal, bacterial, and viral diseases, with viral infections being particularly critical due to the lack of effective chemical control measures. One of the major viral threats to spinach is *Benyvirus necrobetae* (beet necrotic yellow vein virus; BNYVV), the causal agent of "Rhizomania" disease. Although the presence of BNYVV has been documented in several production regions in Türkiye, its status in the spinach-growing areas of Bursa and Balıkesir provinces remains unclear. To address this gap, field surveys were conducted, and 83 spinach plants showing virus-like symptoms were sampled from the Southern Marmara provinces of Çanakkale, Balıkesir, and Bursa. The samples were tested for BNYVV using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with BNYVV-specific antisera. The serological results were further validated by reverse-transcription polymerase chain reaction (RT-PCR) using BNYVV-specific primers. The findings confirmed the presence of BNYVV in all sampled provinces, with 26 out of 83 samples testing positive, indicating an infection rate of 31.32%. These results suggest that BNYVV infection is widespread in spinach cultivation areas of the Southern Marmara Region. Future research should focus on identifying virus isolates at the pathotype level and exploring their interactions with the fungal vector *Polymyxa betae* (Keskin).

Keywords: BNYVV; DAS-ELISA; Rhizomania; RT-PCR, Spinach.

1. Introduction

Numerous biotic agents cause economic losses in spinach (*Spinacia oleracea*) cultivation (Correll et al., 1994). These agents can significantly reduce yields, leading to a decrease or even complete loss of market value. Among these pathogens, viral diseases are particularly concerning due to the lack of effective chemical control methods. One notable virus that infects spinach is *Benyvirus necrobetae* (beet necrotic yellow vein virus; BNYVV).

BNYVV was first discovered in Italy by Canova in 1952 (Canova, 1959). Since then, the virus has been reported in Asia, the Americas, Southern and Central Europe, and Scandinavian countries (Asher, 1999). BNYVV is transmitted by the soil-borne fungus *Polymyxa betae* (Keskin) and is notable for its unique mode of

transmission compared to many other viral agents. It has been documented that BNYVV can remain viable for up to 15 years within the thick-walled resting spores of *P. betae* and can be carried for extended periods once it enters the fungal vector (Abe and Tamada, 1986). Under conditions of low soil moisture and unfavorable temperatures, BNYVV infections caused by *P. betae* may manifest in the later stages of plant development. Disease progression under such conditions can sometimes mitigate yield losses (Putz et al., 1990).

The primary host of BNYVV is sugar beet. Infections in sugar beet result in excessive lateral root formation, giving the roots a characteristic "beard-like" appearance. Due to these distinctive symptoms, the disease is also referred to as "Rhizomania" (Putz et al., 1990). In infected plants, yellowish discoloration, particularly along the veins, is often observed. Moreover, these yellowish areas may become necrotic as the infected leaves age (Whitney and Duffus, 1991).

Studies conducted in Türkiye and globally have documented that various viral diseases cause infections in spinach (Dinant and Lot, 1992; Correll et al., 1994). Among these, BNYVV has been frequently reported in spinach production areas in Türkiye in recent years (Erbay, 2010; Gökdağ, 2014; Bağlan and Korkmaz, 2019). However, research on BNYVV in the context of spinach cultivation remains very limited, and the presence of the virus in spinach production areas of the Southern Marmara Region has not yet been documented. To address this gap, samples were collected from spinach production fields in the Southern Marmara Region, and the presence of the pathogen in spinach was assessed using serological and molecular methods.

2. Materials and Methods

2.1. Field studies

The field studies were conducted between 2015 and 2019 in the South Marmara region of Türkiye, covering the provinces of Çanakkale, Balıkesir, and Bursa, where spinach production is prevalent. Samples were collected from plants displaying typical virus or virus-like symptoms, as well as from plants that appeared different from the others in the field. These samples were transported to the laboratory under cold chain conditions.

2.2. Serological tests

The collected samples were tested for the presence of beet necrotic yellow vein virus (BNYVV) using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) test, following the procedure described by Clark and Adams (1997) and according to the manufacturer's instructions (Bioreba, Switzerland). Samples with absorbance values at 405 nm (Awareness Tech. Ins., USA) that were twice or more than that of the negative control were considered infected with BNYVV.

