

Development of Pink Tomato Genitors Resistant or Tolerant to Tomato Root-Knot Nematode (*Meloidogyne* spp.) and Tomato Spotted Wilt Virus (TSWV) Using Molecular Markers

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Article info

Received: 15.07.2025

Accepted: 24.09.2025

Article type: Research

Key words: MAS, Mi23,
Nematode, Sw5, Tomato, TSWV,

Abstract

Tomato (*Solanum lycopersicum*) production faces increasing threats from biotic stress factors such as Root-knot Nematodes (*Meloidogyne* spp.) and Tomato Spotted Wilt Virus (TSWV), which can cause significant yield losses. In this study, molecular characterization of 154 tomato genotypes was performed to assess their resistance and tolerance to Root-knot Nematodes (*Meloidogyne* spp.) and Tomato Spotted Wilt Virus (TSWV) using Polymerase Chain Reaction (PCR) with SCAR markers of Mi23 and Sw5 specific to the target pathogens. PCR results successfully categorized the genotypes into TSWV resistant (35.06%), heterozygous resistant/tolerant (7.14%), and susceptible groups (12.99%). PCR results successfully categorized the genotypes into Root-knot nematode resistant (14.94%), heterozygous resistant/tolerant (11.69%), and susceptible groups (46.10%). The Mi23 marker detected genotypes carrying the *mi-1.2* gene associated with resistance to Root-knot Nematodes, while the Sw5 marker identified those resistant to TSWV. The results showed that several tomato lines (6.49%) have both resistance and tolerance mechanisms, highlighting their potential value in breeding programs aimed at developing new cultivars with better resistance to multiple biotic stresses. While molecular data offered useful insights, field-based pathogenicity tests are needed to confirm the resistance traits under different environmental conditions. Overall, this study highlights the effectiveness of marker-assisted selection (MAS) in speeding up the identification of resistant lines and significantly advances sustainable tomato breeding strategies to minimize yield losses caused by pathogens.

Citation: Basim, H., Kandil, O., Ökem, C., (2025). Development of Pink Tomato Genitors Resistant or Tolerant to Tomato Root-Knot Nematode (*Meloidogyne* spp.) and Tomato Spotted Wilt Virus (TSWV) Using Molecular Markers. *International Journal of Food, Agriculture and Animal Sciences*, 5 (2), 39-49.

Moleküler İşaretleyiciler Kullanılarak Domates Kök-Ur Nematoduna (*Meloidogyne* spp.) ve Domates Lekeli Solgunluk Virüsüne (TSWV) Dayanıklı veya Toleranslı Pembe Domates Yarı-Yol Materyallerinin Geliştirilmesi

Makale bilgileri

Geliş Tarihi: 15.07.2025

Kabul Tarihi: 24.09.2025

Makale türü: Araştırma

Anahtar kelimeler:

Domates, MAS, Mi23,

Öz

Domates (*Solanum lycopersicum*) üretimi, önemli verim kayıplarına neden olabilen Kök-ur Nematodları (*Meloidogyne* spp.) ve Domates Lekeli Solgunluk Virüsü (TSWV) gibi biyotik stres faktörlerinden kaynaklanan artan tehditlerle karşı karşıyadır. Bu çalışmada, Kök-ur Nematodları (*Meloidogyne* spp.) ve Domates Lekeli Solgunluk Virüsü'ne (TSWV) karşı direnç ve toleranslarını değerlendirmek için 154 domates genotipinin moleküler karakterizasyonu, hedef patojenlere özgü Mi23 ve Sw5 SCAR belirteçleri ile Polimeraz Zincir Reaksiyonu (PCR) kullanılarak gerçekleştirilmiştir. PCR sonuçları TSWV dayanıklı genotipleri (%7.14), heterozigot dayanıklı/toleranslı (%35.06) ve duyarlı gruplara (%12.99) başarıyla kategorize etmiştir. PCR

