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Phenotypic and genotypic characterisation of pepper genotypes for *Tomato* Spotted Wilt Orthotospovirus reaction and resistance

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ABSTRACT

In order to determine the effects of Tomato spotted wilt orthotospovirus (TSWV) on the yield and fruit quality parameters of some common local and commercial pepper (Capsicum annuum L.) genotypes under greenhouse conditions, mechanical inoculations were performed, and virus infections were tested by DAS-ELISA and RT-PCR after the inoculations. Based on the DAS-ELISA results, 95% of the inoculated plants were found to be positive for TSWV, in accordance with the expected amplicon size (276 bp) obtained by RT-PCR results. Infection of TSWV caused significant (p≤0.05) reduction in fruit number (45.97-100%), fruit weight (66.38-98.77%), fruit width (63.77-96.52%), fruit length (31.97-93.514%), flesh thickness (28.64-82.41%), fruit firmness (5.82-94.43%), fruit colour (1.62-7.79%), and total yield (68.62-100%) in infected plants. The best performance against TSWV was observed on cv. Yalova Carliston 341, while the lowest was observed on cv. Bora 77 among the tested pepper cultivars Yalova Çarliston 341, Yalova Tatlı Kıl, Yalova Yağlık 28, Mazamort, Sera Demre 8, Üçburun, Geyikboynuzu, Bor Biberi, Bora 77 and 153-227. Moreover, the incidence of Tsw resistance gene was investigated by molecular analysis using CAPS marker SCAC568 with TaqI restriction enzyme digestion; however, Tsw gene could not be detected in any of the tested cultivars except wild type C. chinense and resistant genotype cv.153-227. This study reveals the effects of TSWV in common pepper genotypes and will be important for virus resistant breeding studies.

1. Introduction

Vegetables play an important role as a source of vitamins and minerals in the nutrition of people. Due to the fact that the genetic centre of many important vegetable crops can be found in Turkey, vegetable production is an important sub-sector of farming in Turkey (Turhan and Korkmaz 2006; Çelik et al. 2018). Pepper is an important crop both for human nutrition and commercially, with its rich nutrient content, wide usage areas and large production volume. Pepper is produced in many regions of the world and additionally pepper production is carried out in Turkey with high production volumes over large areas of production. Turkey is one of the most important pepper suppliers as its production is around 2600000 tons annually and, ranked 3rd in the pepper production after China and Mexico (TÜİK 2020). There are many disease factors that negatively affect pepper production. It is known that viruses cause diseases in pepper as well as many plants and restrict pepper production (Celik et al. 2010). It is reported that among these viral diseases, Tomato spotted wilt orthotospovirus (TSWV) is one of the most important 10 viruses that cause a high loss of yield (Scholthof et al. 2011).

TSWV firstly appeared on tomato plants in Australia Brittlebank (1919). It spread rapidly from Australia and was found in many countries in America, then in Europe, Asia, and Africa (Adkins 2000). In Turkey, TSWV was initially reported in lettuce plants in Mersin (Tekinel et al. 1969), after that, it was detected in Çanakkale, Balıkesir, Manisa, Uşak, Şanlıurfa, Samsun and the Mediterranean Region (Azeri 1981). Worldwide, there are more than 1000 plant species that are the host plants of TSWV, causing the virus to spread rapidly (Margaria et al. 2015). Another factor that causes the spread of TSWV is thrips, the vector of this virus. While TSWV is transmitted both circulatively and propagatively by 9 thrips species of 3 genus (Thrips, Frankliniella, Scirtothrips) belonging to the Thysanoptera order and Thripidae family, Frankliniella occidentalis has been reported as the most important vector of TSWV (Ullman et al. 1992; Şevik 2008). While cultural and chemical management are not effective in controlling TSWV due to the biological structures of viruses, the wide host plants range of TSWV and its transportation via thrips, utilising resistant varieties is the most effective method of controlling its spread. Both classical and molecular breeding methods are used in the mes development of resistant varieties. However, the marker assisted selection (MAS) method, which is one of the molecular breeding methods, is more reliable, faster and is the most widely used alternative and auxiliary method in recent years (Simsek et al. 2015). Tsw gene provides resistance to viral disease caused by TSWV in pepper plants. Lines developed from Capsicum chinense Jacq. show hypersensitivity resistance against TSWV in pepper and are used as a source of resistance in breeding programmes. Pepper varieties which have the Tsw gene do not show symptoms after mechanical inoculation, they show hypersensitivity resistance by shedding their leaves, after forming local lesions (Boiteux 1995). Studies on the *Tsw* gene have shown that this gene is located on the same loci (chromosome 10) of some *Capsicum chinense* Jacq. lines (Black et al. 1991; Boiteux 1995; Moury et al. 1997).

