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Research Article/ Araştırma Makalesi

Comparative Evaluation of Resistance to Tomato Spotted Wilt Virus (TSWV) in Pepper Using Different Restriction Enzymes

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ABSTRACT: *Tomato spotted wilt virus (TSWV) is a serious virus that negatively impacts agricultural production. Since peppers are one of its major hosts, utilizing genetic resistance to prevent virus-related losses is crucial for both environmental and human health. Resistance to TSWV in pepper is controlled by a single dominant gene, Tsw. TSWV is among the top 10 most economically important viruses, and because of its numerous hosts, its spread through inoculation poses a significant risk, using molecular testing of genetic resistance essential. In this study, sixty-four pepper genotypes representing different fruit types (capia pepper, sweet bell pepper, and bristle pointed pepper) were initially amplified by conventional PCR using the SCAC568 cleaved amplified polymorphic sequence (CAPS) molecular marker, followed by digestion with the restriction enzymes TaqI and XbaI. The C. chinense accession PI152225 and the advanced breeding line KY76 (C. annuum) were used as resistant controls, whereas the bell pepper cultivar Uraz 98 served as a susceptible control. Resistant control genotypes produced distinct band patterns (230 bp, 330 bp, and 568 bp) depending on their genetic background and the restriction enzymes used. The tested breeding lines also exhibited different banding patterns with XbaI and TaqI according to fruit type. While capia pepper lines formed more consistent band patterns, differences were observed in bristle pointed pepper and sweet bell pepper breeding lines. In conclusion, using both methods together is beneficial for identifying commercial varieties and breeding lines. Therefore, comparative CAPS marker analysis using XbaI and TaqI is recommended for routine breeding programs.*

Keywords: *Resistance gene, Tsw, dominant, variety, CAPS marker.*

Biberde (TSWV) Domates Lekeli Solgunluk Virüsüne Karşı Dayanıklılığın Farklı Kesim Enzimleriyle Karşılaştırmalı Olarak Değerlendirilmesi

ÖZ: *Domates lekeli solgunluk virüsü (TSWV), tarımsal üretimi olumsuz yönde etkileyen ciddi bir virüstür. Biber, virüsün başlıca konukçularından biri olduğundan, virüs kaynaklı kayıpların önlenmesinde genetik dayanıklılığın kullanılması hem çevre hem de insan sağlığı açısından büyük önem taşımaktadır. Biberde TSWV'ye karşı dayanıklılık, Tsw adı verilen tek baskın bir gen tarafından kontrol edilmektedir. TSWV, ekonomik etkisi bakımından en önemli ilk 10 virüs arasında yer almakta olup, çok sayıda konukçuya sahip olması nedeniyle inokulasyon yoluyla yayılma riski yüksektir; bu durum genetik dayanıklılığın moleküler yöntemlerle test edilmesini gerekli kılmaktadır. Bu çalışmada, farklı meyve tiplerini (kapya biber, dolmalık biber ve sivri biber) temsil eden altmış dört biber genotipi, SCAC568 kesilmiş çoğaltılmış polimorfik dizi (CAPS) moleküler markeri kullanılarak konvansiyonel PCR ile çoğaltılmış ve ardından TaqI ve XbaI restriksiyon enzimleri ile kesilmiştir. C. chinense orijinli PI152225 aksesyonu ile ileri ıslah hattı KY76 (C. annuum) dayanıklı kontrol olarak, Uraz 98 dolmalık biber çeşidi ise hassas kontrol olarak kullanılmıştır. Dayanıklı kontrol genotipleri, genetik arka planları ve kullanılan restriksiyon enzimlerine bağlı olarak 230 bp, 330 bp ve 568 bp büyüklüklerinde farklı bant desenleri oluşturmuştur. Test edilen ıslah hatları da meyve tipine bağlı olarak XbaI ve TaqI enzimleri için farklı bantlanma desenleri sergilemiştir. Kapya biber hatları daha tutarlı bant desenleri oluştururken, sivri biber ve dolmalık biber ıslah hatlarında farklılıklar gözlenmiştir. Sonuç olarak, her iki yöntemin birlikte kullanılması ticari çeşitlerin ve ıslah hatlarının belirlenmesinde faydalı olacaktır. Bu nedenle, rutin ıslah programlarında XbaI ve TaqI kullanılarak yapılan karşılaştırmalı CAPS markör analizinin uygulanması önerilmektedir.*

