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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of Resistance to Tomato Spotted Wild Virus in Pepper Genotypes from Turkey and Nigeria Using Molecular Markers*

Türkiye ve Nijerya Biber Genotiplerinin Moleküler Markörler Kullanılarak Domates Lekeli Solgunluk Virüsüne Dayanıklılıklarının Belirlenmesi

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Abstract

Pepper (*Capsicum* spp.), a key species within the *Solanaceae* family, is extensively grown across the globe for use as both a vegetable and a spice. Pepper like other agricultural crops, are vulnerable to various biotic and abiotic stress factors. To mitigate these threats, they must possess effective defense mechanisms and resistance genes. The *Tsw* gene, found in *Capsicum chinense*, confers resistance to Tomato Spotted Wilt Virus (TSWV) and is expressed as a dominant allele (*Tsw*) in many genotypes. The $SCAC_{568}$ CAPs marker, linked to TSWV resistance, allows for the co-dominant differentiation of resistant and susceptible pepper genotypes (RR, Rr, rr). Due to its close association with the *Tsw* gene, this marker is a valuable tool for marker-assisted selection and for screening pepper germplasm collection in pepper breeding programs. Therefore, in this study the resistance of 60 pepper local genotypes from Turkey and Nigeria to TSWV were evaluated by the $SCAC_{568}$ CAPs marker linked to *Tsw*. The genomic DNA of the samples was amplified with the $SCAC_{568}$ marker PCR protocol and then subjected to digestion with the Taq1 enzyme. The digested products were resolved by electrophoresis on a 2% agarose gel and visualized under UV light. For validation purposes, three control genotypes, including *Capsicum chinense* PI152225 (homozygous resistant) and two *Capsicum annuum* genotypes (homozygous susceptible and heterozygous resistant) were included. In the marker screening, 60 pepper genotypes were evaluated, revealing that 13 of these samples were homozygous resistant (RR), 24 were heterozygous (Rr), and 23 were homozygous susceptible (rr). Amongst the tested genotypes, Nigerian pepper genotypes showed more resistance to TSWV. These results validate the utility of the $SCAC_{568}$ CAPs marker in pepper breeding programme for identifying genotypes with resistance or susceptibility to Tomato Spotted Wilt Virus. In addition, it is shown that, the resistant local pepper genotypes in this study can be used in the TSWV resistance breeding programme.

Keywords: *Capsicum* spp., TSWV, CAPs Marker, Resistance gene, $SCAC_{568}$

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Öz

Biber (*Capsicum* spp.), Solanaceae familyasının önemli bir türü olup, dünya genelinde hem sebze hem de baharat olarak yaygın bir şekilde yetiştirilmektedir. Biber, diğer tarımsal bitki türleri gibi çeşitli biyotik ve abiyotik stres faktörlerine karşı hassastır. Bu stres faktörlerine karşı hassasiyeti azaltmak için, etkili savunma mekanizmalarına ve dayanıklılık genlerine sahip olmaları gerekmektedir. *Capsicum chinense*'de bulunan *Tsw* geni, Domates Lekeli Solgunluk Virüsü'ne (TSWV) karşı dayanıklılık sağlamaktadır. Bu gen birçok genotipte dominant allel (*Tsw*) olarak bulunarak dayanıklılığı kontrol etmektedir. $SCAC_{568}$ CAPs markörü, TSWV dayanımı ile bağlantılı olup, dayanıklı ve hassas biber genotiplerinin (RR, Rr, rr) kodominant ayrımını sağlamaktadır. *Tsw* geni ile yakın ilişkili olan bu markör, biber ıslah programlarında biber genetik materyallerinin taranması ve markör destekli seleksiyon için değerli bir araçtır. Bu nedenle bu çalışmada, Türkiye ve Nijerya'dan 60 yerel biber genotipi TSWV'ye karşı dayanıklılık durumlarını belirlemek için $SCAC_{568}$ CAPs markörü ile değerlendirmiştir. Biber genotiplerinin genomik DNA'sı, $SCAC_{568}$ markör PCR protokolü ile çoğaltılmış ve ardından Taq1 enzimi ile kesilmiştir. Kesilen PCR ürünleri, %2 agaroz jel elektroforezi ile ayrılmış ve UV ışığı altında görüntülenmiştir. Doğrulama amacıyla, *Capsicum chinense* PI152225 (homozigot dayanıklı) ve iki *Capsicum annuum* genotipi (homozigot duyarlı ve heterozigot dayanıklı) dahil olmak üzere üç kontrol genotipi kullanılmıştır. Markör taramasında 60 biber genotipi değerlendirilmiş ve bu örneklerden 13'ünün homozigot dayanıklı (RR), 24'ünün heterozigot dayanıklı (Rr), 23'ünün ise homozigot hassas (rr) olduğu ortaya çıkmıştır. Test edilen genotiplerden, Nijerya biber genotipleri TSWV'ye karşı daha fazla dayanıklılık göstermiştir. Bu sonuçlar, biber ıslahı programlarında Domates Lekeli Solgunluk Virüsüne karşı dayanıklı veya hassas genotipleri belirlemek için $SCAC_{568}$ CAPs markörünün kullanılabileceğini desteklemektedir. Ayrıca, çalışmada belirlenen dayanıklı yerel biber genotiplerinin TSWV'ye dayanıklılık ıslahı programlarında kullanılabileceği de gösterilmiştir.

