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Developing Resistant/Tolerant Charleston Pepper Varieties to *Tomato Spotted Wilt Virus* (*TSWV*) Disease Using Existing Pepper Lines

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Pepper is a vegetable that belongs to the Capsicum genus, which includes 25 species and is part of the Solanaceae family. Several abiotic and biotic stress factors can limit pepper yield in cultivation. One of the top ten viruses that limit pepper production worldwide and cause economic losses is *tomato spotted wilt virus* (TSWV). The use of chemical control along with cultural practices against viral diseases may sometimes not be effective in controlling the disease. However, the use of resistant varieties is considered the most effective and eco-friendly method of control in the cultivation of peppers. In this study, PCR reactions were conducted using SCAC568 primers to evaluate the resistance/tolerance or susceptibility of 27 different hybrid varieties of Charleston peppers grown under cover, as determined by our breeding program. The PCR products obtained were genotypically analyzed after being digested with *Xba*I. Based on the results, it was determined that out of the 27 Charleston pepper varieties studied, 66.6% were tolerant and heterozygous (Rr), while 33.4% were susceptible. This study aims to develop TSWV-resistant Charleston pepper varieties.

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Mevcut Biber Hatları Kullanılarak Domates Benekli Solgunluk Virüsü (*TSWV*) Hastalığına Karşı Dayanıklı/Toleranslı Çarleston Biber Çeşitlerinin Geliştirilmesi

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Öz

Biber, Solanaceae familyasına ait, 25 tür içeren Capsicum cinsine ait bir sebzedir. Biber yetiştiriciliğinde biber verimini sınırlayabilen hem abiyotik hem de biyotik çeşitli stres faktörleri bulunmaktadır. Dünya çapında biber üretimini sınırlayan ve ekonomik kayıplara neden olan ilk on virüsten biri domates benekli dolgunluk virüsüdür (TSWV). Viral hastalıklara karşı kültürel uygulamalarla birlikte kimyasal mücadelenin kullanılması bazen hastalığın kontrolünde etkili olamayabilir. Ancak biber yetiştiriciliğinde dayanıklı çeşitlerin kullanılması en etkili ve çevre dostu mücadele yöntemi olarak kabul edilmektedir. Bu çalışmada, örtü altında yetiştirilen 27 farklı hibrit Charleston biber çeşidinin ıslah programımız tarafından belirlenen direnç/tolerans veya duyarlılığını değerlendirmek için SCAC568 primerleri kullanılarak PCR reaksiyonları gerçekleştirildi. Elde edilen PCR ürünleri *Xba*I ile sindirildikten sonra genotipik olarak analiz edildi. Sonuçlara göre incelenen 27 Charleston biber çeşidinin %66,6'sının toleranslı ve heterozigot (Rr), %33,4'ünün duyarlı olduğu belirlendi. Bu çalışmanın amacı, TSWV'ye dayanıklı/toleranslı Charleston biber çeşitlerinin geliştirilmesidir.

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Introduction

The Capsicum genus, which includes 25 species, belongs to the Solanaceae family, along with vegetables such as tomatoes and eggplants, and is part of the Solanales order (Şalk et al., 2008). In 1492, Christopher Columbus and his companions discovered America and encountered chili peppers. They received credit for discovering chili peppers along with the Americas. Pepper cultivation, which started in the 1600s, gradually became widespread worldwide (Eşiyok, 2006; DeWitt et al., 1990). The species was introduced in our country through trade with Europeans during the mid-16th century, and it gradually spread throughout Anatolia (Vural et al., 2000). *Capsicum annuum L*., *C. frutescens L*., *C. baccatum L*., *C. chinense* Jacq, and *C. pubescens* Ruiz & Pav are among the most important commercially produced peppers (Şalk et al., 2008). *C. annuum* is a plant species with a wide range of adaptability and plays an important role in the economy (Onus, 2001; Fidan & Barut, 2019).