Anahtar kelimeler: *Dayanıklılık geni, Tsw, dominant, çeşit, CAPS markörü.*

INTRODUCTION

Pepper (*Capsicum annuum* L.), a member of the Solanaceae family, ranks seventh among vegetables worldwide and third in Türkiye by production amount (FAOSTAT, 2024). Although pepper originated in South America, Türkiye is one of the centers of diversity in terms of cultivated and consumed pepper varieties and types (Mutlu *et al.* 2009; Binbir and Baş 2010; Kahraman *et al.* 2020). Among the peppers produced, capia, Charleston, stuffed, pointed, block, jalapeño, chili, California Wonder, ornamental, and local peppers are prominent (TTSM,2025).

In recent years, breeding studies related to pepper have increased significantly. According to the Variety Registration and Seed Certification Center (TTSM), 712 pepper varieties were registered in Türkiye in 2024 (TTSM, 2024). Although the first developed varieties were open-pollinated, hybrid varieties have been commercially available since the 1990s. Today, the most important traits targeted in pepper breeding include yield and fruit quality, as well as resistance to diseases and pests, which constitute major biotic stress factors. Most resistance genes controlling diseases and pests show monogenic inheritance and are effectively utilized in breeding programs. Marker-assisted selection (MAS) has been widely applied in breeding studies, as it enables the detection of resistance genes independently of environmental conditions (Abney *et al.* 2025; Anonymous 2025

Virus resistance is a critical parameter in pepper breeding. Tomato spotted wilt virus (TSWV) is a highly destructive virus that negatively affects agricultural production. According to *Molecular Plant Pathology*, TSWV ranks second, after Cucumber mosaic virus (CMV), among the top ten most important phytoviruses in terms of scientific and economic impact (Scholthof *et al.*, 2011). TSWV was first identified in Australia in 1915 (Brittlebank, 1919). The virus is not seed-borne and is not transmitted through plant-to-plant contact. Instead, it is transmitted mechanically and, more importantly, by thrips vectors, primarily tobacco thrips (*Frankliniella fusca* Hinds) and western flower thrips (*F. occidentalis* Pergande).

Symptoms of TSWV infection in pepper vary depending on cultivar susceptibility, plant age at the time of infection, season, and environmental conditions. Common symptoms include stunting, necrosis, chlorosis, ringspots, and ring patterns on leaves, stems, and fruits (German *et al.*, 1992; Mumford *et al.*, 1996). TSWV is difficult to manage due to its wide host range and the presence of numerous thrips vector species. Attempts to control the disease by managing thrips populations are generally unsuccessful. Therefore, integrated disease management strategies focusing on the use of resistant cultivars, disease-free transplants, and appropriate cultural practices are considered the most effective approaches (Adkins *et al.*, 2003; Koike *et al.*, 2009; Cooper and Meadows, 2024).

The use of resistant pepper cultivars against TSWV is also important for human health, as it reduces reliance on chemical control strategies and minimizes pesticide residues in edible plant parts (Aktar *et al.*, 2009; Pretty and Bharucha, 2015).

In pepper and tomato, screening for TSWV resistance during breeding programs has traditionally relied on early selection methods such as mechanical inoculation, symptom evaluation, and serological assays (Stevens *et al.*, 1992; Boiteux and de Ávila, 1994). However, selection for TSWV resistance using inoculation tests has several limitations. Mechanical inoculation requires the maintenance and multiplication of infectious TSWV material and the cultivation of inoculated plants under restricted conditions to prevent virus spread. TSWV causes serious diseases in a range of crop species, including tomato, pepper, lettuce and many ornamental species. Growing only TSWV-resistant varieties will reduce the incidence of the disease (EFSA 2012).

Resistance to TSWV in pepper is controlled by a single dominant gene, *Tsw* (Moury *et al.*, 2000; Jahn *et al.*, 2000). Resistance was first identified in *Capsicum chinense* accessions PI 152225 and PI 159236 and is characterized by a hypersensitive local lesion response (Black *et al.*, 1991; Moury *et al.*, 1997). The *Tsw* gene has been introgressed into *C. annuum* types, including commercial bell pepper cultivars such as ‘Stiletto’. However, this resistance is strain-specific and has been

overcome in certain regions, including Italy (Moury *et al.*, 1997). In addition, *Tsw*-mediated resistance is expressed only in leaves and not in flowers or fruits. Because thrips vectors preferentially feed on flowers, TSWV symptoms may still develop on fruits of plants carrying the *Tsw* gene.