Anahtar Kelimeler: *Capsicum* spp., TSWV, CAPs Markörü, Dayanım geni, $SCAC_{568}$

1. Introduction

Pepper (*Capsicum* spp.) is extensively cultivated around the world as both a vegetable and spice crop, and it serves as a key ingredient in numerous global food industries (Bosland and Votava, 2000). The agricultural sector plays a vital role in facilitating the economic development processes within countries (Grzelak et al., 2019).

Peppers (*Capsicum* spp.) İs a member of the Solanaceae family, encompassing 25 species, and is categorized under the Solanales order (Şalk et al., 2008). Among these, five domesticated species *C. annuum*, *C. baccatum*, *C. frutescens*, *C. chinense,* and *C. pubescens* originated from the New World (McLeod et al., 1982). Regarding pepper production in Turkey, the quantities produced for various types are as follows: 1,602,457 tons of red peppers, 395,441 tons of bell peppers, 939,178 tons of long peppers, and 143,934 tons of chili peppers (TURKSTAT, 2023).

The breeding of new varieties from locally cultivated pepper populations, which are grown for diverse purposes across different regions, along with the production of certified seeds for agricultural utilization, has been investigated (Özalp et al., 2011). Research on local pepper varieties in Turkey has demonstrated that, despite being a predominantly self-pollinating species, peppers exhibit varying degrees of cross pollination. As a result, genetically diverse pepper populations have dispersed across different regions of the country, contributing to extensive genetic variation. By selecting genotypes based on fruit and plant characteristics, valuable lines have been identified, which hold potential as parental material for the development of standard cultivars and hybrid varieties (Bozokalfa, 2009).

Nigeria holds the position of the foremost pepper producer in Africa. *C. annuum* and *C. frutescens* are the most prominent species grown in significant commercial volumes in Nigeria (Falusi and Morakinyo, 2001; Madu and Uguru, 2006). Genetic diversity serves as a vital source of variation in plant breeding programs. However, the increasing use of cultivated *Capsicum* species in Nigeria may lead to the erosion of genetic elements that govern important quality traits in local pepper varieties, thus diminishing overall genetic diversity. Conducting an analysis of the genetic diversity among *Capsicum* species cultivated across various regions is essential for evaluating genetic variation, steering plant breeding initiatives, and safeguarding the sustainable management of genetic resources (Hoisington et al., 1999).

It is important to gather and preserve the germplasm of pepper and to investigate their utility in breeding programs, in particular for resistance to abiotic and biotic stress. Because the climatic factor directly impacts plant growth, influencing the nutritional composition and characteristics of the plant. In particular, precipitation and water facilitate the solubilization of nutrient elements in the soil, which enables their uptake by plants (Karaçal, 2008).

Globally, yield reductions caused by plant diseases are substantial and significant, with plant pathogenic viruses being a major contributing factor (Fauquet et al., 2005; Strange and Scott, 2005). Viral diseases have been identified as key factors that significantly impact both the quality and yield of pepper production in cultivation areas (Anandakumar et al., 2018). Tomato Spotted Wilt Virus (TSWV) has been characterized as a Tospovirus capable of rapidly disseminating in agricultural fields via the vector *Frankliniella occidentalis* (Dal Bo et al., 1999). Due to the widespread distribution of efficient thrips vectors globally, tospoviruses have emerged as a significant pathological threat to numerous vegetables, ornamental, and industrial crops (Goldbach and Peters, 1994). TSWV was initially discovered in Australia and later disseminated rapidly, with its presence subsequently recorded in many countries throughout the Americas, Europe, Asia, and Africa (Cho et al., 1989). It has been observed that this virus induces diseases in more than 900 plant species, such as tomatoes, peppers, lettuce, and tobacco, and that weeds and ornamental plants function as reservoirs for the virus (Gordillo et al., 2008). To mitigate crop losses attributed to TSWV, a range of control strategies is utilized. Nevertheless, the physical, chemical, and biological approaches employed for vector management are both difficult to apply and frequently inadequate (Rosello et al., 1996; Cebolla-Cornejo et al., 2003). Developing resistant varieties is proposed as the most effective strategy for managing TSWV (Floor, 1971). The gene governing resistance to TSWV (*Tsw*) has been located on chromosome 10 in various C. *chinense* genotypes (‘PI 152225’, ‘PI 159236’, ‘CNPH 275’, ‘C00943’, and ‘7204’), and this gene has been introduced into commercial Capsicum *annuum* varieties (Black et al., 1991; Costa et al., 1995; Boiteux, 1995; Moury et al., 1997; Jahn et al., 2000).

Roggero et al. (1996) examined the impact of high temperatures on the resistance of C. *chinense* 'PI 152225.' They observed a significant reduction in genetic resistance after 20 days of exposure to a constant 33°C, particularly in 16- and 30-day-old plants. The researchers hypothesized that this resistance breakdown could be due to epistatic interactions and concluded that elevated temperatures might weaken the long-term resistance of these pepper varieties, potentially affecting their cultivation in warm climates.

It has been observed that the resistant (RI) isolate, which maintains resistance, undergoes mutations over time, resulting in the development of the resistance-breaking (RB) isolate (De Ronde et al., 2013). The global use of pepper varieties containing the *Tsw* gene has facilitated the rapid development of resistance-breaking (RB) TSWV strains. These strains were first reported in the Mediterranean pepper-growing regions of Italy (Roggero et al., 1999; Roggero et al., 2002) and Spain (Margaria et al., 2004). Following this, they have also been identified in Australia (Thomas-Carroll et al., 2003), Turkey (Deligoz et al., 2014), Argentina (Ferrand et al., 2015), and California, USA (Macedo et al., 2019). While TSWV was first documented in Korea in the 2000s (Kim et al., 2004), RB strains emerged less than a decade later (Chung et al., 2012; Hoang et al., 2013).