2.3. Molecular tests

To confirm the presence of BNYVV in samples identified as infected by DAS-ELISA, a more sensitive method, reverse transcription polymerase chain reaction (RT-PCR), was employed. Total RNA was extracted from all BNYVV-positive samples using the CTAB method (Li et al., 2008). The coat protein gene region of 567 base pairs (bp) was amplified by RT-PCR with the primer pair BN2/F1 (ATGTCGAGTGAAGGTAGATATATG) and BN2/R3 (ATTGTCCGGGTGGACTGGTTC) (Schirmer et al., 2005) using a two-step RT-PCR kit (TaKaRa, Japan).

The RT-PCR products were separated on a 1.5% agarose gel at 100 volts alongside 100-5000 bp DNA size markers, stained with ethidium bromide (EtBr), and visualized under ultraviolet light using a Major Science UVDI gel imaging system to detect the 567 bp bands corresponding to the viral coat protein gene.

3. Results and Discussion

During the survey, some fields exhibited spinach plants with clear virus symptoms, while others showed no symptoms. And, only plants showing symptoms were sampled. The samples were collected without distinguishing spinach cultivars. The variation in symptom expression across fields is likely related to differences in disease transmission and the presence of cultivars with varying levels of tolerance or susceptibility.

During the testing of collected samples using DAS-ELISA, infected samples began to yellow within a few minutes after the substrate step, and their absorbance values increased to at least twice that of the negative control after 30 minutes (Table 1). In addition, visible color changes were observed in the wells containing plants infected with BNYVV as a result of the DAS-ELISA tests. Although molecular methods such as RT-PCR and real-time PCR have been increasingly used in recent years for the diagnosis of plant viruses, DAS-ELISA is still effectively employed for large-scale field sampling, particularly for diagnosing BNYVV (Kutluk-Yılmaz and Yanar, 2001). In this context, DAS-ELISA is considered a reliable method for the successful diagnosis of BNYVV.

Table 1. Absorbance values obtained from spinach samples tested against *Benyvirus necrobetae* (beet necrotic yellow vein virus; BNYVV) using DAS-ELISA.

Samples from Bursa Province								
Row	A	B	C	D	E	F	G	H
1	0.395	0.392	1.095*	0.486	0.454	1.384*	0.992*	0.372
2	0.407	0.385	0.976*	0.503	0.386	1.371*	0.951*	0.413
3	0.906*	0.390	0.504	1.105*	0.884*	0.513	0.401	0.365
4	0.960*	0.412	0.541	1.180*	0.908*	0.457	0.465	0.390
5	0.401	0.412	0.985*	0.380	0.397	0.982*	0.374	0.422
6	0.393	0.420	1.120*	0.413	0.425	0.955*	0.412	0.531
7	0.392	0.372	0.452	0.415	1.050*	0.374	0.984**	0.368***
8	0.386	0.394	0.395	0.443	1.020*	0.541	1.079**	0,378***
Samples from Balıkesir Province								
Row	A	B	C	D	E	F	G	H
1	1.205*	0.980*	0.410	0.456	1.120*	1.440*	0.412	0.892*
2	1.154*	1.106*	0.386	0.396	1.183*	1.520*	0.398	0.840*
3	0.384	0.382	0.954*	0.413	0.374	0.412	0.346	0.454
4	0.296	0.346	0.974*	0.442	0.354	0.446	0.364	0.422
5	0.898*	0.984*	1.101*	0.386	0.879*	0.525	0.384	0.342
6	0.924*	0.962*	1.156*	0.354	0.921*	0.498	0.372	0.364
7	0.451	0.384	0.471	0.490	1.104**	0.356***	-	-
8	0.474	0.331	0.420	0.475	1.056**	0,366***	-	-
Samples from Çanakkale Province								
Row	A	B	C	D	E	F	G	H
1	0.426	0.384	1.120*	0.406	0.410	0.397	0.946*	0.406
2	0.457	0.368	1.046*	0.423	0.402	0.405	0.984*	0.387
3	0.389	0.412	0.378	0.394	0.920*	0.395	0.942*	0.504
4	0.405	0.422	0.366	0.405	0.941*	0.412	1.050*	0.412
5	0.984*	0.394	0.356	1.348*	0.451	0.341	0.358	0.378
6	0.945*	0.347	0.384	1.357*	0.412	0.346	0.369	0.348
7	1.312*	1.214**	0.376***	-	-	-	-	-
8	1.324*	1.186**	0,384***	-	-	-	-	-