To facilitate selection for TSWV resistance, Moury *et al.* (2000) identified four RAPD markers linked to the *Tsw* locus using bulked segregant analysis. A closely linked RAPD marker was converted into a co-dominant cleaved amplified polymorphic sequence (CAPS) marker located 0.9 ± 0.6 cM from the *Tsw* gene. PCR products digested with XbaI, TaqI, or HaeIII restriction enzymes were shown to effectively distinguish resistant and susceptible genotypes across different genetic backgrounds. The SCAC568 CAPS marker has been widely used in breeding programs to identify homozygous resistant (RR), heterozygous (Rr), and susceptible (rr) pepper genotypes (Dal Bo *et al.*, 1999; Ikten, 2019; Basım *et al.*, 2024; Hassan and İlbi, 2025).

Traits of interest to pepper breeders include fruit quality, disease resistance, earliness, vigor, yield, stress tolerance, and plant morphology. Significant progress was achieved during the latter half of the 20th century due to increasing consumer demand and the global expansion of the commercial vegetable seed industry. Although hybrid varieties dominate the market today, the importance of open-pollinated varieties should not be underestimated, as they constitute the genetic base of modern cultivars (Prohens and Nuez, 2008). In several countries, including Türkiye, Serbia, and Bulgaria, open-pollinated varieties still account for a substantial proportion of open-field pepper production. The Aegean Agricultural Research Institute (AARI) has conducted pepper breeding programs since the 1970s. To date, nine open-pollinated pepper varieties with different fruit types, shapes, and colors have been registered: Bağcı Charleston (1979), Ege Acı Sivri (1981), Ege 91 (1991), Uraz 98 (1998), Menderes (2000), Seyrek (2010), Aybar (2022), Sare (2023), Arın (2023), and Hürel (2024) (TTSM, 2025).

According to International Seed Federation (ISF) guidelines, the open-pollinated variety 'Yolo Wonder' (registered in 1952) is recommended as a susceptible control, while PI 152225 (*C. chinense*) is used as a resistant control by the seed industry and virus researchers (Deligoz *et al.*, 2014; CPVO, 2019; Arpacı *et al.*, 2020).

The objective of this study was to assess the resistance status of pepper breeding lines to Tomato spotted wilt virus using marker-assisted selection with the SCAC568 CAPS marker and the restriction enzymes XbaI and TaqI.

MATERIAL AND METHOD

The genetic material of *Capsicum* spp. used in this study was obtained from advanced breeding lines maintained in the pepper genepool of the Aegean Agricultural Research Institute (AARI), Vegetable Research Section, Türkiye. Uraz 98 is an early-maturing, sweet, bell-shaped pepper variety with vigorous plant growth and 3–4 lobed fruits that ripen from green to red. Due to its landrace-based genetic background and lack of known virus disease resistance genes, Uraz 98 was used as the susceptible control in this study. KY 76 is an advanced inbred line developed by AARI from *C. annuum*-based resistant germplasm and has been repeatedly tested under laboratory and field conditions.

Three different genotypes were used as controls: two homozygous resistant genotypes (RR), PI 152225 (*C. chinense*) and KY 76 (*C. annuum*), an advanced capia-type inbred line developed by AARI, and one homozygous susceptible genotype (rr), Uraz 98, a bell-type open-pollinated variety registered in 1998 by AARI. Different bant patterns of controls after digested with Xba1 enzyme in 2% agarose gel shown in Figure 1.

A total of 64 genotypes were evaluated, consisting of 19 sweet bell pepper F_2 inbred lines, 6 capia pepper F_4 inbred lines, 33 pointed pepper F_2 inbred lines, two resistant controls (PI 152225 and KY 76), and one susceptible control (Uraz 98). These genotypes were selected from lines exhibiting superior plant growth and fruit characteristics within the AARI pepper breeding program. To ensure the reliability of molecular analyses, control genotypes were included twice in separate positions during the PCR amplification and restriction enzyme digestion stages. For visualization, control samples were loaded into

wells 20–21–22 in the first section of the agarose gel and wells 29–30–31 in the second section (Figures 2 and 3).

Genomic DNA was isolated from fresh young leaf tissues of resistant and susceptible controls and breeding lines using the GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific). DNA concentration and quality were determined using a spectrophotometer, and DNA integrity was confirmed on a 1% agarose gel.