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Nematod, Sw5, TSWV

sonuçları Kök-uru nematoduna dayanıklı genotipleri (%14.94), heterozigot dayanıklı/toleranslı (%11.69) ve duyarlı gruplara (%46.10) başarıyla kategorize etmiştir. Mi23 belirteci, Kök-ur Nematodları'na dirençle ilişkili mi-1.2 genini taşıyan genotipleri tespit ederken, Sw5 belirteci TSWV'ye dirençli olanları belirlemiştir. Sonuçlar, birçok domates hattının (%6.49) hem dayanıklı hem de tolerantlı olduğunu göstererek, çoklu biyotik stres faktörüne karşı daha iyi dayanıklı yeni çeşitlerin geliştirilmesini amaçlayan ıslah programlarındaki potansiyel değerlerini vurgulamaktadır. Moleküler veriler faydalı bilgiler sunarken, farklı çevre koşullarında dayanıklılık özelliklerini doğrulamak için arazi bazlı patojenite testlerine ihtiyaç duyulmaktadır. Genel olarak, bu çalışma, dayanıklı domates hatların belirlenmesini hızlandırmada markör destekli seleksiyonun (MAS) etkinliğini vurgulamakta ve patojenlerin neden olduğu verim kayıplarını en aza indirmede sürdürülebilir domates ıslah stratejilerini önemli ölçüde geliştirebilir.

Atıf: Basım, H., Kandil, O., Ökem, C., (2025). Moleküler İşaretleyiciler Kullanılarak Domates Kök-Ur Nematoduna (*Meloidogyne* spp.) ve Domates Lekeli Solgunluk Virüsüne (TSWV) Dayanıklı veya Toleranslı Pembe Domates Yarı-Yol Materyallerinin Geliştirilmesi. *Uluslararası Gıda, Tarım ve Hayvan Bilimleri Dergisi*, 5 (2), 39-49.

Introduction

Tomato belongs to the *Solanaceae* family and is the second most widely grown vegetable in global food production. They are an important source of nutrition for humans, being rich in minerals, vitamins, and antioxidants. Notably, the high lycopene content in tomatoes plays a significant role in reducing the risks associated with cancer and neurological diseases. Due to these features, tomatoes are considered an indispensable food for both nutrition and health (Giovannucci, 1999).

As of 2022, tomatoes were cultivated on an area of 49.2 million hectares, with total production reaching 186.1 million tons. The largest producing countries are China, India, and Türkiye, which together account for 64% of the world's total tomato production. Türkiye ranks third in the world for tomato production, making this crop strategically important for both domestic consumption and exports. Tomato production increased by 2.3% in 2023, reaching a total of 13.3 million tons (TUIK, 2024).

In agricultural production, biotic and abiotic stress factors can significantly impact both the quantity and quality of crops, particularly in important products like tomatoes. Among the harmful biotic stressors in tomato production are the root-knot nematodes (*Meloidogyne* spp.) and *Tomato Spotted Wilt Virus* (TSWV), which result in substantial economic losses.

Different tomato varieties show varying levels of susceptibility to different *Meloidogyne* species. Additionally, different populations of the same *Meloidogyne* species may display varying pathogenicity levels on the same tomato variety. This variability emphasizes the importance of variety-based strategies in nematode control (Trudgill, 2001). Root-knot nematodes are one of the major biotic stress factors affecting tomato production, but another significant challenge comes from viral diseases. Among these, the *Tomato Spotted Wilt Virus* (TSWV) is particularly harmful, causing substantial losses in tomato crops. TSWV is one of 136 different viral species that affect tomatoes and is regarded as one of the most detrimental.

TSWV (*Tomato Spotted Wilt Virus*) has a wide host range, infecting over 1,090 plant species, including important agricultural crops such as tomatoes, peppers, potatoes, and tobacco. This virus poses significant risks to global agriculture due to its widespread distribution, particularly causing more severe effects in temperate and subtropical regions (Yeturu et al., 2016; Oliver et al., 2016; Sivaprasad et al., 2017).

