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Presence of Tomato Spotted Wilt Virus Between Cress and Pepper Intercropped in Kumluca District of Turkiye

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Abstract: Tomato spotted wilt virus (TSWV) transmits via thrips and causes significant diseases in *Solanaceae* species. In this study, samples were collected to identify TSWV and determine its frequency in cress (*Lepidium sativum*) and pepper (*Capsicum annum*) grown in 27 different greenhouses at Kumluca, Antalya province. More than 102 plant samples of cress and pepper plants were collected in Autumn 2023, these samples showing symptoms of virus-like ring spot and leaf decay, they were tested for the presence of TSWV using RT-PCR and qRT-PCR. Positive cress and pepper samples were discovered and TSWV cases in cress plants were common in 5 greenhouses. Based on the result, this is the first report of TSWV infection in cress. In conclusion, the role of thrips species in virus epidemiology in Turkey should be focused on with the potential of the tested TSWV isolates to break the resistance mechanisms in their hosts increases interspecies epidemic risks.

Key Words: Cress, TSWV, Pepper, Permaculture, PCR

Türkiye'nin Kumluca İlçesinde Yetiştirilen Tere ve Biber Arasında Domates Benekli Solgunluk Virüsünün Varlığı

Öz: Domates benekli solgunluk virüsü (TSWV), thrips yoluyla bulaşmakta ve *Solanaceae* bitkilerinde önemli hastalıklara neden olmaktadır. Bu çalışmada, Antalya ilinde Kumluca'da bulunan 27 farklı serada yetiştirilen tere (*Lepidium sativum*) ve biberde (*Capsicum annum*) TSWV'nin tespit edilmesi ve görülme sıklığının belirlenmesi amacıyla örnekler toplanmıştır. 2023 Sonbaharında tere ve biber bitkilerinden 102'den fazla bitki örneği toplanmıştır. Virüs benzeri halkalı leke ve yaprak bozulması belirtileri gösteren örnekler, TSWV'nin varlığı açısından RT-PCR ve qRT-PCR kullanılarak test edilmiştir. Pozitif saptanan tere ve biber örnekleri ayırt edilmiş ve tere bitkilerinde TSWV vakası 5 serada yaygın olarak görülmüştür. Bulgular ışığında, bu çalışma teredeki TSWV enfeksiyonuna ilişkin ilk rapordur. Sonuç olarak, Türkiye'deki virüs epidemiyolojisinde thrips türlerinin rolüne odaklanılmalı, test edilen TSWV izolatlarının konukçularındaki dayanıklılık mekanizmalarını kırma potansiyeli türler arası epidemik riskleri arttırmaktadır.

Anahtar Kelimeler: Tere, TSWV, Biber, Permakültür, PCR

1. Introduction

Cress (*Lepidium sativum*) is a spicy plant species from the cruciferous family, with its leaves are commonly consumed as a salad. It is massively cultivated in Anatolia, but its origin is native to Egypt and Southwest Asia (Shah et al.2021).

Cress is an important crop in Kumluca province located in the western part of Antalya, Turkiye. Total production of leafy or edible stem vegetables in Turkiye was 2,012,801 tons and Kumluca contributed to 10,222 tons of the production in 2023 (Anonymous, 2023).

One of the most widely spread plant viruses, Tomato spotted wilt virus (*Bunyaviridae, Tospovirus,* TSWV), is economically devastating with reference to yield losses in many vegetable productions. Although TSWV has a wide host range, it has more than 900 plant host (Peters, 1998) consisting of ornamental, vegetable (tomato, pepper, lettuce etc.) and weed. It is mainly transmitted by western flower thrips (*Frankliniella occidentalis*) (Antignus et al., 1997) in a propagative-persistent manner (Wijkamp *et al*., 1993). There are several TSWV incidences reported on tomato (Tekinel, 1973; Azeri, 1994), tobacco (Azeri, 1981), pepper (Yurtmen, 1998) and lettuce (Kamberoglu and Alan, 2011) in Turkiye.

To date, there is limited information about TSWV presence and incidence in cress. The objectives of this

study are to conduct molecular examinations for detecting TSWV, determining incidence in cultivated cress plants through RT-PCR and qPCR analysis and highlighting epidemiology at Kumluca, Antalya, Türkiye.

2. Material and Methods

2.1. Surveys and sample collection

Preliminary examinations in field were conducted in randomly selected cress and pepper growing as mix-cultivated greenhouses in Kumluca region in the autumn of 2023 (Table 1). Samples of leaves from each plant were placed in plastic bags and tagged then placed in a cool box. Sampling process was conducted on affected plants exhibiting both typical TSWV symptoms with distorted leaves, yellowing, brown spots, and wilting and generic virus symptoms, minor mosaic, crinkling, and deformation (Figure 1).

