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Molecular Analysis of Capsicum Breeding Lines for Resistance to *Tomato Spotted Wilt Virus* (TSWV)

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Abstract

This study was conducted to determine the molecular resistance status of 35 different pepper breeding lines against *Tomato Spotted Wilt Virus* (TSWV). PCR amplification was performed using the $SCAC_{568}$ CAPS marker targeting the *tsw* resistance gene. The PCR products were digested with the *Xba*I restriction enzyme and subsequently analyzed by agarose gel electrophoresis. Based on the resulting band patterns, the genotypes were classified, revealing that 22 pepper breeding lines were heterozygous resistant/tolerant (*Rr*), while 13 lines were susceptible (*rr*). The identified resistant pepper breeding lines will be subjected to further selection and pathogenicity testing and integrated into hybrid breeding programs. This study contributes to local seed improvement efforts and demonstrates that marker-assisted selection is an effective and reliable strategy for developing TSWV-resistant *Capsicum* cultivars.

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Biber Hatlarının *Domates Benekli Solgunluk Virüsü* (TSWV)'ne Dayanıklılık Bakımından Moleküler Analizi

Makale bilgileri

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Öz

Bu çalışma, 35 farklı capsicum biber ıslah hattının *Domates Benekli Solgunluk Virüsü*'ne (TSWV) karşı moleküler dayanıklılık durumunu belirlemek amacıyla yürütülmüştür. PCR amplifikasyonu, *tsw* dayanıklılık genini hedef alan $SCAC_{568}$ CAPS markörü kullanılarak gerçekleştirilmiştir. PCR ürünleri *Xba*I restriksiyon enzimi ile kesilmiş ve daha sonra agaroz jel elektroforezi ile analiz edilmiştir. Elde edilen DNA bant örüntülerine dayanarak, genotipler sınıflandırılmış ve 22 biber ıslah hattının heterozigot dayanıklı/tolerant (*Rr*), 13 hattın ise duyarlı (*rr*) olduğu ortaya çıkmıştır. Belirlenen dayanıklı biber ıslah hatları daha ileri seleksiyon ve patojenite testlerine tabi tutulacak ve melez ıslah programlarına entegre edilecektir. Bu çalışma, yerel tohum ıslah çalışmalarına katkıda bulunmakta ve markör destekli seleksiyonun TSWV'ye dayanıklı capsicum çeşitlerinin geliştirilmesi için etkili ve güvenilir bir strateji olduğunu göstermektedir.

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Introduction

Pepper is a widely consumed vegetable, particularly popular in the Mediterranean region and Turkey. In Türkiye, pepper production increased by 11.3% in 2024, reaching a total of 3,428,028 tons (TUIK, 2024). Peppers belong to the Capsicum genus within the Solanaceae family and display a wide range of genetic diversity, including 41 species (Barboza et al., 2020). The most common species in this genus are *Capsicum annuum* L., *C. frutescens* L., *C. chinense* Jacq., *C. baccatum* L., and *C. pubescens*. Production mainly focuses on these species or their hybrids (Ruiz et al., 2005). Pepper is known to have originated in Central and South America and was introduced to Europe by Christopher Columbus at the end of the 15th century. It later spread to Africa, India, and China, reaching Türkiye through trade in the mid-16th century (Pernezny et al., 2003).

Capsicum species are versatile and can be utilized in various forms, whether fresh or dried. Their characteristics, such as fruit shape (which can be isosceles, triangular, horn-shaped, narrow triangular, heart-shaped, round, square, or flat), size, color (including yellow, red, and green), and aroma, contribute to their wide usage (Govindarajan et al., 1987).

The sustainability of pepper production faces threats from various diseases and pests, despite the rising demand. These issues are significant because they cause yield losses and drive more research. Plant diseases cause about 15% of annual yield losses, with viral diseases making up roughly 30% of these losses (Islam et al., 2018). Viral diseases are especially troubling since they pose a major risk to the long-term viability of agricultural production.

Among the viral diseases that threaten sustainable agricultural production, the *Tomato Spotted Wilt Virus* (TSWV) stands out as a significant plant pathogen. It belongs to the Tospovirus group within the Bunyaviridae family and causes substantial losses in various crops worldwide (Uhrig et al., 1999; Tsompana et al., 2005). Initially identified in Australia, TSWV quickly spread to many regions, especially in the Americas, Europe, Asia, and Africa (Brittlebank, 1919; Cho et al., 1986). TSWV infects over 1.000 host plants, including key vegetables like tomatoes, peppers, eggplants, lettuce, and beans (Hanssen et al., 2010). The virus can cause various symptoms such as leaf chlorosis, ring-shaped spots, deformation, top wilt, and ring spots on fruit surfaces, leading to crop losses of 30% to 100% (Cho et al., 1986; German et al., 1992; Rosello et al., 1996; Chiemsombat & Adkins, 2006; Şevik, & Tohumcu, 2010). Thrips, insects from the Thysanoptera order, are crucial in transmitting TSWV. They can carry the virus throughout their lives after infection during their larval stage and can transmit it to host plants they feed on (Jones, 2005). Although strategies like removing host plants, controlling vector populations, and disinfecting infected plants are used to control TSWV, these approaches are often not enough