Utilizing resistant varieties is a crucial strategy in the fight against both root-knot nematodes and TSWV. In this context, understanding the resistance genes associated with these pathogens is vital for developing effective and sustainable solutions. Root-knot nematode resistance in tomatoes was first identified by Bailey in a wild species known as *Lycopersicon peruvianum* (PI 128657). Later, Smith successfully transferred this resistance to the cultivated species *Lycopersicon esculentum* using embryo rescue techniques. Research has shown that this resistance is governed by a single dominant gene called *Meloidogyne incognita*-1 (*Mi-1*), which is located on chromosome 6 of the tomato plant. The *Mi-1* gene offers effective resistance against *M. incognita*, *M. arenaria*, and *M. javanica*, but it is not effective against *M. hapla*. Currently, *Mi-1* serves as the primary source of resistance incorporated into commercial tomato cultivars. This gene activates within two weeks after germination, providing protection for both the roots and leaves of the plants. In addition to *Mi-1*, other resistance genes have also been identified in tomatoes. These genes include *Mi-2*, *Mi-3*, *Mi-4*, *Mi-5*, *Mi-6*, *Mi-7*, *Mi-8*, *Mi-9* and *Mi-HT*, and especially *Mi-2*, *Mi-3*, *Mi-4*, *Mi-5*, *Mi-6*, *Mi-9* and *Mi-HT* genes show heat resistance. *Mi-3* and *Mi-5* genes are located on chromosome 12; *Mi-9* was identified as the homolog of *Mi-1* and mapped on the short arm of chromosome 6. *Mi-HT* is also located on the short arm of chromosome 6. The genetic diversity of wild tomato species provides a mechanism supporting the formation of new resistance traits in the nematode resistance locus (Khalid et al., 2017).

The resistance mechanisms against TSWV have been associated with the *Sw-1a*, *Sw-1b*, *sw-2*, *sw-3*, *sw-4*, *Sw-5*, *Sw-6* and *Sw-7* loci identified in different tomato materials. Among these, *Sw-5* is the most widely used dominant resistance gene that provides effective resistance to a wide range of *Tospoviruses* (Stevens et al., 1995; Brommonschenkel et al., 2000). This gene, originating from *Lycopersicon peruvianum*, was introduced into cultivated tomatoes and mapped in the telomeric region on chromosome 9 (Brommonschenkel et al., 2000). The *Sw-5* locus contains six homologous paralogous genes belonging to a loose gene family: *Sw-5a*, *Sw-5b*, *Sw-5c*, *Sw-5d*, *Sw-5e* and *Sw-5f*. Within this gene family, especially *Sw-5a* is a critical component that provides effective resistance to TSWV and is of great importance in agricultural applications (Okorley et al., 2018), (Kiewnick et al., 2009), (Brito et al., 2007).

Molecular Marker Assisted Selection (MAS) is an effective method that allows the selection of desired traits in plant breeding through molecular markers tightly linked to genes. MAS is based on the use of markers that indirectly indicate the presence of the target gene and stands out as a particularly environmentally friendly and economical solution. Tomato is considered one of the plants where molecular markers are most effectively applied in commercial breeding studies (Garland et al., 2005). Molecular markers linked to the *Mi-1* gene have enabled rapid and effective screening of resistance alleles without the need for nematode inoculation. This technology is gaining importance by providing environmental sustainability and reducing costs. In studies on developing resistance to TSWV (*Tomato Spotted Wilt Virus*) and root-knot nematodes, MAS enables rapid selection and development of resistant varieties.

Obtaining tomato genitors resistant to biotic and abiotic factors in tomato breeding is essential for developing improved tomato varieties, which are considered the most important genetic resource in tomato breeding efforts.