RAPD, SCAR, and CAPS markers are used for the detection of *Tsw* gene (Welsh and McClelland 1990; Williams et al. 1990, Lefebvre et al. 1997). In addition, CAPS markers are the most commonly used markers for detecting *Tsw*. Marker assisted selection is not always possible with RAPDs, because RAPDs designed for one population are not always polymorphic or not reliable for other populations (Paran and Michelmore 1993). It is possible to covert a RAPD piece into a SCAR marker to overcome these problems. SCAR markers are based on the sequencing of RAPD fragments and higher identification of more specific primers. But these identified primers often lead to monomorphic amplifications and loss of polymorphism. To achieve this polymorphism CAPS markers can be obtained by enzymatic restriction of SCAR (Konieczny and Ausubel 1993, Moury et al. 2000).

Various studies have been carried out in Turkey aiming to develop resistant pepper lines to TSWV. Celik et al. (2018) aimed to breed lines that can be used as parents in order to develop new pepper varieties resistant to TSWV. In the study, they used a variety sensitive to TSWV, three genotypes resistant to TSWV, and reported that they obtained 10 lines resistant to TSWV with features that can be used in pepper breeding studies. Simşek et al. (2015) used 12 TSWV resistant pepper genotypes, 6 TMV and PMMoV resistant pepper genotypes, and one superior pepper genotype in terms of quality characteristics. As a result of the study, it was reported that 3 genotypes had the desired resistances, and were determined as candidate varieties.

The aim of this current study, was to determine the effects of tomato spotted wilt orthotospovirus on the yield and fruit quality parameters of some local and commercial pepper genotypes that are widely used in the Central Anatolian Region, and screening for *Tsw* resistance gene incidence.

2. Materials and Methods

2.1. Materials

In this study, the most common local cultivars were selected based on their known superior fruit characteristics and yield in the Central Anatolian and Southern Mediterranean regions. In total ten different pepper genotypes (Capsicum annuum L.) and one wild type of pepper (Capsicum chinense) were used. The cultivars Üç Burun, Yalova Yağlık 28, Bora 77, Mazamort, Sera Demre 8, Yalova Çarliston 341, Yalova Kıl Tatlı are commercially used in the Central Anatolian region, and two of them are local genotypes called Bor pepper and Geyikboynuzu (Samandağ- Southern Mediterranean region). The resistant varieties used in this study were Capsicum chinense L. which is a wild type of pepper genotype and 153-227 which was produced by the company Yüksel® Seeds. In addition, TSWV infected pepper plants were supplied by the T.R. Ministry of Agriculture and Forestry, Ankara Directorate of Agricultural Quarantine and were used as inoculum sources of the virus.

2.2. Methods

2.2.1. Experimental design

This study was conducted under greenhouse conditions during the 2020 summer period in the Niğde province. Seeds were germinated in seed trays and seedlings transplanted to plastic pots after germination. While the seed trays were filled with a mixture of peat and perlite (3:1), a mixture of soil and peatperlite (3:1) was used in the plastic pots. No fertiliser was applied to the plants during the daily irrigation. An equal number of plants were inoculated with TSWV, and mock-inoculated plants were used for each genotype as a control. Each genotype contained a total of 50 plants, including 25 virus inoculated plants and 25 mock-inoculated (buffer inoculated) plants.

2.2.2. Mechanical inoculations of pepper seedlings

Mechanical inoculation of TSWV was performed to the pepper plants twice at 15-day intervals using inoculation buffer (pH: 7.4) including 0.199 g l KH₂PO₄, 1.14 g l Na₂HPO₄, 0.1% Na₂SO₃ and 1% PVP-40 (1:10 sample dilution). The first inoculation was carried out 1 week after transplanting, when the plants were at the 5-6 leaf stage.