Molecular markers linked to the *Tsw* alleles conferring resistance to TSWV have advantage in pepper breeding. To enhance the application of the TSWV resistance gene (*Tsw*) in breeding programs, (Moury et al., 2000) identified a RAPD (Randomly Amplified Polymorphic DNA) marker linked to *Tsw*. This marker was later converted into a CAPS marker $SCAC_{568}$ and has been employed in marker-assisted selection (MAS) approaches (Çelik et al., 2018). Researchers have demonstrated that PCR products of the SCAR marker, after digestion with XbaI, TaqI, or HaeIII enzymes, are effective for distinguishing between TSWV resistant and susceptible genotypes across different genetic backgrounds (Moury et al., 2000).The $SCAC_{568}$ CAPS marker, associated with the TSWV resistance gene, has been used in breeding programs to distinguish between resistant and susceptible pepper genotypes at the co-dominant level (RR, Rr, rr), as demonstrated in previous studies (Dal Bo et al., 1999; İkten, 2019).

But there is limited knowledge on resistance to TSWV of local pepper genotypes in Turkey and Nigeria that can be potential genetic resources for pepper breeding programme. Therefore, this study conducted to distinguish pepper genotypes from Turkey and Nigeria as either resistant or susceptible to Tomato Spotted Wilt Virus (TSWV) and to mitigate yield losses by integrating resistant genotypes into the pepper breeding programs.

2. Materials and Methods

The genetic material for *Capsicum* spp. utilized in this study was sourced from accessions provided by the National Center for Genetic Resources and Biotechnology (NACGRAB) in Nigeria and the Aegean Agricultural Research Institute Gene Bank (AARI) in Turkey. A total of 60 accessions were analyzed, comprising 39 accessions from NACGRAB and 21 from AARI (*Table 1*). These genetic materials were evaluated agromorphologically in the master's thesis study conducted by Anınkan (2022), and this study also aimed to determine the TSWV resistance status of promising genotypes.

In the AARI germplasm collection, 17 were classified as *Capsicum annuum* and 4 as *Capsicum frutescens*. The remaining 39 *Capsicum* spp. genotypes from Nigeria and its sub-branches are unknown. Additionally, in this study three different genotypes were used as controls: PI 152225 (*C. chinense*) from United States Department of Agriculture, USDA (USA), as a resistant homozygous genotype (RR); Demre (*C. annum*) Antalya agriculture (Turkey), a resistant heterozygous genotype (Rr); and HV12 (*C. annum*) from National Institute of Agricultural Research INRAE (INRA) (France) a susceptible homozygous genotype(rr). as illustrated in *Figure 1* below.

DNA isolation from leaf samples of pepper accessions was performed using the CTAB protocol (Doyle and Doyle, 1990; Doyle, 1991). The obtained DNA samples were subjected to agarose gel electrophoresis for quality and concentration assessment, followed by PCR amplification using the CAPs marker ($SCAC_{568}$) developed by (Moury et al., 2000).

In the reaction mixture, 20-30 ng μl^{-1} genomic DNA, 3 µl 10X PCR buffer, 3 µl 2.5 mM dNTP mix, 7.5 µl 25 mM $MgCl_2$, 1.5 µl each of forward (5' GTGCCAGAGGAGGATTTAT 3') and reverse (5' GCGAGGTGGACACTGATACT 3') primers (10 pmol μl^{-1}), and 0.75 µl (5 U μl^{-1}) Taq polymerase enzyme were used. The PCR cycling protocol was as follows: an initial denaturation at 94°C for 3 minutes, followed by 34 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 1

Table 1. List of Turkish and Nigerian genotypes and their collection sources.

Number	Genotypes	Botanical name	Location
1	TR 80002	*Capsicum annuum*	Afyonkarahisar
2	TR 82505	*Capsicum annuum*	Eskişehir
3	TR 69128	*Capsicum annuum*	Aksaray
4	TR 71080	*Capsicum annuum*	Kayseri
5	TR 71412	*Capsicum annuum*	Yozgat
6	TR 71414	*Capsicum frutescens*	Yozgat
7	TR 71425	*Capsicum frutescens*	Kayseri
8	TR 71432	*Capsicum annuum*	Nevşehir
9	TR 77192	*Capsicum frutescens*	Tekirdağ
10	TR 77205	*Capsicum annuum*	Tekirdağ
11	TR 77211	*Capsicum annuum*	İstanbul
12	TR 62538	*Capsicum annuum*	Balıkesir
13	TR 61641	*Capsicum annuum*	Aydın
14	TR 61673	*Capsicum annuum*	Muğla
15	TR 61894	*Capsicum annuum*	Denizli
16	TR 66278	*Capsicum annuum*	Bileçik
17	TR 66404	*Capsicum frutescens*	Bileçik
18	TR 75288	*Capsicum annuum cerasiforme*	Erzurum
19	TR 75326	*Capsicum annuum conoides*	Artvin
20	TR 75332	*Capsicum annuum conoides*	Artvin
21	TR 45440	*Capsicum annuum*	Şanlıurfa
22	NGB 00703	*Capsicum* spp. *	Nigeria gene bank
23	NGB 01864	*Capsicum* spp. *	Nigeria gene bank
24	NGB 02621	*Capsicum* spp. *	Nigeria gene bank
25	NGB 02642	*Capsicum* spp. *	Nigeria gene bank
26	NGB 02634	*Capsicum* spp. *	Nigeria gene bank
27	NGB 00700	*Capsicum* spp. *	Nigeria gene bank
28	NGB 01852	*Capsicum* spp. *	Nigeria gene bank
29	NGB 00624	*Capsicum* spp. *	Nigeria gene bank
30	NGB 00609	*Capsicum* spp. *	Nigeria gene bank
31	NGB 00198	*Capsicum* spp. *	Nigeria gene bank
32	NGB 01851	*Capsicum* spp. *	Nigeria gene bank
33	NGB 01650	*Capsicum* spp. *	Nigeria gene bank
34	NGB 02619	*Capsicum* spp. *	Nigeria gene bank
35	NGB 01854	*Capsicum* spp. *	Nigeria gene bank
36	NGB 00620	*Capsicum* spp. *	Nigeria gene bank
37	NGB 02618	*Capsicum* spp. *	Nigeria gene bank
38	NGB 01848	*Capsicum* spp. *	Nigeria gene bank
39	NGB 00629	*Capsicum* spp. *	Nigeria gene bank