Peppers rank third in vegetable production after tomatoes and cucumbers based on the Turkish Statistical Institute (TÜİK) data released in 2024. The total pepper production quantity was 1,068,884 tons. Peppers are cultivated in almost every region of Türkiye and are processed in various forms in the food industry, including canned, paste, spices, frozen food, pickles, sauces, dyes, and pharmaceuticals (Aybak, 2007). Approximately 8.52% of the total global pepper production, equivalent to 36,286,640 million tons, comes from Türkiye. China is the leading pepper producer followed by Türkiye, Indonesia, Mexico, Spain, Egypt, Nigeria, the USA, and other countries (FAO, 2022). Pepper is a major crop in Türkiye, mainly grown in the Mediterranean, Aegean, and Marmara regions. The most commonly produced types of pepper are Capiya (1.4 million tons), Long Green Pepper (979 thousand tons), Bell Pepper (404 thousand tons), and Charleston (153 thousand tons). Charleston peppers have a thicker fruit structure than pointed peppers. On average, they are around 20-22 cm long with fruit diameters ranging from 5-6 cm (Öztekin, 2019).

Pepper cultivation faces various stressors that can limit productivity, one of which is *tomato spotted wilt virus* (TSWV) disease. This virus causes huge economic losses and poses a danger to pepper output worldwide. (Goldbach & Peters, 1994; Parrella, 2003). According to Dal Bo et al. (1999), TSWV is a type of Tospovirus that spreads rapidly in production areas through the *Frankliniella occidentalis* vector. TSWV was initially reported in Australia and can infect a wide variety of plants, including tomatoes, peppers, lettuce, potatoes, papayas, peanuts, and tobacco (German et al., 1992; Parrella et al., 2003). In Türkiye, TSWV was first detected in lettuce plants by Tekinel et al. (1969) and later found in tomatoes as well (Tekinel, 1973; Fidan 1993; Fidan, 1995; Azeri, 1994; Güldür ve ark., 1995; Güldür, 1997; Arlı-Sökmen ve Sevik, 2006; Turhan ve Korkmaz, 2006; Yardımcı ve Kılıç, 2009), tobacco (Azeri, 1994), and peppers (Yurtmen et al., 1999).

Tomato spotted wilt virus (TSWV) is an extensively studied virus (Goldbach & Peters, 1994; Parrella, 2003). It can cause various symptoms in peppers, which may differ depending on factors such as plant species, variety, age, growth stage (seedling, flowering, fruiting stage), nutrition, and ecological conditions (temperature, light, etc.). Common symptoms include stunted growth, yellowing (chlorosis), wilting, chlorotic streaks, necrotic spot-like mosaic formations (leaf deformation), and necrotic streaks that spread to the terminal shoots and occur throughout the stem (Fidan, 1993; Soler et al., 1998; Kim et al., 2004).

"In mature fruits, concentric yellow rings or necrotic streaks may be observed, and severe virus infections can lead to plant death (Güldür et al., 1995; Kim et al., 2004; Arli-Sokmen et al., 2005; Yardımcı & Kılıç, 2009; Scholthof et al., 2011; Atakan et al., 2013). In addition to cultural practices in the control against viral diseases in pepper cultivation, chemical control against their vectors is also

frequently used. However, these methods are sometimes ineffective in managing the spread of diseases. The most effective and environmentally friendly approach is using resistant varieties. Therefore, developing resistant varieties is an important aspect of plant breeding. In addition to improving quality and yield, using resistant cultivars is a sustainable and environmentally friendly approach (Qi et al., 2022; Boiteux, 1995; Moury et al., 1997; Duman et al., 2020). The production of vegetables in protected environments primarily involves using hybrid varieties, especially for vegetables such as tomatoes, peppers, eggplants, cucumbers, and watermelons. Hybrid varieties are increasingly popular due to their superior qualities compared to standard varieties. They provide higher quality and more consistent products, ensure high yield and early harvest, and are resistant to various diseases and pests. Additionally, they have high adaptability (Yanmaz, 2006). In the field of vegetable breeding, researchers give priority to external quality traits like yield, fruit color, and fruit size, and also work on improving resistance to diseases and pests. However, it's important to note that consumers highly value the richness of vegetables in phytochemicals, taste, and aroma compounds. Hence, there are ongoing efforts to prioritize these characteristics as breeding targets (Abak, 2022). Improvement of vegetable species will increase consumption and production (Salles, 2008). Marker-assisted selection (MAS) streamlines breeding studies, yielding results quicker and with less labour than traditional methods, while also reducing the necessary population size (Gupta & Rustgi, 2004).