All control genotypes and advanced pepper breeding lines were screened for the presence of the *Tsw* resistance gene using the SCAC568 CAPS marker (Moury *et al.*, 2000). PCR amplifications were carried out in a total reaction volume of 15 µL, containing 6 µL PCR master mix (DreamTaq Green Master Mix, Thermo Fisher Scientific), 1 µL each of forward and reverse primers (10 pmol), 3 µL genomic DNA (15–20 ng), and 6 µL nuclease-free water. The PCR conditions consisted of an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at the primer-specific temperature for 30 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 7 min.

PCR products obtained from the 64 genotypes were first separated on a 1.5% agarose gel to confirm amplification. Subsequently, 7.5 µL of PCR products amplified with the SCAC568 marker were digested separately with TaqI and XbaI restriction endonucleases according to the manufacturer's instructions (Thermo Fisher Scientific). The digested products were separated on a 2% agarose gel and visualized using a GelCapture MiniBIS UV imaging system.

Table 1. Sequence of Primer SCAC 568 and Band sizes obtained after digestion with XbaI and Taq1.

SCAC 568 primer sequence (Moury *et al.* 2000)	Forward (F): 5' - GTGCCAGAGGAGGATTTAT -3' Reverse (R) 5'- GCGAGGTGGACACTGATAC- 3'	568 bp
RE Enzymes	TaqI	568 bp (resistant) 350 bp (susceptible) 250 bp (susceptible)
RE Enzymes	XbaI	280 bp (resistant)- 568 bp (susceptible)

RESULTS AND DISCUSSION

PCR analysis using the SCAC568 primer produced a 568 bp DNA fragment in all pepper genotypes, confirming the successful amplification of the Tsw-specific region. All PCR products were subsequently digested with two restriction enzymes, XbaI and TaqI. The results of XbaI digestion, visualized on a 2% agarose gel (Figure 1), showed distinct patterns among the controls. The susceptible control, Uraz 98, remained undigested, displaying a single 568 bp band. In contrast, the resistant controls exhibited different banding patterns due to their varying genetic backgrounds. The wild-type resistant control, *C. chinense* PI152225, produced bands of 500 bp and 568 bp, whereas the capia-type resistant advanced inbred line, KY76, showed bands of 280 bp and 568 bp. These results confirm the co-dominant inheritance of the Tsw gene, as previously reported by Moury *et al.* (2000). The differences in digestion patterns among resistant genotypes reflect their genetic background variation. The gel image illustrating these patterns is presented in Figure 1.

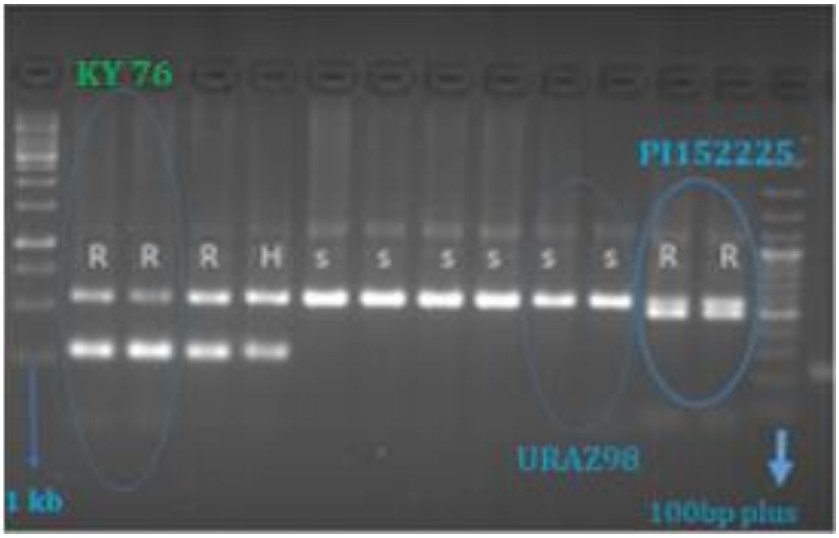


Figure 1. Status of controls after digested with Xba1 enzyme in 2% agarose gel.
R: KY 76 (advance inbred line, resistant, *C.annuum*), S: Uraz 98 (susceptible, variety, *C.annuum*) R: PI152225 (Accession, resistant *C. chinense*)

According to the XbaI restriction enzyme results, 19 sweet bell pepper F_2 inbred lines from AARI's gene pool exhibited wide variation: 9 lines were susceptible (rr), 6 were heterozygous (Rr), and 4 were homozygous resistant (RR), yielding bands of approximately 568 bp (RR), 280 bp and 568 bp (Rr), and 568 bp (rr).The observed segregation ratio deviated from the expected 1:2:1 ratio for a co-dominant marker in a typical F_2 population, consistent with the findings of Ikten (2019).