2.2.3. Confirmation of TSWV infections

The DAS-ELISA method was performed 30 days after inoculations to check TSWV infections according to Clark and Adams (1977) and instructions of the antisera's manufacturer for the monoclonal antisera of TSWV. After the ELISA test, the plate was read by the ELISA reader, and numerical results of the ELISA test were obtained. In addition to the serological detection, molecular tests were also performed to confirm the presence of TSWV in the tested pepper plants. A Plant/Fungi RNA Isolation Kit was used for isolation and purification of total RNA, according to the manufacturer's instructions (Norgen Biotek Corp., Canada). cDNA was synthesized by reverse transcription of obtained total RNAs using random hexamer primers and the cDNA synthesis Kit instructions (OneScript plus cDNA synthesis Kit, abm good, Canada). RT-PCR was performed using the obtained cDNA samples and PCR was carried out with 2 µl of cDNA, 0.5 µl of 10 mM dNTP, 1 µl of 25 mM MgCl₂, 2.5 μ l of 5x PCR buffer and 0.5 μ l of 10 μ M of each virus specific primers (TSWV RdRp, F: 5'-ATCAGTCGAAATGGTCGGCA-3', R: 5'-AATTGCCTTGCA ACCAATTC-3', amplicon size: 276 bp, Perez et al. (2014), with 0.25 µl of 5 units µl Taq DNA polymerase. PCR was performed under the following conditions: denaturation 94°C 5 min, 40 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 1 min; and a final extension for 10 min at 72°C. The PCR products were visualised under UV light after electrophoresis on 1.5% agarose gel and stained with ethidium bromide under a UVtransilluminator.

2.2.4. Molecular screening of TSWV resistance in pepper varieties

The cetyl trimethylammonium bromide (CTAB) extraction method (Doyle and Doyle 1987) was used to extract DNA from pepper leaves to check the incidence of resistance genes. The Tsw CAPS markers (SCAC568: resistance gene F٠ GTGCCAGAGGAGGATTTAT. R: GCGAGGTGGACACTG ATACT were used for PCR analysis (Moury et al. 2000). The PCR was carried out with 2 µl of diluted DNA, 1 µl of 10 µM dNTP mix, 2 µl of 25 mM MgCl₂, 2.5 µl of 10X PCR buffer, and 0.5 µl of 10 µM of each primer with 0.2 µl of 5 U µl Taq DNA polymerase. Reactions were incubated at 94°C for 5 min and following 40 amplification cycles (30 s at 94°C, 45 s at 50°C, and 1 min at 72°C) was performed and a final extension for 10 min at 72°C. The final PCR products were visualised under UV light after electrophoresis on ethidium bromide-stained 3% agarose gels. The obtained PCR amplicons were used for digestion. The mixture prepared by using 1 μ l *Taq*I enzyme, 1 μ l of 10x Cutting Buffer, 2 μ l of nuclease-free sterile water and 7 μ l of PCR product was incubated for 2 hours at 65°C (Moury et al. 2000). The final products were visualised under UV light after electrophoresis on ethidium bromide-stained 3% agarose gels.

2.2.5. Fruit quality and yield analysis

Fruits of both the infected and uninfected plants were harvested after maturation. All fruit quality traits were evaluated for ripen fruits per plant. Fruit number (FN) of each plant was recorded. Fresh weight (FW) (g) of each fruit was measured by a precise scale. Fruit width (FWth) (mm) and flesh thickness of fruits (FT) were determined by caliper. Fruit length (FL) (mm) of each fruit was measured by ruler. Also, fruit colours (FC) were determined, and different measurements were taken from two different parts of each fruit by the colorimeter during this process. Firmness of fruits (FF) was measured with a penetrometer by taking two different measurements from two different faces of each fruit.

2.2.6. Statistical analysis

Analysis of variance (ANOVA) statistical tests were performed using the statistical package JMP 16 (SAS, USA). Duncan multiple comparison test is used to compare the differences between the averages which are statistically significant according to the variance analysis results.

3. Results and Discussion

3.1. Virus symptoms

Plants were inoculated twice with an interval of 15 days using TSVW isolates. Although symptoms were observed as a result of

the first inoculation in some plants, they were obtained after the second inoculation in most of the plants. TSWV symptoms varied according to genotypes, and they were mostly observed on leaves. Symptoms such as necrotic ringspot, concentric ringspot, chlorotic ringspot, yellowish or brownish ringspots with bronzing, mosaic, mottle, leaf curving and deformity were observed on the leaves of the pepper plants. No symptoms were observed on the fruits, but some symptoms were visible on the stems. Common symptoms of TSWV such as stunting, wilting, and die back at the tips of shoots were observed on the pepper plants.

While symptoms such as necrotic, chlorotic, and concentric ringspots, wilting, stunting and die back were observed in all genotypes, in addition to these symptoms, bronzing in Geyikboynuzu and Yalova Çarliston 341, and leaf deformity in Üçburun were also observed (Figure 1).