Table 1. All collected samples and positive found samples with TSWV, they were collected from different greenhouses located in Antalya province.
Çizelge 1*. Antalya'nın farklı lokasyonlarından toplanan örnekler ve bu örneklerde TSWV pozitif bulunanlar.*

Antalya Locations	Collected Samples	TSWV (+) Pepper	TSWV (+) Cress
Kumluca	27	11	5
Finike	11	6	-
Gazipaşa	13	4.	-
Serik	14	6.	-
Aksu	12	3	-
Total	**77**	**30**	**5**

Figure 1. Minor mosaic and spots on pepper (left side), crinkling and leaf deformation on cress (right side) due to TSWV infection.
Şekil 1. *TSWV enfeksiyonuna bağlı biberde küçük mozaik ve lekeler (solda), tere yaprağında buruşma ve deformasyon (sağda).*

2.2. Molecular tests

The RT-PCR and qRT-PCR molecular analyses were used to detect viruses on pepper and cress (testing was repeated thrice). Pepper viruses were not the focus of this study, however, Tomato yellow leaf curl virus (TYLCV), Tomato mosaic virus (ToMV), Tomato mottle mosaic virus (ToMMV), Pepper mild mottle virus (PMMoV), Tobacco mosaic virus (TMV), Tobacco rattle virus (TRV), Cucumber mosaic virus (CMV), Potato virus Y (PVY) and Alfalfa mosaic virus (AMV) were tested for in cress.

2.3. Total RNA extraction

Total RNA was extracted from fresh leaves of TSWV infected cress and pepper plants using GeneJet RNA Purification Kit (Thermo Fisher Scientific, USA), according to manufacturer's instructions.

2.4. RT-PCR

One step RT-PCR reactions were performed as described below; the complementary DNA (cDNA) strands of a portion of cDNA were synthesized with specific amplification of the TSWV N (nucleocapsid) gene by one-step RT-PCR using the TSWV N gene specific primers, TSWV L1 AATTGCCTTGCAACCAATTC; TSWV L2 ATCAGTCGAAATGGTCGGCA (Mumford et al., 1996). They were amplified with expected product size of 276 bp.

A total of 25 µL mixture was used including of 2 µL of RNA template, 1 µL of each specific primer, 0.25 µL of Verso enzyme mix, 1.25 µL of RT Enhancer, and 12.5 µL 1-Step PCR Hot-Start Master Mix (Thermo Science) and 7 µL of nuclease-free water were used for amplification. The cDNA stage was performed at 50 °C for 15 minutes and the reaction was terminated at 95 °C for 2 minutes. Then PCR steps were followed with 35 cycles of denaturation at 95°C for 45 seconds, 52 °C annealing for 30 seconds, 72 °C extension for 45 seconds, and then final elongation at 72 °C for 10 minutes (Fidan and Koç, 2019; Fidan et al., 2019). The amplified products were run on 1.5% agarose gel during gel electrophoresis. The Verso 1-step RT-PCR Kit (Thermo Fisher Scientific, USA) was used to conduct a one-step qRT-PCR test with a final volume of 25 µL, adhering to the manufacturer's recommendations. Using the Bio-Rad Realtime PCR Detection System (Bio-Rad, Germany), 2 µL of total RNA isolated from collected samples was used as a template for each reaction.

A probe and primary designs required for qRT-PCR were carried out using TSWV-F (5'-GCTTGTTGAGGAAACTGGGAATT- 3') as forward, and TSWV-R (5'-AGCCTCACAGACTTTGCATCATC-3') as reverse

primer. The fluorescent dye-labelled probe (5' 6FAM-AAATCTAAGATTGCTTCCCACCCTTTGATTCAA -TAMRA 3') was also used in qPCR reactions (Roberts et al., 2000).

The reporter dye's (FAM) and quencher dye's (TAMRA) fluorescence intensities were measured during the amplification process. The threshold cycle (Ct value) refers to the number of amplification cycles required for a significant increase in the reporter's fluorescence. The Bio-Rad (Hercules, California /USA) Real Time PCR Detection System Program was used to examine their data.

3. Results

The survey and sample collection were carried out in a total of 27 greenhouses in Kumluca where mainly cress growing fields with 3800 plants per decare (da). More than 273,600 cress plants were monitored in 27 greenhouses where these 5 da of them were showing virus-like symptoms were tested using RT-PCR and qPCR analyses. The rate of incidence of TSWV in collected cress samples was found as 18.5% at Kumluca district. The expected fragment size of 276 bp was obtained after electrophoresis of RT-PCR products in 1.5% agarose gel (Figure 2). plants. The qRT-PCR analyzes exhibited Ct values in 8th cycles for pepper and 14th cycles for cress plants respectively (Figure 3). A Ct value was obtained at 24th cycles for positive control but there was no Ct value for negative control (Figure 3).