(Roditakis et al., 2001). Therefore, more effective and sustainable methods are needed to reduce TSWV's impact. The most effective way to control TSWV is to select resistant varieties. Therefore, developing pepper varieties resistant to TSWV should be a key goal in plant breeding. To start breeding studies, breeders need access to a large pool of genetic resources. Currently, local varieties, which are declining, are some of the most valuable sources of genetic diversity and can be essential for breeding. However, traditional breeding methods can take many years, resulting in significant losses of time and resources. Molecular marker techniques such as RFLP (Restriction Fragment Lenght Polymorphism), RAPD (Random amplified Polymorphism), AFLP (Amplified Fragment Lenght Polymorphism), SNP (Single Nucleotide Polymorphism), and SRAP (Sequence-Related Amplified Polymorphism) have greatly improved research in breeding studies, enabling researchers to reach their goals more efficiently (Li & Quiros, 2001). The molecular markers developed in recent years have not only decreased the time and cost associated with plant breeding, but they have also offered significant advantages. In pepper breeding, the use of molecular markers that provide resistance to various pathogens has greatly helped in developing resistant lines and creating new varieties (Moury et al., 2000). For example, four different RAPD markers linked to the *tsw* gene were identified using interspecific hybrid populations of *Capsicum chinense* PI 152225 and *Capsicum frutescens* PI 195301. These markers were later converted into a common CAPS marker called SCAC568, which is now widely used for detecting the *tsw* gene.

The aim of this study is to identify genotypes of capia-type pepper breeding lines that are resistant or tolerant to the *Tomato Spotted Wilt Virus* (TSWV) through molecular analyses conducted as part of the R&D programs at İstanbul Tarım A.Ş. Additionally, the study aims to develop hybrid capia-type pepper varieties derived from these genotypes. To reduce the economic losses caused by TSWV, we will identify genes that confer resistance or tolerance to this virus using the specified molecular marker. This research seeks to accelerate market diversification and reduce costs, protect local genetic resources, and support sustainable agriculture and plant breeding by developing new capia-type pepper varieties with improved resistance.

Material and Method

This study was conducted within the framework of a breeding program at Istanbul Tarım A.Ş. to determine the molecular resistance status of 35 different *Capsicum* pepper breeding lines against Tomato Spotted Wilt Virus (TSWV). Genomic DNA was extracted from young leaf tissues using Cetylrimethylammonnium bromide (CTAB) (Doyle and Doyle, 1990), and PCR amplification was performed using the $SCAC_{568}$ CAPS (Cleaved Amplified Polymorphic Sequence) marker targeting the *Tsw* resistance gene. PCR reactions for TSWV were optimized to a total volume of 10.5 µL. The reaction

components for one sample included 1.2 µL of DNA (50 ng/µL), 1.25 µL of 10X Dream Taq buffer (containing 20 mM MgCl2), 1 µL of dNTPs (2.5 mM), 0.25 µL of Taq polymerase (5 U), 0.25 µL of forward and reverse primers (10 mM), and deionized water. PCR reactions were completed in; initial denaturation: 94°C for 3 min. 35 cycles of denaturation: 94°C for 30 sec. Annealing: 53°C for 1 min. Extension: 72°C for 1 min. Final extension: 72°C for 10 min. The obtained PCR products were loaded onto 1.5% agarose gel prepared in 1% X TAE buffer, and gel electrophoresis was performed at 100 volts for 100 minutes. A 100 bp ladder was used to determine the band sizes. DNAs were stained with ethidium bromide (0.5 mg/mL) and Analyzed using a Vilber Lourmat (France) ultraviolet (UV) imaging system.

The PCR products were digested with the *Xba*I restriction enzyme and then analyzed by agarose gel electrophoresis (Thermo Fisher Scientific, USA). Based on the resulting band patterns, the genotypes were classified, revealing that 22 pepper lines were heterozygous resistant (*Rr*), while 13 pepper lines were susceptible (*rr*)

Results and Discussion

The identified resistant individuals will undergo further selection and pathogenicity testing and will be integrated into hybrid breeding programs. This study contributes to local seed improvement efforts and shows that marker-assisted selection is an effective and reliable strategy for developing TSWV-resistant Capsicum varieties.