Roggero et al. (2002) reported that TSWV causes chlorotic and necrotic ringspots, wilting of the shoots and deformity, while Ferrand et al. (2019) reported that TSWV causes concentric and chlorotic ringspots, mosaic, mottling and deformity. These symptoms reported as a result of different studies are similar to the symptoms obtained in our study.

To show the effect of TSWV on pepper plants, a scale was created by ranking the plants from mild to severe (1 to 5). While creating the disease scale, the least affected plant was numbered as 2, while the most affected plant was numbered as 5. Plant number 1 was chosen from mock-inoculated (healthy) plants that did not show stunting, had green leaves, and produced fruit. Plant number 2 was chosen from infected plants that did not show stunting, yellowed leaves, and produced less or no fruit. Plant number 3 was selected from infected plants with stunting, hardly any TSWV symptoms on leaves, and no fruit. Plant number 4 was selected from infected plants with stunting, TSWV symptoms frequently on leaves, and no fruit. Plant number 5 was selected from infected plants with stunting, complete deformation of leaves and die back on shoot tips (Figure 2).

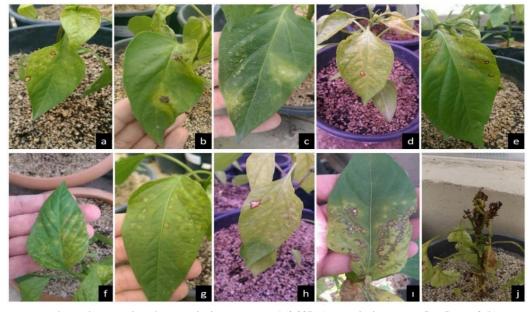


Figure 1. Symptoms are observed as necrotic and concentric ringspots on cv. 153-227 (a), necrotic ringspots on Sera Demre 8 (b), concentric ringspots on Bora 77 (c), bronzing and necrotic ringspots on Geyikboynuzu (d), concentric ringspots on Yalova Yağılık 28 (e), concentric and chlorotic ringspots on Bor (f), concentric and chlorotic ringspots on Mazamort (g), bronzing, concentric and necrotic ringspots on Yalova Çarliston 341 (h), concentric and necrotic ringspots with deformity of leaves on Üçburun (I), wilting, stunting and die back at the tips of shoots on Yalova Kıl Tatlı (j).

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3.2. TSWV infections

All samples were tested by the DAS-ELISA method to detect the presence of TSWV. Test results were evaluated both visually and numerically. The evaluation was made according to the value of the negative control. While values greater than twice the value of the negative control were considered positive. As a result of the DAS-ELISA test, 95% of the inoculated plants were determined as TSVW positive. Also, TSWV infections were molecularly detected by the RT-PCR analysis. The test was performed with two samples from each pepper genotype and a TSWV-specific primer, which amplify a region of 276 bp in size. As a result, amplicons with the expected size were obtained from the samples tested by the primer pairs used (Figure 3). The presence of TSWV in mechanically inoculated pepper plants was confirmed via RT-PCR. These results, obtained from the RT-PCR process, agree with studies of Bozdoğan and Kamberoğlu (2015) and Keleş Öztürk and Baloğlu (2019) and show that symptoms detected on inoculated plants were caused by TSWV infection.



Figure 2. The effects of TSWV infections on pepper plants. The scale created by ranking the plants from healthy and mild to severe (left to right); Yalova Yağlık 28 (a) and Mazamort (b).

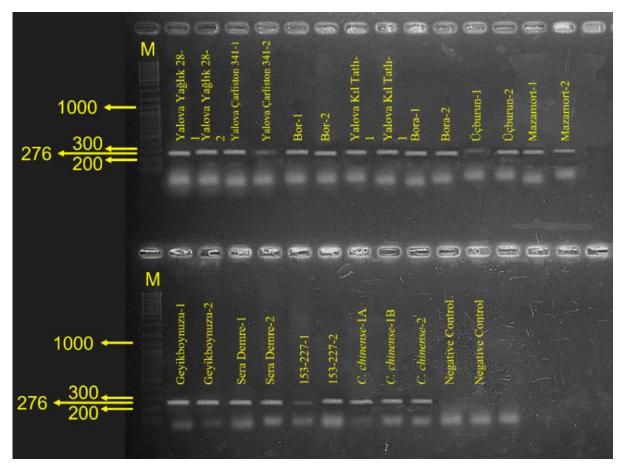


Figure 3. Results of PCR test using TSWV-specific primers (TSWV RdRp, 276 bp). As a result, bands with the expected size (276 bp) were obtained from the samples tested by the primer pairs used. The presence of TSWV in mechanically inoculated pepper plants was molecularly confirmed with these results. M: DNA Ladder.