All capia-type advanced inbred lines were resistant, similar to the capia-type resistant control KY76, which displayed 568 bp and 280 bp bands (Figure 2). When evaluated with both enzymes, among the 19 bell pepper lines, 9 were susceptible (rr), 7 heterozygous (Rr), and 3 homozygous resistant (RR). All capia-type breeding lines were identified as resistant. According to the XbaI results, bristle-pointed pepper inbred lines were susceptible to Tomato Spotted Wilt Virus (TSWV) due to carrying the susceptible (rr) allele of the monogenic *Tsw* gene (Figure 2, Table 2).

The results obtained with the TaqI restriction enzyme were generally similar to those with XbaI, although some differences were observed in two bell pepper inbred lines (ET14 and ET19) and in the bristle-pointed pepper inbred lines. Based on TaqI gel electrophoresis, homozygous resistant (RR) capia pepper inbred lines produced PCR products of 568 bp that were not cleaved, retaining their original size. Heterozygous (Rr) genotypes showed cleavage of the susceptible alleles, resulting in two bands of 230 bp and 330 bp, whereas homozygous susceptible (rr) genotypes were fully cleaved, yielding bands of approximately 230 bp and 330 bp (Moury *et al.*, 2000; Hassan and Ilbi, 2025). Heterozygous resistant (Rr) individuals exhibited three bands (230 bp, 330 bp, and 568 bp).

Although XbaI and TaqI generally produced similar digestion patterns, certain bell pepper lines (ET14 and ET19) displayed distinct profiles. These differences may result from sequence variation at restriction sites or adjacent regions. Similar observations were reported by Ikten (2019), who attributed variations in F_2 populations from different genetic backgrounds to polymorphisms near the *Tsw* locus. Hassan and Ilbi (2025) also noted that the three-band pattern in heterozygotes is a reliable marker-based diagnostic feature.

Comparison of both enzymes showed that while both effectively detected the *Tsw* gene, XbaI provided clearer band distinction in heterozygous individuals. Ikten (2019) demonstrated that XbaI digestion accurately identifies resistant genotypes at a dominant level, whereas TaqI digestion may sometimes produce misleading results. In this study, bristle-pointed pepper inbred lines exhibited completely different band patterns with XbaI and TaqI digestion. The superior

reliability of XbaI for assessing TSWV resistance has been reported by Moury *et al.* (2000) and Ikten (2019). Therefore, XbaI-based CAPS marker analysis is recommended for routine breeding programs aimed at developing TSWV-resistant cultivars.

Polat *et al.* (2012) reported that the co-dominant CAPS marker combination (SCAC568 + XbaI and TaqI) segregated perfectly in 118 F_2 long-type pepper individuals and is useful for introgressing the *Tsw* gene into agronomically important *C. annuum* backgrounds. Similar reliability of XbaI + TaqI for assessing TSWV resistance has been reported by Moury *et al.* (2000), Polat *et al.* (2012), and Ikten (2019). Therefore, comparative CAPS marker analysis using XbaI + TaqI is recommended for routine breeding programs to develop TSWV-resistant cultivars.

Overall, these findings demonstrate that, within AARI's pepper gene pool, marker-assisted selection (MAS) enables rapid and reliable identification of TSWV-resistant genotypes. This approach provides significant advantages over conventional phenotypic screening, being independent of environmental influences and facilitating the development of resistant open-pollinated varieties and new resistant hybrids.