*Samples found infected with BNYVV , **Positive control, ***Negative control

In the RT-PCR tests, bands of the expected size were obtained from the samples that tested positive for BNYVV infection in the DAS-ELISA assays (Figure 1). This confirmed the presence of BNYVV infection in the collected samples.

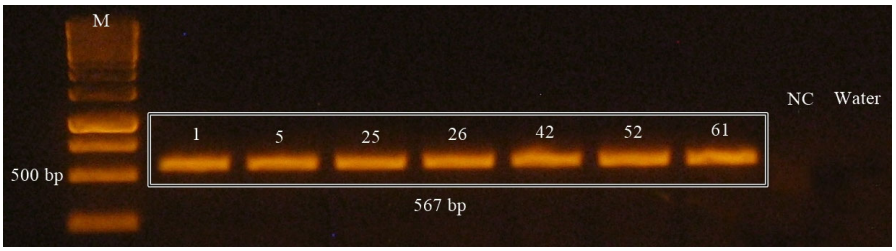

Figure 1. Molecular test results of spinach samples found infected with *Benyvirus necrobetae* (beet necrotic yellow vein virus; BNYVV) in serological tests (1, 5, 25, 26, 42, 52, 61: sample number; NC: Negative control; M: Marker).

DAS-ELISA and RT-PCR testing of field samples provided insights into the symptoms associated with BNYVV infection. Infected plants commonly showed mild yellowish-green spots, leaf narrowing, and tapering (Figure 2). The observation that this type of symptom has previously been identified as indicative of BNYVV infection further supports the results of this study (Bağlan and Korkmaz, 2019). In some cases, these symptoms were severe enough to significantly reduce the market value of the produce. Previous studies conducted in Türkiye have also reported that these symptoms are frequently observed in spinach plants (Erbay, 2010; Güngör et al., 2017; Gökdağ et al, 2014; Kurtoğlu, 2019).

Figure 2. Light yellowish mosaic symptoms on leaves of spinach plant infected with *Benyvirus necrobetae* (beet necrotic yellow vein virus).

The results indicated a BNYVV infection rate of 31.32% in the collected samples. At the provincial level, the highest infection rate was found in Balıkesir at 35%, while the lowest was in Çanakkale at 28%. The infection rate in Bursa was determined to be 30% (Table 2). Although all samples exhibited symptoms indicative of viral infection, only 31.32% of the collected samples were infected with BNYVV. This may suggest that the remaining samples were infected with other viruses that cause disease in spinach. Indeed, other studies conducted in spinach production areas in Türkiye have also identified infections with, turnip mosaic virus, cucumber mosaic virus, and beet western yellow virus (Gümüş et al., 2014; Güngör et al., 2017; Kurtoğlu and Korkmaz, 2018; Sertkaya, 2015).

Table 2. Numbers and rates of beet necrotic yellow vein virus infection in collected samples.

Provinces	Infected/Collected Sample Numbers	Infection Rates (%)
Çanakkale	7/25	28.00
Balıkesir	10/28	35.00
Bursa	9/30	30.00
Total	26/83	31.32

4. Conclusions

This study identified the presence of BNYVV in spinach production areas of the Southern Marmara Region using serological and molecular methods. The results indicated that BNYVV infection is present at a considerable level. Future studies should prioritize resistance breeding and cultivar improvement efforts to address viruses causing infections in spinach cultivation.

Conflicts of Interests
Authors declare that there is no conflict of interests

Financial Disclosure
Authors declare no financial support.

Statement contribution of the authors
A. K. collected samples, perfomed the laboratuary analyses. All authors wrote the draft and approved the final version.

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