3.3. Genotypic Characterisation

In order to determine the resistance level of the genotypes against TSWV, T_{SW} gene was investigated in these genotypes. PCR test was performed using DNAs isolated from these genotypes and using a CAPS marker (SCAC₅₆₈). PCR products were digested by using TaqI restriction enzyme.

As a result of PCR, amplicons approximately 568 bp in size were obtained and these samples were digested by the *TaqI* enzyme. Two amplicons were obtained approximately 200 and 300 bp in size in cvs. Yalova Yağlık 28, Yalova Çarliston 341, Yalova Kıl Tatlı, Bora 77, Bor, Üçburun, Mazamort, Geyikboynuzu and Sera Demre, while only a single amplicon 568 bp in size was obtained as expected for the resistant variety 153-227 and wild type *C. chinense* (Figure 4). The results were in accordance with the previous results in which the SCAC₅₆₈ marker was developed (Moury et al. 2000). In addition, completely similar results were obtained with the results of the studies conducted by Polat et al. (2012), Silvar and García-González (2017), Çelik et al. (2018) and İkten (2019).

Based on these results, *Tsw* gene was detected in cv. 153-277 pepper genotypes which is known to be a resistant variety. Although 153-227 carries the resistance gene, it was found to be infected by TSWV and showed a weak reaction according to our phenotypic analysis. The reason for these contradictory results obtained from molecular and phenotypic analysis could be due to the mechanical inoculations with a high concentration of the virus. Also, it is known that single resistance genes have a mostly temperature-dependent manner. The resistance gene *Tsw* does not provide resistance at high temperature conditions (Moury et al. 1997); Roggero et al. 1996, 2002).

3.4. Phenotypic Characterisation

According to the results of variance analysis for yield and quality parameters of pepper genotypes, it was determined that there are significant differences ($P \le 0.05$) between all mock-

inoculated and infected plants. Infected plants of Yalova Yağlık 28, Mazamort, Geyikboynuzu and Bor genotypes did not produce fruit and data could not be obtained from these groups. These groups were not included in the evaluations except for total yield and fruit number.

No fruit was obtained in the genotypes of Yalova Yağık 28, Mazamort, Geyikboynuzu and Bor infected with the TSWV. The highest fruit number decrease was obtained in these genotypes with 100%. Bora 77 (96.37%) was the group with the highest decrease after the groups that did not produce fruit. The least decrease was observed in Yalova Çarliston 341 with 45.97% (Table 1). The highest total yield loss was obtained from Yalova Yağlık 28, Mazamort, Geyikboynuzu and Bor with 100%. After the groups that did not produce fruit, the highest percent reduction was calculated for Bora 77 (98.85%), while the lowest percent reduction was calculated for Yalova Carliston 341 (68.62%) (Figure 5 and Table 1). The highest fruit weight reduction was obtained in genotype Bora 77 with 98.77%, and the lowest reduction was obtained in genotype Yalova Carliston 341 with 66.38% (Table 1). The genotype with the highest fruit width decrease was Bora 77 (96.52%) and the genotype with the lowest decrease was Yalova Çarliston 341 with 63.77% (Table 1). The highest percent fruit length reduction was calculated for Bora 77 (93.51%), while the lowest percent reduction was calculated for Yalova Carliston 341 (31.97%) (Table 2). 153-227 (82.41%) was the group with the highest flesh thickness decrease, and the least decrease was seen in Sera Demre 8 with 28.64% (Table 2). The genotype with the highest fruit firmness decrease was Bora 77 (94.43%), while the genotype with the lowest decrease was Yalova Çarliston 341 with 5.82% (Table 2). While the highest fruit colour (L value) decrease was obtained in Bora 77 (7.79%), the lowest decrease was obtained in Yalova Carliston 341 with 1.62% (Table 2). In addition, according to the results obtained from the Yalova Carliston 341 in fruit firmness and fruit colour, no significant difference was found between the infected and mock-inoculated groups.