Table 1 Continued

40	NGB 00704	*Capsicum* spp. *	Nigeria gene bank
41	NGB 00702	*Capsicum* spp. *	Nigeria gene bank
42	NGB 00596	*Capsicum* spp. *	Nigeria gene bank
43	NGB 01850	*Capsicum* spp. *	Nigeria gene bank
44	NGB 00597	*Capsicum* spp. *	Nigeria gene bank
45	NGB 01853	*Capsicum* spp. *	Nigeria gene bank
46	NGB 02730	*Capsicum* spp. *	Nigeria gene bank
47	NGB 04600	*Capsicum* spp. *	Nigeria gene bank
48	NGB 00701	*Capsicum* spp. *	Nigeria gene bank
49	NGB 01858	*Capsicum* spp. *	Nigeria gene bank
50	NGB 03222	*Capsicum* spp.	Nigeria gene bank
51	NGB 02620	*Capsicum* spp. *	Nigeria gene bank
52	NGB 00617	*Capsicum* spp. *	Nigeria gene bank
53	NGB 02633	*Capsicum* spp. *	Nigeria gene bank
54	NGB 00586	*Capsicum* spp. *	Nigeria gene bank
55	NGB 02655	*Capsicum* spp. *	Nigeria gene bank
56	NGB 00590	*Capsicum* spp. *	Nigeria gene bank
57	NGB 01472	*Capsicum* spp. *	Nigeria gene bank
58	NGB 00564	*Capsicum* spp. *	Nigeria gene bank
59	NGB 00600	*Capsicum* spp. *	Nigeria gene bank
60	NGB 02641	*Capsicum* spp. *	Nigeria gene bank

* Pepper genotypes obtained from the National centre for genetic resources and biotechnology (NACGRAB) İn Nigeria and the Aegean agricultural research institute gene bank (AARI) in Turkey.
* Subspecies unknown

minute. This was followed by a final extension at 72°C for 5 minutes. To verify whether the PCR products were amplified, they were separated on a 1.5% agarose gel.

Subsequently, each PCR product visualized on the gel was digested with TaqI enzyme in separate tubes. For this procedure, 10 µl of the PCR product was transferred to one tube, 2 µl of TaqI enzyme (10 U μl^{-1}) was added, then received 2 µl of 10X digestion buffer and 6 µl of nuclease-free sterile water.

The digested products were subsequently visualized using electrophoresis on a 2% agarose gel. The Gene Ruler 100 bp Plus DNA Ladder (Thermo Scientific) was used as a molecular standard.

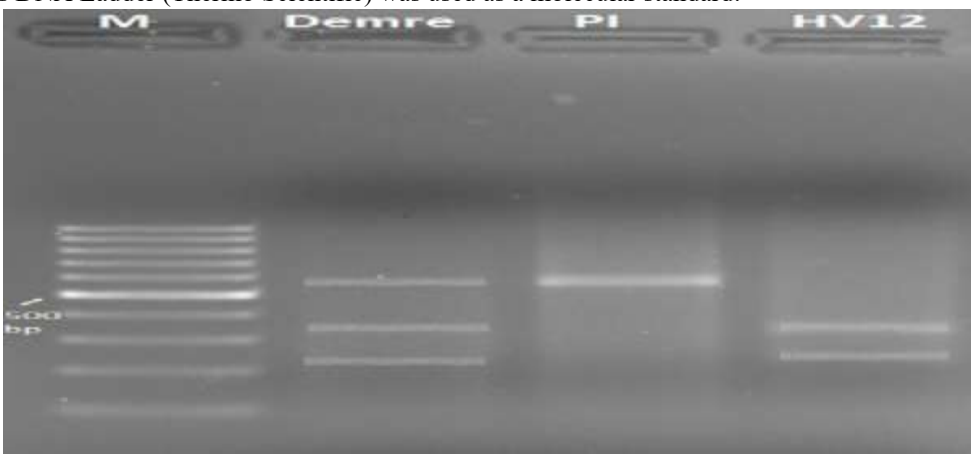

Figure 1. Gel image showing the PCR products of Demre, PI, and HV12 after digestion with Taq1, M: 100 bp DNA marker.

3. Results and Discussion

In this research, pepper genotypes originating from Turkey and Nigeria were evaluated for their resistance to Tomato Spotted Wilt Virus (TSWV) using by the codominant CAPS marker, $SCAC_{568}$ which is linked to the *Tsw* gene as identified by (Moury et al., 2000). This marker was successfully used to screen the genotypes for TSWV resistance. After isolating the pepper genotypes, DNA samples were subjected to amplification using the $SCAC_{568}$ primer through PCR reactions. The gel electrophoresis results of the PCR products from a subset of the 60 pepper genotypes, prior to digestion with the Taq1 enzyme, are shown in *Figure 2*.