The aim of this study is to develop tomato genitors that are resistant or tolerant to Root-Knot Nematode and TSWV disease using molecular marker assisted selection methods developed in line with advances

provided by modern biotechnology. This approach aims to improve both disease management and agricultural productivity within the scope of the breeding program.

Material and Method

In this study, young leaf samples were collected from 154 different tomato varieties grown in greenhouses owned by İstanbul Tarım A.Ş. DNA isolation was carried out using the CTAB protocol (Doyle and Doyle, 1990), and the quantity and quality of the extracted DNA were measured with a Nabi Micro Volume Spectrophotometer. The Polymerase Chain Reaction (PCR) method was used to determine the resistance/tolerance status, and specific primers were employed for various target genes (Table 1).

Table 1. Molecular marker and primer sequences used in study

Primer name	Disease name	Sequence (5'-3')	Molecular size of PCR product	References
<i>Mi23</i>	Root-Knot Nematode	Forward Primer TGGAAAAATGTTGAATTTCTTTTG	430 bp (recessive)	Seah et al., 2007
		Reverse Primer GCATACTATATGGCTTGTTTACC	380 bp (resistant)	
<i>Sw5</i>	TSWV	Forward Primer AATTAGGTTCTTGAAGCCCATCT	574 bp (resistant)	Dianese et al., 2010
		Reverse Primer TTCCGCATCAGCCAATAGTGT	470 bp (recessive)	

PCR reactions for Root-Knot Nematode and 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. For the *Mi23* assay; 1 cycle of 94°C for 3 min, 35 cycles of 94°C for 30 s, 57°C for 1 min, and 72°C for 1 min, and 72°C for 10 min. PCR reactions were completed in 1 cycle for 10 min. For TSWV, PCR reactions were completed in 35 cycles at 94°C for 3 min., 1 cycle at 94° C for 30 sec., 1 min at 53° C for 1 min at 72° C for 1 min. and 1 cycle at 72°C for 10 min. with a final extension. 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.

Results and Discussion

In this study, we assessed the genetic resistance levels of tomato varieties against root-knot nematodes and *Tomato spotted wilt virus* (TSWV) using PCR analyses with *Mi23* and *Sw5* markers. The results from using the *Mi23* marker showed two distinct band patterns. Bands of 430 bp indicated resistant or

tolerant genotypes (14.94%), whereas bands of 380 bp signified susceptible genotypes (46.10%). Individuals (11.69%) showing both bands were classified as heterozygous resistant or tolerant (Figure 1, Table 2). Similarly, PCR analyses using the Sw5 marker also showed two band profiles. Bands of 574 bp were associated with resistant/tolerant genotypes (7.14%), while bands of 470 bp indicated susceptible genotypes (12.99%). Individuals (35.06%) displaying both bands simultaneously were considered heterozygous resistant/tolerant (Figure 1, Figure 2, Table 2). Overall, the PCR results showed that the genetic resistance levels of the evaluated tomato genotypes could be accurately classified using both markers. As a result, the individuals were successfully divided into three categories: susceptible, resistant/tolerant, and heterozygous resistant/tolerant.