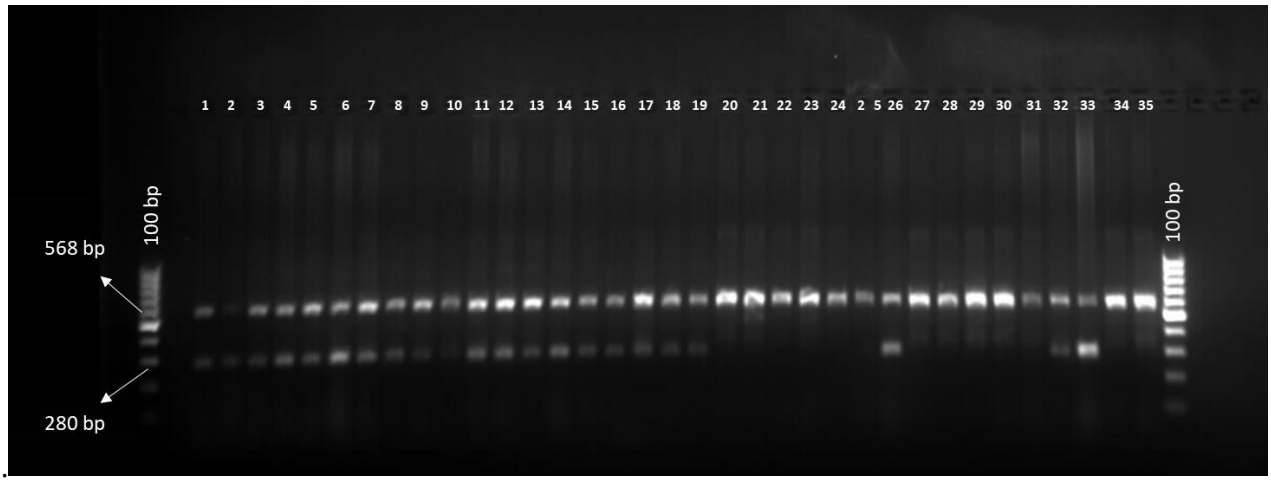
Table 1. Molecular marker and restriction enzyme sequences used in study.

Primer Name	Restriction Enzyme	Primer Sequence (5′-3′)	Molecular Size of PCR Product	References
$SCAC_{568}$	*Xba*I	Forward Primer 5’ GTGCCAGAGGAGGATTTAT 3’ Reverse Primer 5’ GCGAGGTGGACACTGATAC 3’	280 bp (resistant) 568 bp (ressesive)	Moury et al. 2000

The expanding global population continuously increases the demand for food, along with the need for agricultural production. However, one major challenge to agricultural productivity is plant diseases. While some diseases can be managed using chemical methods, there is currently no effective chemical

control for certain viral diseases, such as TSWV. TSWV can spread quickly through seeds, seedlings, host plants, and vectors, causing significant economic losses in agriculture. Therefore, developing TSWV-resistant or tolerant plant varieties is among the most effective strategies to control this disease.

In this study, we performed PCR analyses using primers developed by Moury et al. (2000) for the TSWV resistance gene (*tsw*). Our results showed different genotypic band patterns in capia pepper populations, based on cutting reactions with the *Xba*I enzyme. The PCR products of sensitive genotypes (*rr*) were not cut by *Xba*I, producing a single band of 568 bp. Meanwhile, heterozygous genotypes (*Rr*) yielded two bands: one at 280 bp, which was cut, and another at 568 bp, which remained uncut (see Figure 1). Analysis of the gel results identified 13 capsicum varieties as susceptible (*rr*) and 22 as heterozygous resistant/tolerant to TSWV (see Figure 1 and Table 2).



2).

Figure 1. The gel image after cutting with *Xbal* enzyme (280 bp, 568 bp: Rr, 568 bp: rr, 100 bp ladder)

Table 2. Genotypic Characteristics of Capia Pepper Varieties

Test No	*TSWV*	Test No	*TSWV*	Test No	*TSWV*
1	*Rr*	13	*Rr*	25	*Rr*
2	*Rr*	14	*Rr*	26	*Rr*
3	*Rr*	15	*Rr*	27	*Rr*
4	*Rr*	16	*Rr*	28	*Rr*
5	*Rr*	17	*Rr*	29	*Rr*
6	*Rr*	18	*Rr*	30	*Rr*
7	*Rr*	19	*Rr*	31	*Rr*
8	*Rr*	20	*rr*	32	*Rr*
9	*Rr*	21	*rr*	33	*Rr*
10	*Rr*	22	*rr*	34	*Rr*
11	*Rr*	23	*rr*	35	*Rr*
12	*Rr*	24	*rr*		

***Rr*:** Hereterozygous Resistant/tolerant ***rr*:** Susceptible

Molecular marker-assisted selection is an essential tool for developing capsicum varieties that are resistant or tolerant to TSWV. This approach enables quick and accurate identification of individuals with resistance genes and desirable agronomic traits, helping to create new varieties. Additionally, the primers $SCAC_{568}$ used in our study have been successfully applied in various research projects focused on developing resistance or tolerance to viral diseases. Research by Şimşek (2014), Özkaynak et al. (2014), and Polat et al. (2012) shows that the $SCAC_{568}$ marker effectively contributes to creating TSWV-resistant pointed pepper and Charleston hybrid pepper varieties. Moreover, Şimşek et al. (2015) achieved significant success using this marker to develop TSWV resistance in bell pepper varieties.