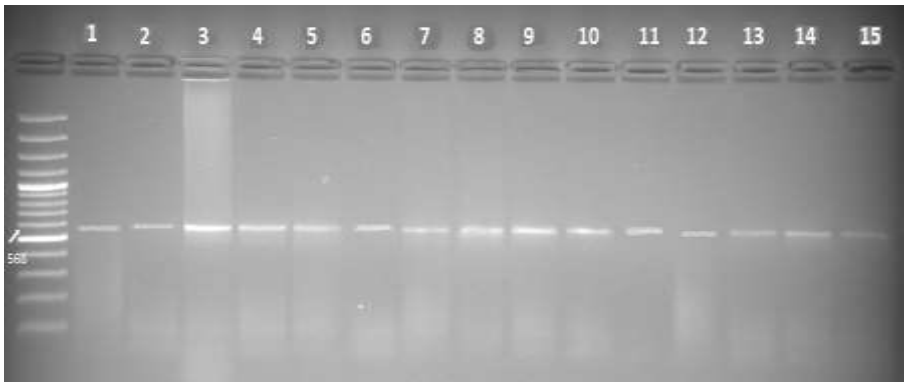

Figure 2. Representative gel image showing 568 bp PCR products generated with the SCAC 568 primer before cutting with Taq1 for some of the 60 genotypes. M: 100 bp DNA marker.

PCR products from 60 pepper genotypes were individually processed in separate tubes to evaluate each genotype's resistance and susceptibility. These products were then subjected to digestion with the restriction enzyme Taq I to distinguish the genotypes based on their resistance to Tomato Spotted Wilt Virus (TSWV). Based on the gel electrophoresis results, homozygous resistant (RR) individuals exhibited PCR products of 568 bp that were not cleaved and thus retained their original size. In contrast, heterozygous individuals showed cleavage of the sensitive alleles, producing two distinct bands at 230 bp and 330 bp, while the resistant allele remained uncut at 568 bp, resulting in a total of three DNA bands on the agarose gel. In homozygous sensitive (rr) individuals, the PCR products were completely cleaved, yielding bands of approximately 230 bp and 330 bp as (Moury et al., 2000) indicated. The study confirms the efficacy and precision of Taq I digestion in differentiating between resistant and susceptible genotypes (*Figure 3*).

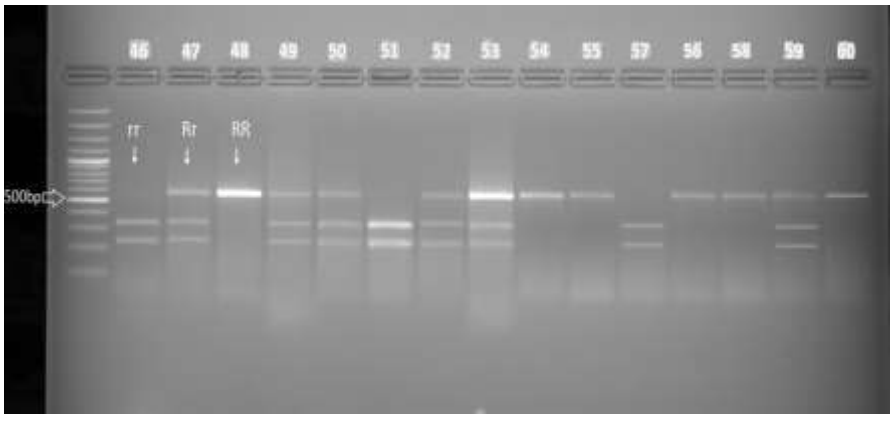

Figure 3. Gel electrophoresis images of PCR products from 60 pepper genotypes digested with Taq I enzyme. M: 100 bp DNA marker.

The classification of genotypes as resistant or susceptible, based on the Taq I digestion outcomes and the gel electrophoresis images of the PCR products presented in *Table 2*. The analysis identified 13 of the 60 genotypes as homozygous resistant (RR), 24 as heterozygous resistant (Rr), and 23 as homozygous susceptible (rr).

Table 2 The outcomes of PCR product digestions performed with Taq I enzyme.

Genotypes	Taq1	Genotypes	Taq1	Genotypes	Taq1	Genotypes	Taq1	Genotypes	Taq1
TR 80002	rr	TR 61641	rr	NGB 02642	rr	NGB 02618	Rr	NGB 01858	Rr
TR 82505	rr	TR 61673	rr	NGB 02634	RR	NGB 01848	Rr	NGB 03222	Rr
TR 69128	rr	TR 61894	Rr	NGB 00700	Rr	NGB 00629	RR	NGB 02620	rr
TR 71080	rr	TR 66278	Rr	NGB 01852	Rr	NGB 00704	RR	NGB 00617	Rr
TR 71412	rr	TR 66404	Rr	NGB 00624	rr	NGB 00702	rr	NGB 02633	Rr
TR 71414	rr	TR 75288	rr	NGB 00609	Rr	NGB 00596	Rr	NGB 00586	RR
TR 71425	rr	TR 75326	rr	NGB 00198	rr	NGB 01850	Rr	NGB 02655	RR
TR 71432	rr	TR 75332	rr	NGB 01851	Rr	NGB 00597	RR	NGB 00590	rr
TR 77192	rr	TR 45440	RR	NGB 01650	Rr	NGB 01853	RR	NGB 01472	RR
TR 77205	Rr	NGB 00703	Rr	NGB 02619	Rr	NGB 02730	rr	NGB 00564	RR
TR 77211	rr	NGB 01864	Rr	NGB 01854	Rr	NGB 04600	Rr	NGB 00600	Rr
TR 62538	rr	NGB 02621	Rr	NGB 00620	RR	NGB 00701	RR	NGB 02641	RR

* Pepper genotypes obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB) in Nigeria and the Aegean Agricultural Research Institute Gene Bank (AARI) in Turkey were digested with the Taq1 enzyme.