The transfer of traits important for agriculture, controlled by multiple genes or loci can be effectively achieved using MAS (Marker-Assisted Selection). A study conducted by Moury et al. (2000) aimed to develop markers for TSWV and identified four RAPD (Random Amplified Polymorphic DNA) markers linked to the Tsw gene. This was achieved using interspecific hybrid populations of *C. chinense* PI 152225 and *C. frutescens* PI 195301. These markers were then converted into a specific co-dominant CAPS marker (SCAC568), used for molecular breeding studies. The marker facilitates the backcrossing of the *Tsw* gene and transfers it to agronomically superior varieties and lines using MAS. Although the homolog of *Sw-5* has not been mapped, phenotypically similar genes exist in tomatoes, and many *Sw-5* homologs have been found in similar regions in both tomatoes and peppers. The gene (*Tsw*) responsible for the resistance to *TSWV* has been identified in several *C. chinense* pepper genotypes such as 'PI 152225,' 'PI 159236,' 'CNPH 275,' 'C00943,' and '7204.' It has been mapped on chromosome 10 and transferred to cultivated varieties of *Capsicum annum* (Black et al., 1991; Costa et al., 1995; Boiteux, 1995; Moury et al., 1997; Jahn et al., 2000). The T*sw* gene can be detected using RAPD, SCAR, and CAPS markers (Welsh & McClelland, 1990; Williams et al., 1990; Lefebvre et al., 1997). Markers for detecting *Tsw* are commonly CAPS, while *RAPD*s often lack polymorphism, making marker-assisted selection unreliable (Paran & Michelmore, 1993). To address certain challenges, it is possible to transform a RAPD fragment into a SCAR (Sequence Characterized Amplified Region) marker. These markers are created from the sequence of RAPD fragments and provide higher resolution with more specific primers. However, the primers used can often result in monomorphic amplifications and the loss of polymorphism. To obtain this polymorphism, CAPS (Cleaved Amplified Polymorphic Sequence) markers can be created through the enzymatic restriction of SCAR (Konieczny & Ausubel, 1993; Moury et al., 2000).

The purpose of this study is to develop TSWV-resistant Charleston pepper varieties using molecular-assisted selection with SCAC568 markers in hybrid varieties identified through our breeding program.

Material and Method

Twenty seven different hybrid Charleston pepper varieties determined by our breeding program were used in the genotyping analysis. We obtained leaf samples from each genotype and stored them in sterile 1.5 ml Eppendorf tubes. DNA was isolated from the leaf samples using the CTAB protocol (Doyle and Doyle, 1990). The concentration and quality of DNA were assessed using a spectrophotometer. PCR

reactions were carried out using SCAC568 primers to assess the resistance/tolerance or susceptibility of 27 different hybrid varieties of Charleston peppers. PCR reactions were conducted using specific forward (5'GTGCCAGAGGAGGATTTAT 3') and reverse (5'GCGAGGTGACACTGATACT 3') primers designed for the *Tsw* gene by Moury et al. (2000). For each sample, a PCR reaction mixture was prepared which included 1.3 µl DNA (50 ng/µl), 5.36 µl ddH2O, 0.87 µl dNTP Mix (10 mM, 2.5 mM each), 0.7 µl 10x DreamTaq Buffer (including 20 mM MgCl2), 0.17 µl forward primer, 0.17 µl reverse primer, and finally, 0.043 µl DreamTaq DNA polymerase (500 U, 5U/µl). The PCR cycling conditions had already been optimized, with 30 cycles consisting of an initial denaturation at 94°C for 3 minutes, followed by denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The PCR products underwent digestion using Thermo Scientific FastDigest *Xba*I. To perform the digestion reaction, a reaction mixture containing 6 µl of ddH_2O, 0.66 *Xba*I (1500 U, 10U/µl), and 0.66 10x buffer Tango (with BSA) was prepared and then incubated in a water bath at 37°C overnight. The resulting digestion products were loaded onto a 1.5% agarose gel prepared in 1xTAE (Tris-Acetate-EDTA) buffer with 0.5 mg/ml ethidium bromide. The electrophoresis was run at 115 volts for approximately 120 minutes. Finally, the results were visualized using a UV transilluminator. On agarose gel, susceptible (*rr*) genotypes of the pepper varieties showed up as a single band, roughly 568 bp in length, while heterozygous (*Rr*) lines appeared as two bands, one cut and one uncut, approximately 280 bp and 568 bp in length, respectively.