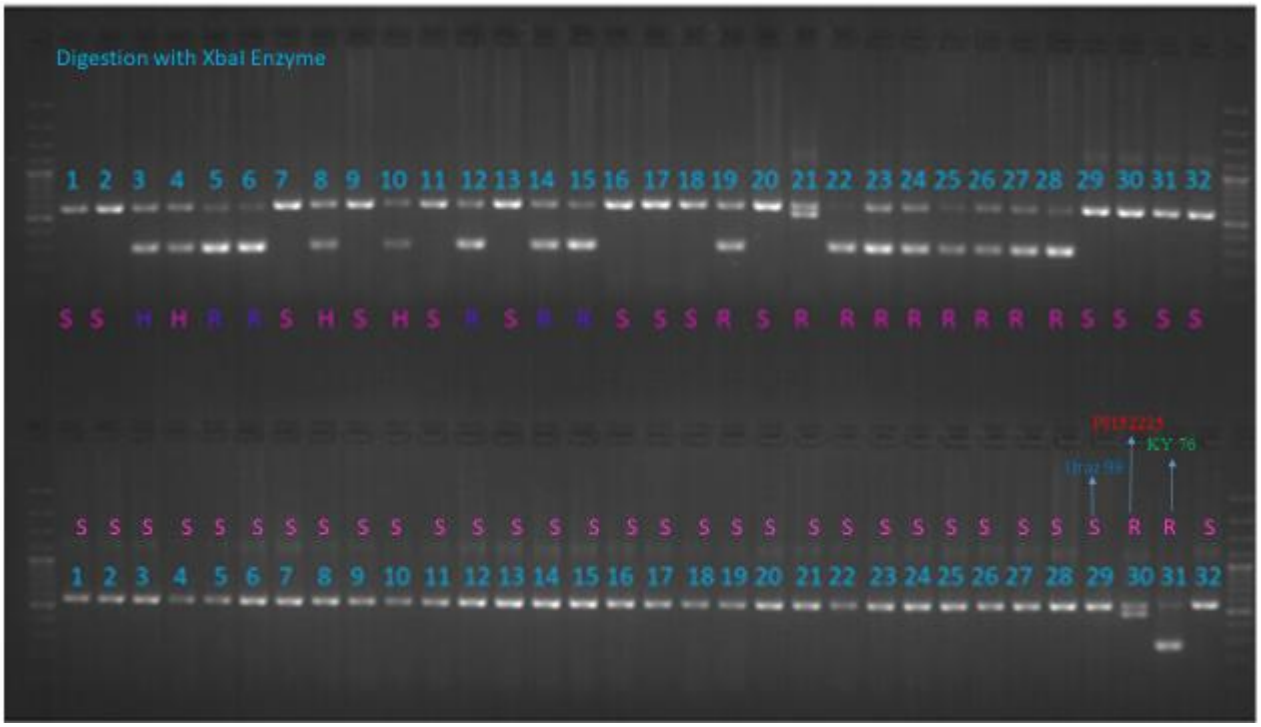


Figure 2. Status of all genotypes after digestion with XbaI enzyme in 2% agarose gel. S: susceptible, H: heterozygous, R: resistant. The Gene Ruler 100 bp Plus DNA Ladder (Thermo Scientific) was used.

Gel description:
a) 1–32 genotypes, first part of the gel: sweet bell pepper breeding lines 1–19 (ET1–ET19).
b) Lane 20: Uraz 98 susceptible control variety.
c) Lane 21: PI152225, wild-type *C. chinense* resistant control.
d) Lane 22: KY76, advanced breeding line resistant control.
e) Lanes 23–28: capia pepper breeding lines, resistant, with band patterns similar to KY76.
f) Lanes 29–32: bristle-pointed pepper breeding lines (ET26–ET58).
g) 1–32 genotypes, second part of the gel: bristle-pointed pepper breeding lines 1–28 (all susceptible).
Lane 29: Uraz 98 susceptible control
Lane 30: PI152225 wild-type *C. chinense* resistant control
Lane 31: KY76 advanced breeding line resistant control
Lane 32: bristle-pointed pepper breeding line (susceptible)