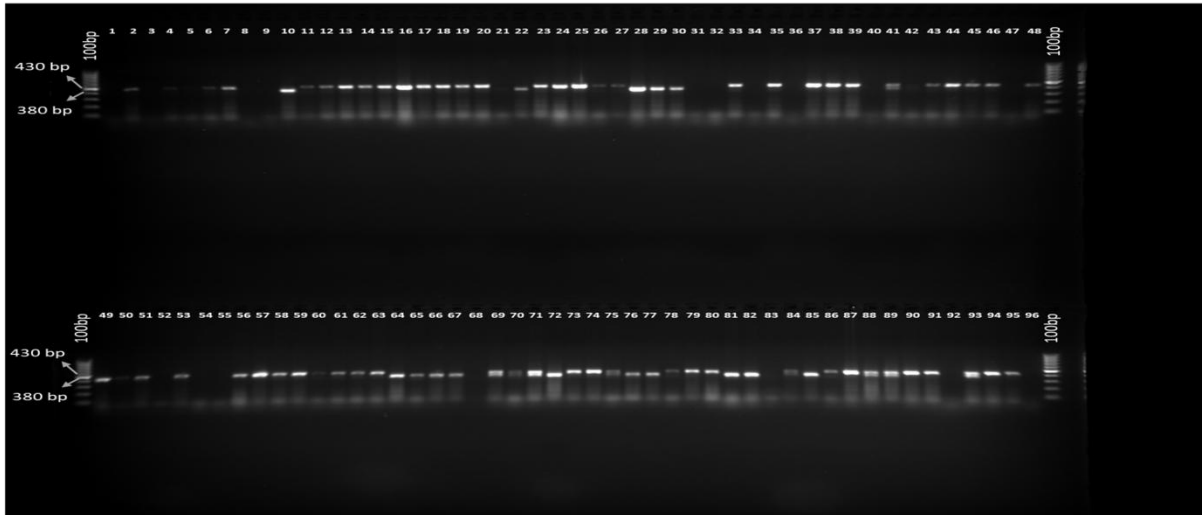


Figure 1. PCR results of different tomato lines for *Mi23*. 1-96: Tomato lines.

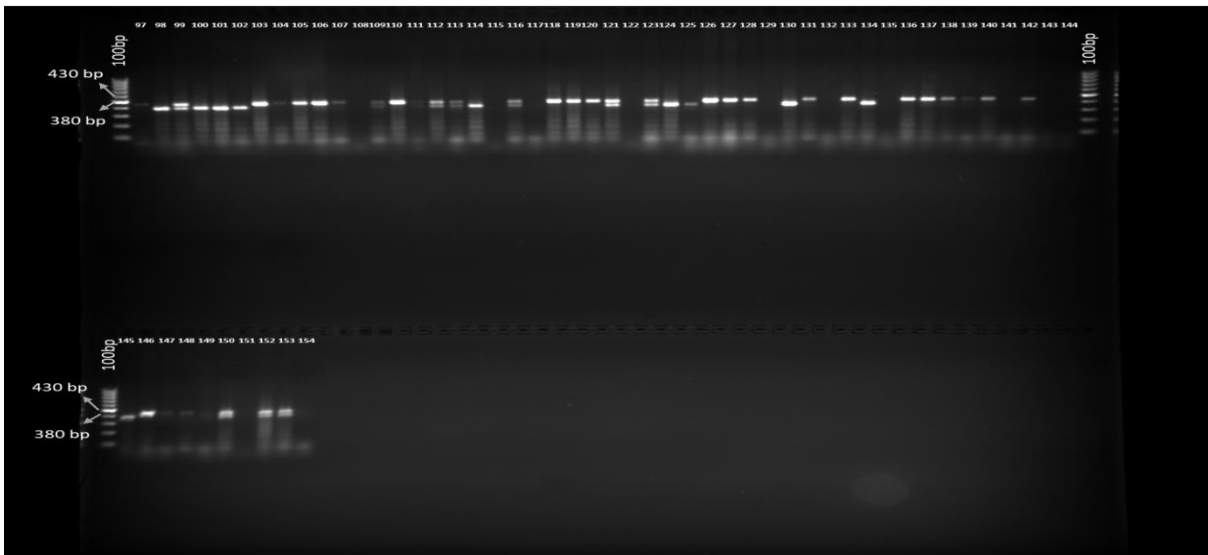


Figure 2. PCR results of different tomato lines for *Mi23*. 97-154: Tomato lines.