Results and Discussion

In this study, PCR reactions were carried out using SCAC568 primers to assess the resistance/tolerance or susceptibility of 27 different hybrid varieties of Charleston peppers grown under cover, as determined by our breeding program. The PCR products obtained were genotypically analyzed after being digested with *Xba*I. On agarose gel, susceptible (*rr*) genotypes showed up as a single band, roughly 568 bp in length, while heterozygous (*Rr*) lines appeared as two bands, one cut and one uncut, approximately 280 bp and 568 bp in length, respectively. Based on the results, it was determined that out of the 27 Charleston pepper varieties studied, 66.6% were tolerant and heterozygous (*Rr*) while 33.4% were susceptible (as shown in Figure 1 and Table 1).

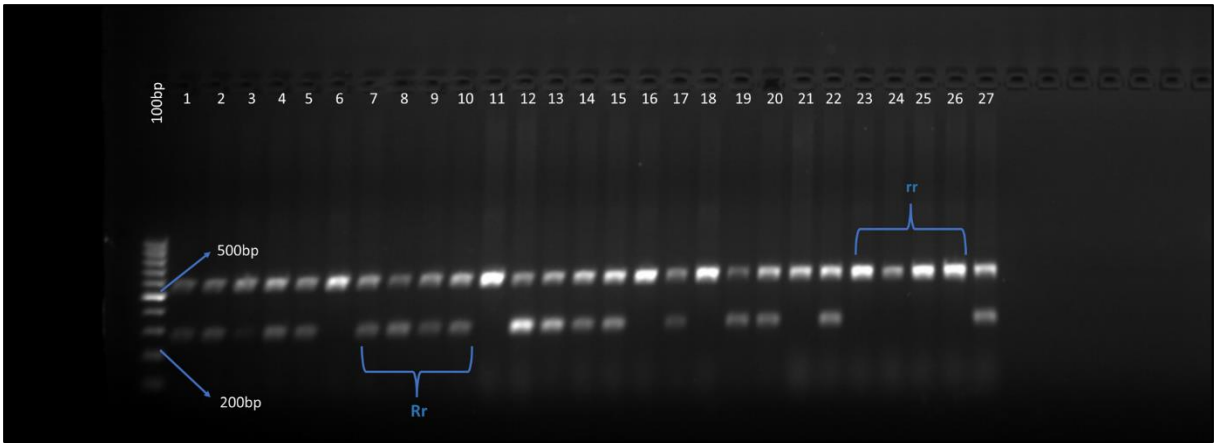

Figure 1. PCR of the susceptibility of hybrid Charleston peppers to TSVW.
Rr: heterozygote (568 bp+ 280 bp), *rr*: recessive homozygote (580 bp).

Table1. PCR of Susceptibility of Hybrit Charleston Peppers to *TSVW.*

Test No	*TSWV*	Test No	*TSWV*	Test No	*TSWV*
1	*Rr*	10	*Rr*	19	*Rr*
2	*Rr*	11	*rr*	20	*Rr*
3	*Rr*	12	*Rr*	21	*rr*
4	*Rr*	13	*Rr*	22	*Rr*
5	*Rr*	14	*Rr*	23	*rr*
6	*rr*	15	*Rr*	24	*rr*
7	*Rr*	16	*rr*	25	*rr*
8	*Rr*	17	*Rr*	26	*rr*
9	*Rr*	18	*rr*	27	*Rr*

Rr:heterozygote (568 bp+ 280 bp), *rr*: recessive homozygote (580 bp).

Tomato spotted wilt virus (TSWV) is ranked among the top 10 viruses that cause the most damage in cultivated vegetables (Goldbach and Peters, 1994; Griep et al., 2000). Because of its economic significance, TSWV is one of the most intensively studied plant viruses today (Parrella et al., 2003). Crops significantly affected by TSWV include tomatoes, peppers, eggplants, lettuce, beans, artichokes, celery, and tobacco (Rosello et al., 1996).