Out of the total of 21 local genotypes from Turkey, five have been identified as resistant to the Tomato Spotted Wilt Virus (TSWV). These genotypes are TR 45440 (RR) *Capsicum annuum* from Şanlıurfa, TR 77205 (Rr) *Capsicum annuum* from Tekirdağ, TR 61894 (Rr) *Capsicum annuum* from Denizli, TR 66278 (Rr) *Capsicum annuum* from Bilecik, and finally, TR 66404 (Rr) *Capsicum frutescens* from Bilecik.

In the study, 39 pepper genotypes from Nigeria were researched (see Table 1). Among these genotypes, 32 had resistant gene see (Table 2). In these genotypes 12 Nigerian local genotypes (NGB 02634, NGB 00620, NGB 00629, NGB 00704, NGB 00597, NGB 01853, NGB 00701, NGB 00586, NGB 02655, NGB 01472, NGB 00564, and NGB 02641) were classified as resistant homozygous (RR). Meanwhile, the rest 20 resistant Nigerian genotypes (NGB 00703, NGB 01864, NGB 02621, NGB 00700, NGB 01852, NGB 00609, NGB 01851, NGB 01650, NGB 02619, NGB 01854, NGB 02618, NGB 01848, NGB 00596, NGB 01850, NGB 04600, NGB 01858, NGB 03222, NGB 00617, NGB 02633 and NGB 00600) were classified as resistant heterozygous (Rr). It can be stated that these resistant Nigerian and Turkish pepper local genotypes are highly important for breeding programs.

The higher resistance of Nigerian pepper genotypes to Tomato Spotted Wilt Virus (TSWV) can be attributed to several key factors. These include greater genetic diversity, which enhances the potential for natural resistance, species-level differences within *Capsicum* that may result in varying resistance levels, and better adaptation to local environmental conditions, including viral pressures.

Furthermore, the selection of plant species and varieties for cultivation should prioritize those that are well-adapted to the region's dominant transitional climate conditions (Atmaca, 2023). Such factors, including climate and soil type, play a crucial role in the plants' ability to resist viruses by less breaking the resistant gene. It can be also said the less introduction of hybrid pepper varieties from abroad to Nigeria resulted in less introduction of TSWV and higher maintain the genetic background of the germplasm. The development of resistant pepper cultivars through targeted breeding programs plays a vital role in promoting sustainable agricultural practices and

strengthening food security at the national level. The development of pepper varieties resistant to the Tomato Spotted Wilt Virus (TSWV) is crucial in mitigating the negative impacts of the virus, which poses a threat to farmers' livelihoods and leads to production losses.

In the breeding programs, developing pepper varieties resistant to Tomato Spotted Wilt Virus (TSWV) are of significant global importance. TSWV causes severe yield losses and economic damage in pepper production, making the development of resistant varieties crucial for food security and sustainable agriculture. Moreover, for sustainable economic development in Turkey, it is essential to prioritize the agricultural sector. Equally important is the protection of agricultural lands and the encouragement of value-added production practices. This approach will contribute to a reduction in environmental pollution and foster a positive trajectory (Çetin et al., 2020). These programs help reduce pesticide use, thereby minimizing environmental impact, and provide varieties that are resilient to increased virus spread due to climate change. Additionally, they improve farmers' livelihoods and contribute to the preservation of genetic diversity within *Capsicum* species, facilitating the development of new varieties resistant to emerging diseases. Therefore, the local pepper genotypes from Turkey and Nigeria are significant importance, particularly in the context of incorporating resistant genotypes into the breeding programs of both countries.

4. Conclusions

Our research emphasizes the genetic resistance potential of pepper genotypes to Tomato Spotted Wilt Virus (TSWV). The resistant genotypes identified from Turkey and Nigeria (13 homozygous RR and 24 heterozygous Rr) hold significant promise for inclusion in breeding programs aimed at developing new TSWV-resistant varieties. Hybridization with commercial pepper varieties could not only strengthen TSWV resistance but also enhance agronomic traits, ultimately contributing to increased food production. The robust resistance observed in Nigerian genotypes could be further investigated through genome-wide association studies (GWAS) or whole-genome sequencing. Such genetic analyses would facilitate a deeper understanding of the genetic mechanisms underpinning TSWV resistance, thereby guiding future breeding efforts. Incorporating TSWV resistance alongside other climate resilience traits, such as drought tolerance, is critical in the context of sustainable agriculture and the escalating challenges posed by climate change. Since climate change may exacerbate viral outbreaks, the development of pepper varieties that are both disease-resistant and climate-resilient is crucial for ensuring the sustainability of agricultural systems. Sharing these findings on a global platform could benefit pepper breeding initiatives in other regions. The use of the $SCAC_{568}$ marker for identifying resistance to additional pathogens and assessing the durability of genotypes under field conditions could further support these efforts. Lastly, establishing a long-term monitoring system in collaboration with agricultural institutions to observe the performance of resistant varieties over time and identify any potential loss of resistance would be a crucial step toward ensuring sustained agricultural productivity and resistance management.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: ILBI, H.; Design: ILBI, H.; Data Collection or Processing: HASSAN, A. A.; Statistical Analyses: ILBI, H.; Literature Search: HASSAN, A. A.; Writing, Review and Editing: ILBI, H., HASSAN, A. A.