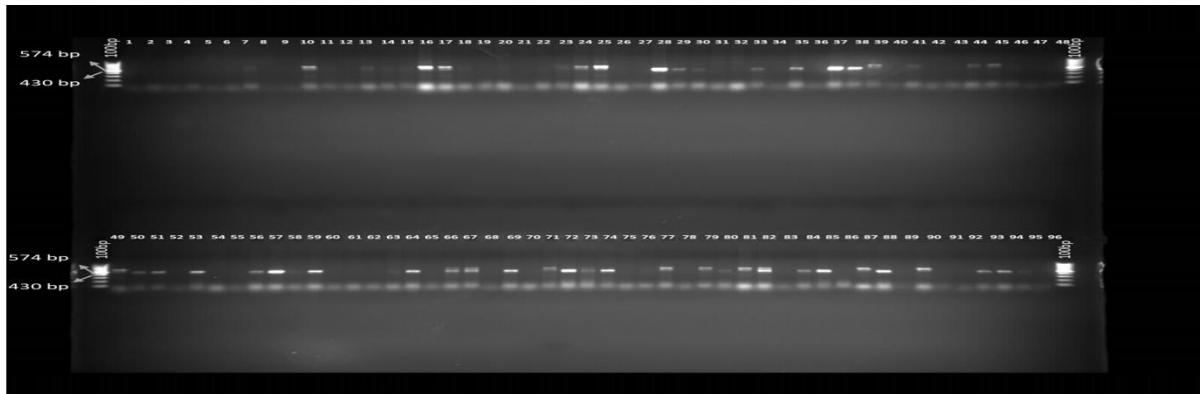


Figure 3. PCR results of different tomato lines for Sw5-2. 1-96: Tomato lines.

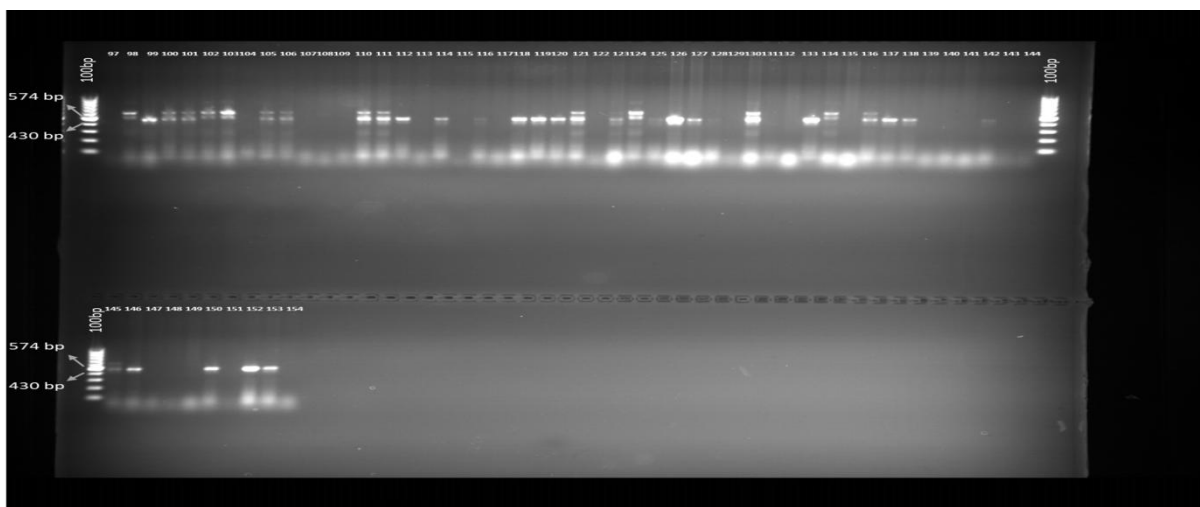


Figure 4. PCR results of different tomato lines for Sw5-2. 97-154: Tomato lines.