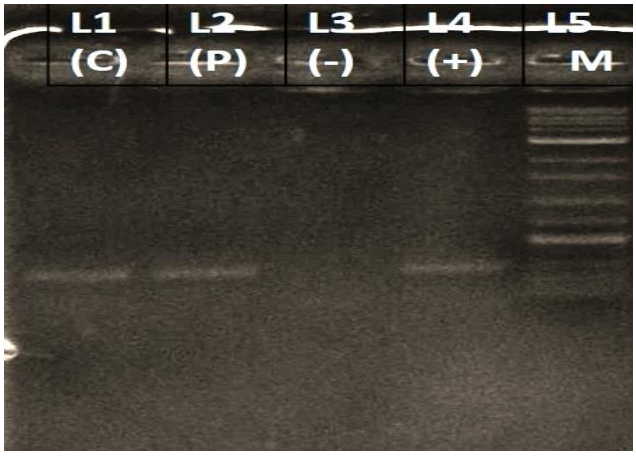

Figure2. A 276 bp RT-PCR product of TSWV was amplified and visualized on agarose from collected samples at Kumluca district. Lane 5- M: contain 100 bp DNA marker; Lane 1, 2: typical symptomatic cress and pepper plants respectively; Lane 3: negative control, Lane 4: positive control.

Şekil 2. *TSWV'nin RT-PCR ürünü olan 276 bp fragment Kumluca ilçesinden toplanan örneklerden elde edildi ve agaroz jel elektroforezinde görüntülendi. Kulvar 5-M:100 bp DNA moleküler marker içermektedir; Kulvar 1, 2: sırasıyla tipik semptomatik tere ve biber bitkileri; Kulvar 3: negatif kontrol; Kulvar 4: pozitif kontrol içermektedir.*

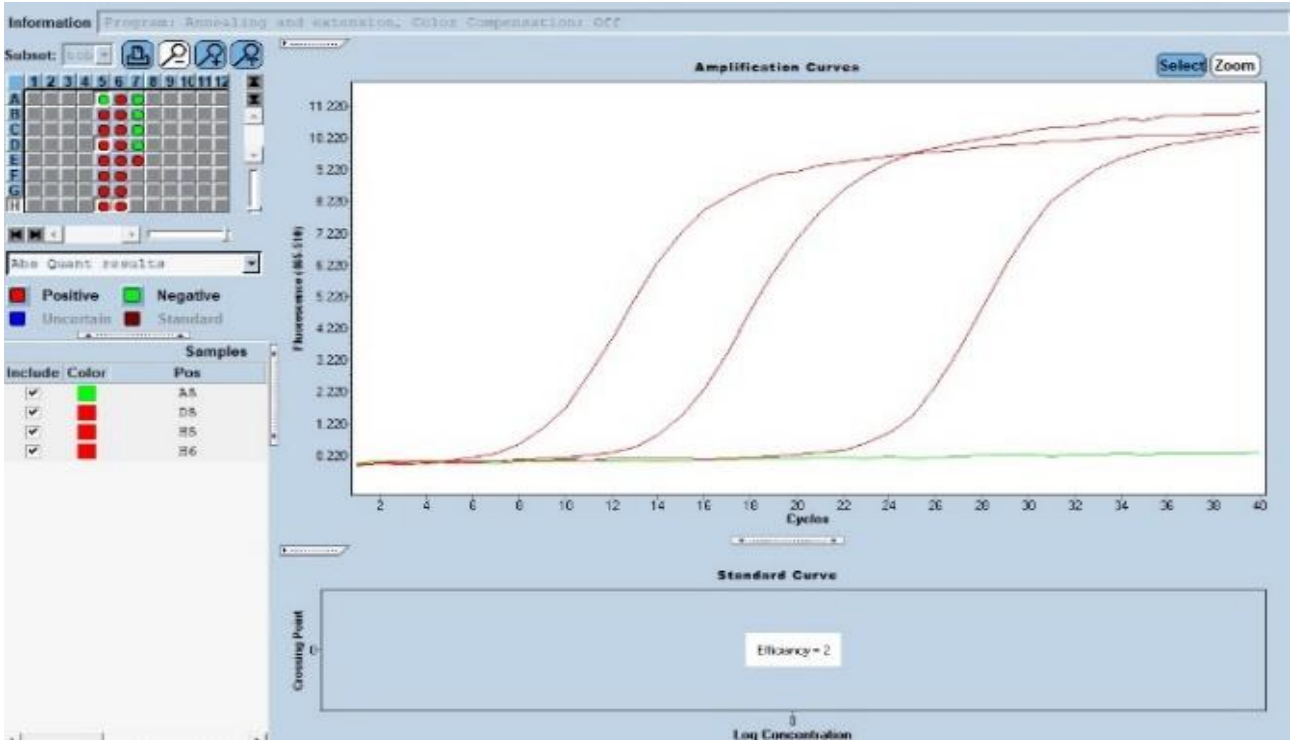

Figure 3. Bio-Rad RealTime PCR Detection System has revealed amplification curves from cress, pepper plants and positive control but there was not any curve for negative control.