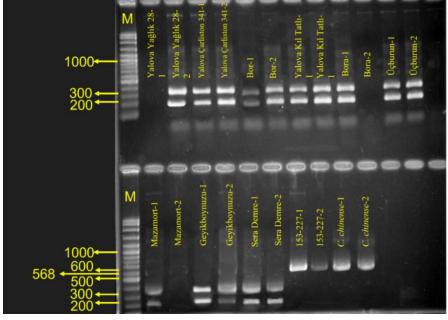


Figure 4. *Tsw* resistance gene screening results. Agarose gels indicate two bands each approximately 200 and 300 bp in cvs. Yalova Yağlık 28, Yalova Çarliston 341, Yalova Kıl Tatlı, Bora 77, Bor, Üçburun, Mazamort, Geyikboynuzu and Sera Demre, while a single band was obtained at 568 bp as expected for the resistant variety 153-227 and wild type *C. chinense*. M: DNA Ladder.

Genotypes	Total yield			Fruit Number			Fruit Weight (g)		
	TSWV Infected	Mock- Inoculated	Effect of TSWV (%)	TSWV Infected	Mock- inoculated	Effect of TSWV (%)	TSWV Infected	Mock- inoculated	Effect TSW (%)
Yalova Çarliston 341	9.58d	30.53c	-68.62	0.47def	0.87bcd	-45.97	3.19cde	9.49bc	-66.3
Yalova Tatlı Kıl	2.89d	31.42c	-90.80	0.40def	1.93a	-79.27	0.98de	9.39bc	-89.5
Yalova Yağlık 28	0d	93.50a	-100	Of	0.93bcd	-100	0e	27.02a	-100
Mazamort	0d	26.26c	-100	0f	0.73cdef	-100	0e	8.75bcd	-100
Sera Demre 8	3.97d	31.53c	-87.40	0.67cdef	1.40abc	-52.14	0.97de	8.51bcd	-88.6
Üç Burun	3.67d	50.89b	-92.78	0.27def	1.53ab	-82.35	1.22de	13.39b	-90.8
Geyikboynuzu	0d	24.86c	-100	Of	1.27abc	-100	0e	8.29bcd	-100
Bor Biberi	0d	29.98c	-100	Of	1bcd	-100	0e	11.66b	-100
Bora 77	0.32d	27.84c	-98.85	0.07ef	1.93a	-96.37	0.10e	8.19bcd	-98.7
153-227	5.7d	43.75b	-86.97	0.07ef	0.80bcde	-91.25	1.92cde	14.58b	-86.8
Std. Deviation	23.707			1.083			11.362		

Table 1. Effects of TSWV infections on total yield, fruit number, fruit weight and fruit width of pepper genotypes

Genotypes	Fruit Length (cm)			Flesh Thickness (mm)			Fruit Firmness (kg)		
	TSWV Infected	Mock- Inoculated	Effect of TSVW (%)	TSWV Infected	Mock- inoculated	Effect of TSVW (%)	TSWV Infected	Mock- inoculated	Effect TSVV (%)
Yalova Çarliston 341	10cd	14.70ab	-31.97	1.22fg	2.57ab	-52.52	5.66bc	6.01bc	-5.82
Yalova Tatlı Kıl	6.70ef	14.64ab	-54.23	0.80gh	1.66def	-51.80	3.13de	6.59bc	-52.5
Yalova Yağlık 28	0g	13.5abcb	-100	Oi	3.12a	-100	Of	6.54bc	-100
Mazamort	0g	8.16de	-100	Oi	1.91cde	-100	Of	6.70bc	-100
Sera Demre 8	8.04de	16.02a	-49.81	1.32efg	1.85def	-28.64	5.39bcd	6.82bc	-20.9
Üç Burun	3.12fg	9.10de	-65.71	0.81gh	2.51abc	-67.72	2ef	6.29bc	-68.2
Geyikboynuzu	0g	11.32bcd	-100	Oi	1.64def	-100	Of	4.80cd	-100
Bor Biberi	0g	8.52de	-100	Oi	2bcd	-100	Of	7.62ab	-100
Bora 77	1.03g	6.78ef	-93.51	0.41hi	2.25bcd	-81.77	0.51f	9.16a	-94.4
153-227	3.09fg	14.12abc	-78.11	0.54hi	3.07a	-82.41	0.91ef	7abc	-87
Std. Deviation	5.247			1.108			3.400		

Table 2. Effects of TSWV infections on fruit length, fruit thickness, fruit firmness and fruit colour of pepper genotypes

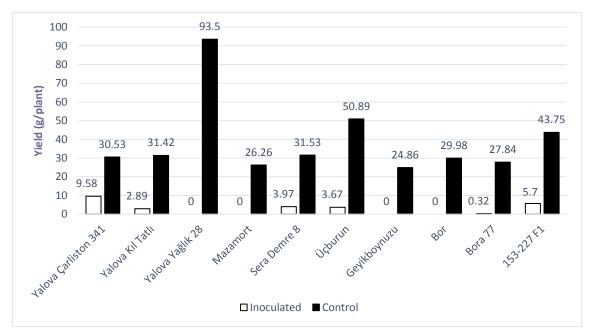


Figure 5. Total yield of TSWV infected and mock-inoculated pepper plants. The bars show the mean values. The difference between inoculated and mock-inoculated groups of each variety is shown.