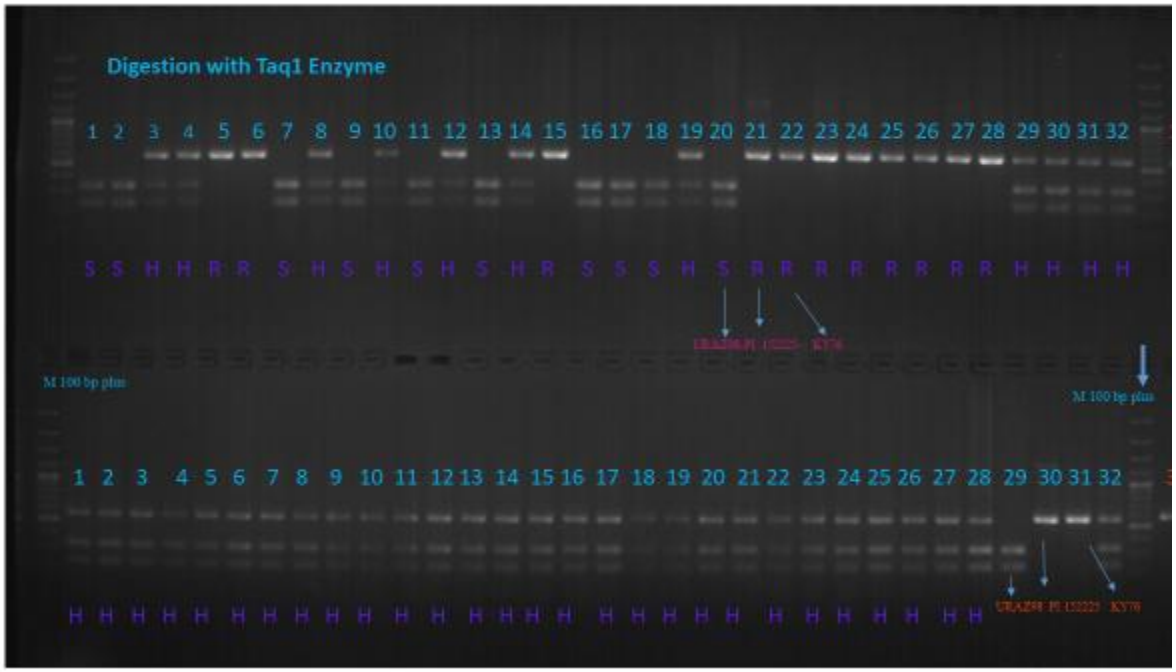


Figure 3. Status of all genotypes after digestion with TaqI enzyme in 2% agarose gel. S: susceptible, H: heterozygous, R: resistant. The Gene Ruler 100 bp Plus DNA Ladder (Thermo Scientific) was used. Gel description:
a) Lanes 1–32, first part of the gel: sweet bell pepper breeding lines 1–19 (ET1–ET19).
b) Lane 20: Uraz 98 susceptible control variety.
c) Lane 21: PI152225, wild-type *C. chinense* resistant control.
d) Lane 22: KY76, advanced breeding line resistant control.
e) Lanes 23–28: capia pepper breeding lines, resistant, with band patterns similar to KY76.
f) Lanes 29–32: bristle-pointed pepper breeding lines (ET26–ET58).
g) Lanes 1–32, second part of the gel: bristle-pointed pepper breeding lines 1–28 (all heterozygous).
Lane 29: Uraz 98 susceptible control
Lane 30: PI152225 wild-type *C. chinense* resistant control
Lane 31: KY76 advanced breeding line resistant control
Lane 32: bristle-pointed pepper breeding line (heterozygous), carrying different alleles of the *Tsw* gene

The *C. chinense* accession PI152225 and the advanced breeding line KY76 (*C. annuum*) were used as resistant controls, whereas the bell pepper cultivar Uraz 98 served as a susceptible control. Uraz 98 exhibited a consistent banding pattern following digestion with both restriction enzymes, indicating its potential use as a susceptible control in future molecular assays aimed at identifying the TSWV resistance gene *Tsw*. Fruit types of susceptible and resistant controls used in the study shown in Figure 4.



Figure 4. Fruit images of susceptible and resistant controls used in the study: a) susceptible control variety Uraz 98 (original) b) Resistant Control *Capsicum chinense* PI152225 (https://doi.org/10.20900/cbgg20200006) c) Resistant Control KY76 (original)

Table 2. Results of pepper genotypes after digested with XbaI and TaqI enzymes according to *Tsw* resistance gene.