In greenhouses where pepper is grown, the plant's green color can attract insect vectors. This leads to the spread of viral diseases through transmission by insect vectors such as aphids, thrips, and whiteflies. The most effective and environmentally friendly solution to control these viral diseases is to develop pepper varieties that are resistant or tolerant to them. There is no chemical control for viruses including TSWV. Control of its thrips vector is also difficult.

It is crucial to properly identify and select resistant and susceptible plants for breeding studies on resistance. This allows for the testing of a large number of breeding materials in a short time using a more reliable method. Marker development studies for important diseases continuously develop new markers alongside existing ones to enhance resistance genes (Moury et al., 2000; Shi et al., 2011; Yang et al., 2012; Nevame et al., 2018).

In a study conducted by Çelik et al. (2018), pepper breeding lines were tested for resistance to This allows for the testing disease using molecular markers. A total of 210 plant seedlings were screened with the SCAC568 marker, revealing that 44 plants were susceptible to the disease, while 166 plants were found to be heterozygous resistant. Molecular tests were applied to plants at the F1 and F5 stages to confirm mechanical inoculation. The researchers used the SCAC568 marker to determine resistance at the DNA level, demonstrating its effective use for selection purposes in TSWV resistance.

In another study, İkten (2019) compared the results of cutting F2 pepper genotypes obtained from different genetic sources for *tomato spotted wilt virus* (TSWV) disease using two different enzymes, *Xba*I and *Taq*I, with the SCAC568 marker. It was observed that F2 pepper genotypes cut with *Xba*I predominantly produced two bands: one approximately 280 bp for homozygous resistant (*RR*) genotypes and two bands at approximately 568 bp and 280 bp for heterozygous (*Rr*) genotypes. Susceptible (*rr*) genotypes remained uncut, forming a single band at 568bp, which matches our study's results obtained

using *Xba*I. It has been found that using the *Xba*I enzyme, along with other genetic markers, combined with PCR analysis, has successfully determined the sensitivity and resistance of 27 pepper varieties to TSWV. In a study by Şimşek et al. (2015), resistance-associated molecular markers L3, L4, and *Tsw* alleles were utilized to develop pepper lines and varieties resistant to virus diseases such as PMMoV, TMV, and TSWV. Many studies showed that the use of molecular markers in plant breeding programs is fast, easy, and beneficial. However, it's important to note that genotypes developed through molecular marker-assisted selection may show different results in field conditions due to the complexity of pathogens, the emergence of new variants, and variability in virulence. A genotype that is resistant in one region may be sensitive in another. Therefore, after exposure to region-specific pathogens, it's vital to confirm both the reliability of the marker and the genotype's resistance.

Following this study, we plan to conduct additional research to verify the resistance and tolerance of the resulting pepper varieties This will involve performing pathogenicity tests using pathogens from different geographical regions to confirm the resistance/tolerance. Furthermore, we will assess the sensitivity of TSWV-resistant/tolerant varieties to different biotic markers separately. This approach will help us develop resistance against various biotic factors in the pepper varieties obtained from this study in the future. By using this method, we will be able to test a larger number of materials in our pepper-related resistance breeding studies. Ultimately, this will streamline the process and improve our success rate in achieving our research goals.

Conclusions

After completing this research, we will select heterozygous resistant/tolerant hybrid Charleston peppers from the susceptible and heterozygous genotypes obtained against TSWV disease. These selected peppers will then undergo pathological testing. In the upcoming season, we will plant the seeds of these selected peppers, and they will be used in breeding experiments to develop resistant/tolerant varieties and lines against TSWV disease in Charleston pepper. Our breeding process will combine molecular and conventional techniques. This study demonstrates that we can develop resistant/tolerant varieties to TSWV disease in Charleston pepper by integrating molecular and traditional breeding methods. This method will allow us to test a wider range of materials and shorten the duration of breeding programs, ultimately increasing the success rate. Modern breeding methods will enable us to hybridize individuals with desired traits among the selected heterozygous individuals.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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