Table 2. Genotypic features of tomato lines analysed by PCR

Test No	TSWV	Root-Knot Nematodes	Test No	TSWV	Root-Knot Nematodes	Test No	TSWV	Root-Knot Nematodes
1	X	X	53	<i>Rr</i>	<i>rr</i>	105	<i>Rr</i>	<i>rr</i>
2	X	<i>rr</i>	54	X	X	106	<i>Rr</i>	<i>rr</i>
3	X	X	55	X	X	107	X	<i>rr</i>
4	X	<i>rr</i>	56	<i>Rr</i>	<i>rr</i>	108	X	X
5	X	<i>rr</i>	57	<i>Rr</i>	<i>rr</i>	109	X	<i>Rr</i>
6	X	<i>rr</i>	58	<i>Rr</i>	<i>rr</i>	110	<i>Rr</i>	<i>rr</i>
7	<i>Rr</i>	<i>rr</i>	59	<i>Rr</i>	<i>rr</i>	111	<i>Rr</i>	<i>Rr</i>
8	X	X	60	X	<i>rr</i>	112	<i>rr</i>	<i>Rr</i>
9	<i>Rr</i>	X	61	X	<i>rr</i>	113	X	<i>Rr</i>
10	<i>Rr</i>	<i>RR</i>	62	X	<i>rr</i>	114	<i>rr</i>	<i>RR</i>
11	X	<i>rr</i>	63	<i>Rr</i>	<i>rr</i>	115	X	X

12	X	rr	64	Rr	RR	116	rr	Rr
13	Rr	rr	65	X	RR	117	X	X
14	Rr	rr	66	Rr	RR	118	rr	rr
15	Rr	rr	67	Rr	RR	119	rr	rr
16	Rr	rr	68	X	X	120	rr	rr
17	Rr	rr	69	Rr	Rr	121	Rr	Rr
18	Rr	rr	70	X	Rr	122	X	X
19	X	rr	71	RR	Rr	123	rr	Rr
20	X	rr	72	Rr	RR	124	Rr	RR
21	X	rr	73	Rr	rr	125	rr	RR
22	X	rr	74	Rr	rr	126	rr	rr
23	Rr	RR	75	X	Rr	127	rr	rr
24	Rr	RR	76	X	RR	128	X	rr
25	Rr	rr	77	Rr	RR	129	X	X
26	X	rr	78	X	rr	130	Rr	RR
27	X	rr	79	Rr	rr	131	X	rr
28	Rr	rr	80	RR	rr	132	X	X
29	Rr	rr	81	X	RR	133	rr	rr
30	Rr	rr	82	X	RR	134	Rr	RR
31	X	X	83	X	X	135	X	X
32	X	X	84	Rr	Rr	136	Rr	rr
33	Rr	RR	85	Rr	RR	137	rr	rr
34	X	X	86	X	Rr	138	rr	rr
35	Rr	rr	87	RR	rr	139	X	rr
36	X	rr	88	Rr	Rr	140	X	rr
37	Rr	rr	89	X	Rr	141	X	X
38	Rr	rr	90	RR	Rr	142	rr	rr
39	RR	rr	91	X	rr	143	X	X
40	X	X	92	X	X	144	X	X
41	RR	Rr	93	rr	Rr	145	Rr	RR
42	X	X	94	rr	rr	146	rr	rr
43	X	Rr	95	rr	rr	147	X	rr
44	RR	Rr	96	X	X	148	X	rr
45	RR	Rr	97	X	RR	149	X	Rr
46	X	Rr	98	Rr	RR	150	rr	Rr
47	X	X	99	Rr	Rr	151	X	X
48	X	rr	100	RR	RR	152	rr	Rr
49	RR	RR	101	RR	RR	153	Rr	Rr
50	Rr	rr	102	Rr	RR	154	X	X
51	Rr	Rr	103	Rr	rr			
52	X	X	104	X	rr			

RR: Resistant/Tolerant

Rr: Heterozygous Resistant/tolerant

rr: Sensitive

X: Undetectable

Root-knot nematodes (*Meloidogyne* spp.) and Tomato spotted wilt virus (TSWV) are major biotic stress factors that cause significant yield losses in tomato (*Solanum lycopersicum*) production. Developing resistant lines against these pathogens is essential for maintaining sustainable agricultural practices. In recent years, besides traditional breeding methods, the opportunities offered by molecular marker technologies have greatly accelerated the identification of resistance genes and the breeding process. Specifically, marker-assisted selection (MAS) has enabled quick and reliable identification of resistant plants (Consuegra et al., 2015; Gómez et al., 2004; Mejía et al., 2007; Rani et al., 2009).