Şekil 3*. Bio-Rad RealTime PCR Tespit Sistemiyle analiz edilen tere, biber bitkileri ve pozitif kontrolde parobol eğrisi üretiliyorken negatif kontrolde herhangi bir parabolik eğri elde edilmemiştir.*

A portion of small RNA of TSWV was targeted to amplify using total RNA extracted from symptomatic plants with virus specific primers in qRT-PCR analyses. The qRT-PCR analyzes confirmed the presence of TSWV infection in interspecies and also confirmed transmissibility of the virus between cress and pepper

After eradicating the diseased plants in the affected greenhouses, they were sterilized through solarization and exposed to disinfectant applications during preparations for the next crop season.

4.Discussion

Cress is one of the important crops cultivated in winter and early spring in Kumluca district, Antalya, Türkiye. The surveys were conducted in greenhouses close to main pepper growing areas during the early autumn in Kumluca. Cho et al. (1987) reported that high incidence of TSWV was attributed to elevating population of *Thrips tabaci* L and *Frankliniella occidentalis* species influenced by local microclimatic conditions like rain fall, minimum and maximum temperature. For example, in research conducted by Atakan and Sarı (2010), *F. occidentalis* and *T. tabaci* were detected on lettuce with reduced and winter time (October-March) in the Cukurova region where has similar climatic condition to Kumluca. Atakan and Sarı, (2010) also indicated that the collected thrips species were female and no larval thrips belonging to either thrips species were collected from lettuce fields. The male population of thrips transmitted TSWV at a higher rate compared to females (Rotenberg et al., 2009; Van de Wetering et al., 1998) also reported that a higher proportion of females in the thrips population has a negative impact on plant damage and virus transmission, and thus spread of TSWV. This is attributed to the lower mobility and higher consumption rate of females in comparison to their male counterparts. The low presence of thrips and the predominance of female thrips during the winter and early spring could explain the low occurrence of Tomato spotted wilt virus (TSWV) in other cress like leafy vegetables as lettuce plants in the Cukurova region (Kamberoglu and Alan, 2011). Wilson (1998) indicated that the percentage of TSWV-infected lettuce plants varied depending on the farm location and season, with more infections more prevalent in late summer and early fall, resulting in losses of 25-65% during fall harvests in southern Tasmania. Moreno et al. (2004) reported that TSWV epidemics were much more frequent in autumn compared to spring, which they attributed to the absence of virus vectors.

Real-time diagnosis of local thrips species should be detailed in such local or limited production areas, especially in places where perm culture is carried out. Thus, the role of local subspecies of thrips on local or regional epidemics will be better understood. In this way, control methods can be shaped more effectively. The role of culture of aromatic plant species, which serve as intermediate reservoir host potential, on virus ecology and the effect of auxiliary arguments are among the main topics that need to be investigated. Distribution of reservoir plants for the virus and insect vectors, efficiency of transmission from these hosts to cultivated crops, determination, and characterization of Turkish local isolates of TSWV from different locations needs to be investigated in Turkey. Thus, the collection of TSWV isolates originating from a wide range of both wild and cultivated forms through different vectors in common host pools at certain periods may constitute a step for the formation of new sub-isolates that can break the resistance in plants. Both the impact factor on agro-ecology and the economic damage to agricultural areas of new races that can develop based on mixed infections and mutation cannot be predicted. To follow effective control strategies, the potential for the formation of new strains and their compatibility with vectors should be reviewed in detail. The effects of global warming on both vectors and pathogen behavior have not yet been predicted, and host plant adaptations are an unknown phenomenon. For this reason, it is considered exclusive to focus on changes in virus ecology and isolate behavior according to host species. In addition to variable global warming, changes in the ability of viral agents and vectors to expand the host spectrum, which can be triggered by regional droughts, are a separate threat. In recent past, local outbreaks of Tomato brown rugose fruit virus (ToBRFV), Tomato Yellow Leaf Curl New Delhi Virus and similar diseases have turned into continental threats in similar ways. Furthermore, genetic recombinant TSWV and ToBRFV isolates were shown to have ecologically selective advantage over the original virus. The challenges of virus disease control are becoming increasingly difficult with the constant emergence of new breeds of existing viruses or completely new viruses. Viruses have a great potential to adapt to the pressure of natural selection for reasons such as large populations, the absence of repair mechanisms that facilitate genetic variation in their genomes, and their ability to reproduce in a short time.

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Conflict of interest All authors declare that they have no conflict of interests.

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