No.	Name	Fruit Type	Genotype	XbaI Enzyme	TaqI Enzyme	
1	ET1	Sweet bell pepper	Inbred Line	susceptible	susceptible	
2	ET2	Sweet bell pepper	Inbred Line	susceptible	susceptible	
3	ET3	Sweet bell pepper	Inbred Line	heterozygous	heterozygous	
4	ET4	Sweet bell pepper	Inbred Line	heterozygous	heterozygous	
5	ET5	Sweet bell pepper	Inbred Line	resistant	resistant	
6	ET6	Sweet bell pepper	Inbred Line	resistant	resistant	
7	ET7	Sweet bell pepper	Inbred Line	susceptible	susceptible	
8	ET8	Sweet bell pepper	Inbred Line	heterozygous	heterozygous	
9	ET9	Sweet bell pepper	Inbred Line	susceptible	susceptible	
10	ET10	Sweet bell pepper	Inbred Line	heterozygous	heterozygous	
11	ET11	Sweet bell pepper	Inbred Line	susceptible	susceptible	
12	ET12	Sweet bell pepper	Inbred Line	resistant	heterozygous	
13	ET13	Sweet bell pepper	Inbred Line	susceptible	susceptible	
14	ET14	Sweet bell pepper	Inbred Line	resistant	heterozygous	*
15	ET15	Sweet bell pepper	Inbred Line	resistant	resistant	
16	ET16	Sweet bell pepper	Inbred Line	susceptible	susceptible	
17	ET17	Sweet bell pepper	Inbred Line	susceptible	susceptible	
18	ET18	Sweet bell pepper	Inbred Line	susceptible	susceptible	
19	ET19	Sweet bell pepper	Inbred Line	resistant	heterozygous	*
20	Uraz98	Bell pepper variety	Variety	susceptible	susceptible	
21	PI152225	Wild type genotype	*C. chinense*	resistant	resistant	
22	KY76	Capia pepper	A.Inbred Line	resistant	resistant	
23	ET20	Capia pepper	A.Inbred Line	resistant	resistant	
24	ET21	Capia pepper	A.Inbred Line	resistant	resistant	
25	ET22	Capia pepper	A.Inbred Line	resistant	resistant	
26	ET23	Capia pepper	A.Inbred Line	resistant	resistant	
27	ET24	Capia pepper	A.Inbred Line	resistant	resistant	
28	ET25	Capia pepper	A.Inbred Line	resistant	resistant	
29	ET26	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
30	ET27	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
31	ET27	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
32	ET29	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
33	ET30	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
34	ET31	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
35	ET32	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
36	ET33	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
37	ET34	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
38	ET35	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
39	ET36	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
40	ET37	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
41	ET38	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
42	ET39	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
43	ET40	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
44	ET41	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
45	ET42	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*

Table 2. Continued.

No.	Name	Fruit Type	Genotype	XbaI Enzyme	TaqI Enzyme	
46	ET43	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
47	ET44	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
48	ET45	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
49	ET46	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
50	ET47	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
51	ET48	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
52	ET49	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
53	ET50	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
54	ET51	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
55	ET52	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
56	ET53	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
57	ET54	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
58	ET55	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
59	ET56	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
60	ET57	Bristle pointed pepper	Inbred Line	susceptible	heterozygous	*
61	Uraz98	Bell pepper variety	Variety	susceptible	susceptible	
62	PI152225	Wild type genotype	*C.chinense*	resistant	resistant	
63	KY76	Capia pepper	A.Inbred Line	resistant	resistant	
64	ET58	Bristle pointed pepper	Inbred Line	susceptible	heterozygous*	

* Differences were observed in two bell pepper inbred lines (ET14 and ET19). None of the 33 genotypes were cleaved by the XbaI enzyme, and all retained the original fragment size, indicating a homozygous susceptible genotype (ET29-ET58), digestion with the TaqI enzyme produced two additional bands, suggesting a heterozygous genotype (Rr).

CONCLUSION

In the present study, resistance to Tomato spotted wilt virus (TSWV) was molecularly characterized in pepper (*Capsicum annuum* L.) genotypes using cleaved amplified polymorphic sequence (CAPS) marker analysis with the restriction enzymes TaqI and XbaI.

A total of 64 pepper genotypes were evaluated. Capia pepper lines showed consistent banding patterns, and all capia genotypes (ET20–ET25) were identified as resistant. In the sweet bell pepper population, only three genotypes (ET4, ET5, and ET15) were homozygous resistant. In the *, all 33 genotypes were not cleaved by the XbaI enzyme and retained the original fragment size, indicating a homozygous susceptible genotype (rr). However, digestion with the TaqI enzyme produced two additional bands, suggesting a heterozygous genotype (Rr).

Based on these results, comparative CAPS marker analysis using both XbaI and TaqI enzymes is recommended for routine application in pepper breeding programs. Overall, the findings demonstrate that within AARI's pepper gene pool, marker-assisted selection (MAS) enables rapid and reliable identification of TSWV-resistant genotypes. These genetic resources and germplasm collections represent valuable materials for the development of TSWV-resistant pepper cultivars and future pepper breeding programs.

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