In conclusion, the findings of this study align with the results obtained by Moury et al. (2000) and other related research. Thus, it has been reaffirmed that the $SCAC_{568}$ marker is a valuable tool for developing TSWV-resistant capsicum varieties.

The effectiveness of the $SCAC_{568}$ marker in capia-type peppers has also been supported by the work of Şavkan et al. (2025), who evaluated 120 S2 generation capia pepper lines using this marker. Their study identified 13 lines as homozygous resistant (*RR*), 17 lines as heterozygous resistant (*Rr*), and 90 lines as susceptible (*rr*). This research emphasized that marker-assisted selection offers a faster and more cost-effective alternative compared to phenotypic resistance screening. Additionally, Şavkan et al. (2025) contributed significantly to the limited literature demonstrating the reliability of the $SCAC_{568}$ marker in *Capia* peppers. In this respect, their findings are consistent with our results and confirm the practical applicability of the $SCAC_{568}$ marker in breeding resistant *capia* pepper varieties.

Furthermore, the utility of the $SCAC_{568}$ marker across diverse genetic backgrounds has been validated in international studies. For instance, AUSVEG (2006), reported the identification of TSWV-resistant *C. annuum* lines in Australia using the $SCAC_{568}$ marker, and these molecular findings were verified through pathogenicity assays under greenhouse conditions. The study concluded that the marker was highly specific for TSWV resistance and did not show any correlation with resistance to *Capsicum Chlorosis Virus* (CaCV), underscoring the marker's specificity and reliability. This international evidence supports the global applicability of our findings.

Although the $SCAC_{568}$ marker is widely used in resistance breeding, it is important to note that molecular marker data alone may not guarantee durable resistance. As highlighted in recent literature, pathogenicity testing remains essential to validate resistance levels, particularly against diverse pathogen genotypes. Breeding lines identified as resistant through molecular screening should also be tested against different isolates of TSWV from various geographic regions to ensure broad and stable

resistance. The combined use of molecular markers and pathogenicity tests not only strengthens the reliability of resistance data but also enhances the sustainability of breeding programs.

Taken together, the results of this study demonstrate that the $SCAC_{568}$ marker is not only consistent with previously reported findings but also serves as a reliable and efficient tool for identifying TSWV-resistant individuals in *capia* pepper populations. These findings support its continued use in breeding programs aimed at developing disease-resistant cultivars.

Conclusions

In this study, the resistance and tolerance levels of various pepper lines to TSWV were evaluated, and the molecular characteristics of resistant genotypes were examined. PCR analyses revealed that out of 35 genotypes, 22 were heterozygous resistant/tolerant (*Rr*) and 13 were recessive susceptible (*rr*). These findings demonstrate the successful identification of individuals carrying resistance-associated alleles against *TSWV*. However, phenotypic validation under field conditions using different TSWV isolates remains essential to confirm the molecular resistance traits observed.

The results highlight the potential of identifying TSWV-resistant or tolerant capsicum breeding lines for the development of new cultivars capable of withstanding multiple biotic stress factors. Understanding the genetic basis of resistance is essential for improving disease management and increasing the sustainability of pepper production. In this context, incorporating the identified resistant genotypes into hybrid breeding programs could lead to higher yields and less dependence on expensive disease control measures.

This study provides a valuable framework for reducing economic losses caused by TSWV and for promoting the development of agricultural products with greater resistance to biotic stress. However, successfully integrating these genotypes into commercial farming requires thorough field trials and detailed analysis of resistance mechanisms under different environmental conditions. These efforts will help create a sustainable foundation for breeding genetically resistant capsicum cultivars.

Importantly, the marker-assisted selection approach demonstrated in this study offers wide-ranging benefits not only for the current breeding program but also for other research institutions and seed companies using similar techniques. Early identification of resistant genotypes allows for shorter breeding cycles, reduces the need for labor-intensive phenotypic screening, and supports sustainable agriculture by minimizing chemical inputs. Additionally, the systematic use of molecular tools improves institutional research capacity and speeds up the development of candidate cultivars suitable for official registration.

In conclusion, this study presents scientifically strong findings consistent with existing literature, while also offering practical, application-focused insights that support ongoing efforts in the public and private sectors to develop TSWV-resistant capsicum varieties through molecular breeding technologies.

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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