References

Anandakumar, L., Bagyalakshmi, K., Nithya, K., Parameswari, B. and Viswanathan, R. (2018). Reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay for rapid diagnosis of Sugarcane yellow leaf virus in sugarcane. *Sugar Tech,* 20(6): 708-716.

Anınkan, A. S. (2022). *Molecular and morphological characterization of Nigerian and Turkish pepper genotypes*. (Msc Thesis) Ege University, Institute of Science, İzmir, Türkiye.

Atmaca, B. (2023). Fertility characteristics of soils in different stream beds under transitional climate conditions. *Journal of Tekirdag Agricultural Faculty,* 20(4): 898-917.

Black, L. L., Hobbs, H. A. and Gatti, J. M. (1991). Tomato spotted wilt virus resistance in *Capsicum chinense* 'PI 152225' and 'PI 159236'. *Plant Disease,* 75: 863.

Boiteux, L. S. (1995). Allelic relationships between genes for resistance to Tomato spotted wilt tospovirus in *Capsicum chinense*. *Theoretical and Applied Genetics,* 90: 146–149.

Bosland, P. W. and Votava, E. J. (2000). Peppers. Vegetable and spice *Capsicums*, CABI Publishing, New York, U.S.A.

Bozokalfa, M. K. (2009). *Characterization of some local pepper genotypes and investigation of their suitability for industrial processing*. (PhD Thesis) Ege University Institute of Science, İzmir, Türkiye.

Cebolla-Cornejo, J., Soler, S., Gomar, B., Soria, D. M. and Nuez, F. (2003). Screening *Capsicum* germplasm for resistance to Tomato spotted wilt virus (TSWV). *Annals of Applied Biology,* 143(2): 143-152.

Çelik, İ., Özalp, R., Çelik, N. and Polat, İ. (2018). Development of pointed pepper lines resistant to Tomato spotted wilt virus (TSWV). *Derim,* 35(1): 27-36.

Çetin, M., Saygın, S. and Demir, H. (2020). The impact of the agricultural sector on environmental pollution: An analysis of cointegration and causality for the Turkish economy. *Journal of Tekirdag Agricultural Faculty, 17* (3): 329-345.

Cho, J. J., Mau, R. F. L., German, T. L., Hartmann, R. W., Yudin, L. S., Gonsalves, D. and Provvidenti, R. (1989). A multidisciplinary approach to management of Tomato spotted wilt virus in Hawaii. *Plant Disease, 73*: 375-383.

Chung, B. N., Choi, H. S., Yang, E. Y., Cho, J. D., Cho, I. S., Choi, G. S. and Choi, S. K. (2012). Tomato spotted wilt virus isolates giving different infection in commercial *Capsicum annuum* cultivars. *The Plant Pathology Journal,* 28: 87-92. https://doi.org/10.5423/PPJ.NT.09.2011.0169

Costa, J., Catalá, M. S., Lacasa, A., Díez, M. J. and Nuez, F. (1995). Introduction of plant genetic resistance to TSWV from *C. Chinense* PI 159236' in different pepper genetic backgrounds. In *First International Symposium on Solanacea for Fresh Market*, March 28–31, *Acta Horticulturae,* 412: 523–532, Malaga, Spain.

Dal Bo, E., Chiarrone, G., Rolleri, J. and Ronco, L. (1999). Tospovirus in ornamental crops in La Plata. *Journal of the Faculty of Agronomy*, 104(1): 35-40. (In Spanish).

De Ronde, D., Butterbach, P., Lohuis, D., Hedil, M., Van Lent, J. W. and Kormelink, R. (2013). *Tsw* gene-based resistance is triggered by a functional RNA silencing suppressor protein of tomato spotted wilt virus. *Molecular Plant Pathology,* 14(4): 405-415.

Deligoz, I., Arli Sokmen, M. and Sari, S. (2014). First report of resistance breaking strain of Tomato spotted wilt virus (Tospovirus; Bunyaviridae) on resistant sweet pepper cultivars in Turkey. *New Disease Reports,* 30: 26. https://doi.org/10.5197/j.2044-0588.2014.030.026

Doyle, J. (1991). DNA protocols for plants. In Molecular techniques in taxonomy, Eds: Hewitt, G. M., Johnston, A. W. B. and Young J. P. W., pp. 283-293, Springer, Berlin, Germany.

Doyle, J. J. and J. L. Doyle (1990). Isolation of plant DNA from fresh tissue. *Focus,* 12:13–15.

Falusi, O. A. and Morakinyo, J. A. (2001). Pollen and hybridization studies in some Nigerian species of peppers. *Nigerian Journal of Technology, Education*, 1 and 2: 40-43.

Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U. and Ball, L. A. (2005). Virus Taxonomy. VIIIth Report of the International Committee on Taxonomy of Viruses, Elsevier Academic Press.

Ferrand, L., García, M. L., Resende, R. O., Balatti, P. A. and Dal Bó, L. (2015). First report of a resistance-breaking isolate of Tomato spotted wilt virus infecting sweet pepper harboring the *Tsw* gene in Argentina. *Plant Disease,* 99: 1869. https://doi.org/10.1094/PDIS-02-15-0207-PDN

Floor, H. H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology,* 9: 275-296.

Goldbach, R. and Peters, D. (1994). Possible causes of the emergence of tospovirus diseases. *Seminars in Virology,* 5: 113–120.