4. Conclusions

According to the results of this study, it was determined that there were significant decreases in total yield and fruit quality parameters in plants infected with TSWV compared to uninfected controls. No fruit was obtained from the infected groups of Yalova Yağlık 28, Mazamort, Geyikboynuzu and Bor genotypes, and these genotypes were determined as the genotypes most sensitive to TSWV infection. It was determined that Bora 77 was the genotype with the highest decrease in yield, fruit number, fruit weight, fruit width, fruit length and fruit firmness, after the genotypes without fruit, and the genotype that was most affected by TSWV infection. Yalova Carliston 341 was the genotype with the least decrease in yield, fruit number, fruit weight, fruit width, fruit length and fruit firmness, and it was determined as the least affected genotype among the genotypes. In conclusion, the results have shown that the performance of Bora 77 was poor among the genotypes and that this genotype was highly susceptible to TSWV. The best performing Yalova Yağlık 28 suggests that it could be tolerant to TSWV. In addition, Tsw gene that provides resistance to TSWV in pepper was not detected in common genotypes tested in this study.

Although the selected genotypes used in this study have superior fruit characteristics and high yields, they were found to be sensitive to TSWV. TSWV is the most destructive viral disease in pepper plants. According to these results, in order to use the superior characteristics of these varieties and to obtain products with the desired characteristics, it is recommended to apply the virus control methods completely in order to prevent the transmission or spread of virus infection, or to transfer the resistance gene to these varieties with the breeding programmes, which are the most effective methods against viruses. The results of this study will also assist in the development of sustainable virus resistant varieties through screening/selection of tolerant/resistant varieties for the management of TSWV.

References

- Adkins S (2000) Tomato spotted wilt virus positive steps towards negative success. Molecular Plant Pathology 1(3): 151-157.
- Azeri T (1981) Preliminary report of tomato spotted wilt virus (TSWV) and its epidemiology on tobacco in the Çanakkale region of Turkey. Journal of Turkish Phytopathology 10(2-3): 79-87.
- Black LL, Hobbs HA, Gatti Jr JM (1991) Tomato spotted wilt virus resistance in *Capsicum chinense* PI 152225 and 159236. Plant Disease 75(8): 863.
- Boiteux LS (1995) Allelic relationships between genes for resistance to tomato spotted wilt tospovirus in *Capsicum chinense*. Theoretical and Applied Genetics 90(1): 146-149.
- Bozdoğan V and Kamberoglu MA (2015) Incidence and distribution of tomato spotted wilt tospovirus (TSWV) in vegetable crops in Antalya province of Turkey. Turkish Journal of Phytopathology 44(1-3): 39-50.
- Brittlebank CC (1919) Tomato diseases. Journal of Agriculture Victoria 27: 231-235.
- Clark MF, Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34(3): 475-483.
- Çelik İ, Özalp R, Polat İ, Çelik N (2010) Biberde domates lekeli solgunluk virus (tomato spotted wilt virus-TSWV) hastalığı, yayılışı ve dayanıklılık çalışmaları. In: VIII. Sebze Tarımı Sempozyumu. Van, Türkiye, pp. 477-484.
- Çelik İ, Özalp R, Çelik N, Polat İ, Sülü G (2018) Domates lekeli solgunluk virüsü (TSWV)'ne dayanıklı sivri biber hatlarının geliştirilmesi. Derim 35(1): 27-36.
- Doyle J, Doyle JL (1987) Genomic plant DNA preparation from fresh tissue-CTAB method. Phytochem Bull 19(11): 11-15.
- Ferrand L, Almeida MMS, Orílio AF, Dal Bó E, Resende RO, García ML (2019) Biological and molecular characterization of tomato spotted wilt virus (TSWV) resistance-breaking isolates from Argentina. Plant Pathology 68(9): 1587-1601.