In studies focus on developing resistance to root-knot nematodes in tomatoes, the Mi23 SCAR marker has proven to be very important for detecting the Mi-1.2 gene. Research by Reddy et al. (2016) showed two distinct band patterns (380 bp for resistant/tolerant and 430 bp for susceptible plants) using the Mi23F and Mi23R primers across various tomato genotypes. These results demonstrated the high accuracy of this marker in distinguishing resistant and susceptible plants. Furthermore, a more recent study by (Kumar (2020) confirmed that individuals carrying the Mi-1.2 gene exhibited lower levels of root galling and highlighted a strong correlation between molecular data and phenotypic observations. Based on these findings, the Mi23 marker is considered an effective molecular tool for identifying resistant individuals and accelerating the selection process in modern breeding programs. Similarly, in studies focused on improving resistance to TSWV in tomatoes, the Sw-5 gene and its related markers are essential. The groundbreaking work by Stevens et al. (1995) identified the genomic location of the sw-5 gene and observed its low recombination rate due to the introgression of *Solanum peruvianum* DNA, which helps in tracking the Sw-5 locus and efficiently selecting resistant lines. Recent research has shown that within the sw-5 gene cluster, only the sw-5b gene provides broad-spectrum resistance against TSWV and various other Orthotospovirus species. Specifically, the sw-5b gene, introduced from wild *Solanum peruvianum*, has been reported to directly interact with the viral NSm protein to trigger resistance. It is also widely used in marker-assisted selection programs (de Oliveira et al., 2018).

Our study successfully demonstrated the effectiveness of Mi23 and Sw-5 markers in selecting for resistance against both root-knot nematodes and TSWV. Molecular analyses with the Mi23 marker enabled accurate differentiation between resistant and susceptible individuals, while the Sw-5 marker allowed for the reliable detection of genotypes resistant to TSWV.

Overall, molecular marker technologies provide much faster, more cost-effective, and reliable results compared to traditional phenotypic screening methods in breeding programs focused on developing resistance to both root-knot nematodes and TSWV. Our findings emphasize the valuable contributions of marker-assisted selection to scientific research and practical breeding applications, offering a useful reference for future breeding efforts.

Conclusions

In this study, the findings indicate that resistance to multiple pathogens can be combined within the same tomato genotype, which holds significant potential for the development of new tomato cultivars. Particularly, the use of lines resistant to multiple biotic stress factors offers important advantages in terms of enhancing yield stability and reducing the need for chemical control in production systems.

Although valuable molecular data were obtained in this study, it is necessary to validate the identified resistance/tolerance traits through phenotypic assessments under field conditions against various pathogens. Future research should focus on conducting comprehensive pathogenicity tests on these lines and supporting the molecular findings with practical applications. This step will be crucial for incorporating the selected genotypes into hybrid breeding programs and will directly contribute to the development of superior tomato cultivars. Thus, this study not only provides robust scientific data for improving resistance to harmful agents such as TSWV and *Meloidogyne* spp., but also highlights the critical role of molecular breeding technologies in achieving sustainability and increased productivity in tomato production. The findings offer positive contributions to breeding programs by reducing disease-related economic losses, promoting the development of resistant varieties, and ensuring the long-term security of agricultural production.

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.

Acknowledgements

The authors give thanks to İstanbul Tarım A.Ş. for funding this research.

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