Gordillo, L. F., Stevens, M. R., Millard, M. A. and Geary, B. (2008). Screening two *Lycopersicon peruvianum* collections for resistance to Tomato spotted wilt virus. *Plant Disease,* 92(5): 694-704.

Grzelak, A., Guth, M., Matuszczak, A., Czyzewski, B. and Brelik, A. (2019). Approaching the environmental sustainable value in agriculture: How factor endowments foster eco-efficiency. *Journal of Cleaner Production,* 241: 1-9.

Hoang, N. H., Yang, H.-B. and Kang, B.-C. (2013). Identification and inheritance of a new source of resistance against Tomato spotted wilt Virus (TSWV) in *Capsicum*. *Scientia Horticulturae,* 161: 8–14. https://doi.org/10.1016/j.scienta.2013.06.033

Hoisington, D., Khairallah, M., Reeves, T., Ribaut, J.-M., Skovmand, B., Taba, S. and Warburton M. (1999). Plant genetic resources: contribute toward what can they increased crop productivity? *Proceedings of the National Academy of Sciences of the United States of America*, 96: 5937e5943.

İkten, H. (2019). Molecular analysis of TSWV resistance in F_2 pepper genotypes (*Capsicum annuum* L.) derived from different genetic sources. *Mediterranean Agricultural Sciences,* 32(1): 43-48.

Jahn, M., Paran, I., Hoffmann, K., Radwanski, E. R., Livingstone, K. D., Grube, R. C., Aftergoot, E., Lapidot, M. and Moyer, J. (2000). Genetic mapping of the *Tsw* locus for resistance to the Tospovirus Tomato spotted wilt virus in *Capsicum* spp. and its relationship to the *Sw-5* gene for resistance to the same pathogen in tomato. *The American Phytopathological Society,* 13(6): 673-682.

Karaçal, İ. (2008). Soil Fertility. Nobel Publishing, No: 1335, p. 222, Ankara, Türkiye.

Kim, J. H., Choi, G. S., Kim, J. S. and Choi, J. K. (2004). Characterization of Tomato spotted wilt virus from paprika in Korea. *The Plant Pathology Journal, 20*: 297-301. https://doi.org/10.5423/PPJ.2004.20.4.297

Macedo, M. A., Rojas, M. R. and Gilbertson, R. L. (2019). First report of a resistance-breaking strain of Tomato spotted wilt orthotospovirus infecting sweet pepper with the *Tsw* resistance gene in California, U.S.A. *Plant Disease,* 103: 1048. https://doi.org/10.1094/PDIS-07-18-1239-PDN

Madu, E. A. and Uguru, M. I. (2006). Inter-relations of growth and disease expression in pepper using principal component analysis (PCA). *African Journal of Biotechnology*, 5(11): 1054-1057.

Margaria, P., Ciuffo, M. and Turina, M. (2004). Resistance breaking strain of Tomato Spotted wilt virus (Tospovirus; Bunyaviridae) on resistant pepper cultivars in Almería, Spain. *Plant Pathology,* 53: 795–795. https://doi.org/10.1111/j.1365-3059.2004.01082.x

McLeod, M. J., Sheldon, I. G. and Eshbaugh, W. I. (1982). Early evolution of chilli peppers. *Economic Botany,* 36: 361-368.

Moury, B., Palloix, A., Gebre Selassie, K. and 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: 45–52.

Moury, B., Pflieger, S., Blattes, A., Lefebvre, V. and Palloix, A. (2000). A CAPS marker to assist selection of Tomato spotted wilt virus (TSWV) resistance in pepper. *Genome,* 43: 137–142.

Özalp, R., Çelik, İ. and Coşkun, A. (2011). Investigation of Yield and Yield Components of Candidate Pickling Ornamental Pepper Varieties. *In Proceedings of the IV. Seed Congress*- Volume I, Ondokuz Mayıs University, Faculty of Agriculture. June 14-17, pp. 43-48, Samsun, Türkiye.

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). *Phytopath Medit,* 35: 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: 950–954. https://doi.org/10.1094/PDIS.2002.86.9.950

Roggero, P., Melani, V., Ciuffo, V., Tavella, L., Tedeschi, R. and Stravato, V. M. (1999). Two field isolates of Tomato spotted wilt Tospovirus overcome the hypersensitive response of a pepper (*Capsicum annuum*) hybrid with resistance introgressed from *C. Chinense* PI152225. *Plant Disease,* 83: 965–965. https://doi.org/10.1094/PDIS.1999.83.10.965A

Rosello, S., Diez, M. J., Lacasa, A., Jorda, C. and Nuez, F. (1996). Testing resistance to TSWV introgressed from *Lycopersicon peruvianum* by artificial transmission techniques. *Euphytica,* 98: 93-98.

Şalk, Arın., L., Deveci, M. and Polat, S. (2008). Özel sebzecilik, Namık Kemal Üniversitesi Ziraat Fakültesi, Tekirdağ, s:448.

Strange, R. N. and Scott, P. R. (2005). Plant disease: A threat to global food security. *Annual Review of Phytopathology,* 43(1): 83-116.

Thomas-Carroll, M. L. and Jones, R. A. C. (2003). Selection, biological properties and fitness of resistance-breaking strains of Tomato spotted wilt virus in pepper. *Annals of Applied Biology,* 142: 235–243. https://doi.org/10.1111/j.1744-7348.2003.tb00246.x

TURKSTAT Corporate. (2023). Crop Production Statistics 2023. https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-istatistikleri-2023- (Accesed Date: 29.12.2023).