- İkten H (2019) Farklı genetic kaynaklardan elde edilen F2 biber genotiplerinde (*Capsicum annuum* L.) TSWV'ye dayanıklılığın moleküler analizi. Mediterranean Agricultural Sciences 32(1): 43-48.
- Keleş Öztürk P, Baloğlu S (2019) Doğu Akdeniz Bölgesi'nde açık alanda yetiştirilen biberlerde bazı virüslerin serolojik ve moleküler tanısı. Alatarım 18(1): 1-11.
- Konieczny A and Ausubel FM (1993) A procedure for mapping Arabidopsis mutations using codominant ecotype-specific PCRbased markers. Plant Journal 4: 403-410.
- Lefebvre V, Caranta C, Pflieger S, Moury B, Daubèze AM, Blattes A, Ferrière C, Phaly T, Nemouchi G, Ruffinato A, and Palloix A (1997). Updated intraspecific maps of pepper. Capsicum and Eggplant Newsletter 16: 35-41.
- Margaria P, Ciuffo M, Rosa C, Turina M (2015) Evidence of a tomato spotted wilt virus resistance-breaking strain originated through natural reassortment between two evolutionary-distinct isolates. Virus Research 196: 157-161.
- Moury B, Palloix A, Selassie KG, Marchoux G (1997) Hypersensitive resistance to tomato spotted wilt virus in three *Capsicum chinense* accessions is controlled by a single gene and is overcome by virulent strains. Euphytica 94(1): 45-52.
- Moury B, Pflieger S, Blattes A, Lefebvre V, Palloix A (2000) A CAPS marker to assist selection of tomato spotted wilt virus (TSWV) resistance in pepper. Genome 43(1): 137-142.
- Paran I and Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theoritical and Applied Genetics 85: 985-993.
- Perez YV, Mejias A, Rodriguez-Roman E, Avilan D, Zambrano KA, Gomez JC, Olachea JE, Marys EE (2014) Identification of Tomato spotted wilt virus associated with fruit damage during a recent virus outbreak in pepper in Venezuela. Plant Disease 99(6): 896-896.
- Polat I, Celik I, Celik N and Ozalp R (2012) Biological and molecular determination for resistance to tomato spotted wilt virus (TSWV) in F2 population of long-type pepper (*Capsicum annuum* L.). In: International Symposium on Biotechnology and Other Omics in Vegetable Science. Antalya, Turkey, pp. 115-120.

- Roggero P, Lisa V, Nervo G and Pennazio S (1996) Continuous high temperature can break the hypersensitivity of *Capsicum chinense* 'PI 152225' to tomato spotted wilt tospovirus (TSWV). Phytopathologia Mediterranea 35(2): 117-120.
- Roggero P, Masenga V and Tavella L (2002) Field isolates of tomato spotted wilt virus overcoming resistance in pepper and their spread to other hosts in Italy. Plant Disease 86(9): 950-954.
- Scholthof KBG, Adkins S, Czosnek H, Palukaitis P, Jacquot E, Hohn T, Hohn B, Saunders K, Candresse T, Ahlquist P, Hemenway C, Foster GD (2011) Top 10 plant viruses in molecular plant pathology. Molecular Plant Pathology 12(9): 938-954.
- Silvar C and García-González CA (2017) Screening old peppers (*Capsicum* spp.) for disease resistance and pungency-related traits. Scientia Horticulturae 218: 249-257.
- Şevik M (2008) Thrips (Thripidae: Thysanoptera) türleri ile taşınan bitki virüsleri. Derim 25(1): 1-11.
- Şimşek D, Pınar H, Mutlu N (2015) Moleküler ıslah yöntemleri kullanılarak Tospovirus ve Tobamoviruslere dayanıklı yeni dolmalık biber (*Capsicum annum* L.) hat ve çeşitlerinin geliştirilmesi. Alatarım 14(1): 1-8.
- Tekinel N, Dolar MS, Sağsöz S, Salcan Y (1969) Mersin bölgesinde ekonomik bakımdan önemli bazı sebzelerin virüsleri üzerinde araştırmalar. Bitki Koruma Bülteni 9(1): 37-49.
- Turhan P, Korkmaz S (2006) Çanakkale ilinde domates lekeli solgunluk virüsünün serolojik ve biyolojik yöntemlerle saptanması. Tarım Bilimleri Dergisi 12(2): 130-136.
- TÜİK (2020) https://www.tuik.gov.tr/. Accessed 2 November, 2021.
- Ullman DE, Cho JJ, Mau RF, Westcot DM, Custer DM (1992) A midgut barrier to tomato spotted wilt virus acquisition by adult western flower thrips. Phytopathology 82: 1333-1333.
- Welsh J, and McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213-7218.
- Williams JGK, Kubelik AR, Livak KJ, Rafalsky A, and Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.