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Molecular Resistance Determination of Different Eggplant (*Solanum melongena* L.) Genetic Resources and Breeding Lines against Fusarium Wilt(§)

Ayşe KAHRAMAN[1*] **Seyfullah BİNBİR**[1] **Tamer BAYTIN**[1]

[1] Horticulture Department, Vegetable Section Aegean Agricultural Research Institute, PO Box 9 35661, İzmir, Türkiye

[*] Corresponding author e-mail: aysekahraman2@gmail.com

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ABSTRACT

Eggplant (*Solanum melongena* L.) is a major vegetable crop with great potential for genetic improvement owing to its large and mostly untapped genetic diversity. Türkiye is the 4th country with the highest production amount among the world's eggplant producing countries. The most important factor limiting eggplant production is its susceptibility to soil-borne diseases such as Fusarium and Verticillium wilt. Resistance to Fusarium wilt in eggplant is monogenic. In the Aegean Agricultural Research Institute, vegetable genetic resources are used extensively in both open-pollinated and hybrid variety breeding studies. In the present study, a total of 48 eggplant genotypes, including F_4 breeding lines and landraces from the institute's gene pool, were screened for resistance to Fusarium wilt using the PCR-based SCAR426 molecular marker. All landraces and 23 breeding lines lacked the resistance-associated allele and were classified as susceptible based on marker analysis. Among the tested materials, only one inbred line ETA5 was found to carry the resistance allele. As classical phenotypic screening was not performed in the present study, the resistance indicated by the SCAR426 marker for ETA5 requires confirmation through classical disease resistance assays. This genotype could represent an alternative source of resistance and may be valuable for future eggplant breeding programs.

Keywords: SCAR426, molecular evaluation, *Solanum melongena*, fusarium wilt, sources

Introduction

Eggplant (*Solanum melongena* L.) is one of the four most important *Solanaceous* crops and is widely cultivated and consumed in Asia, the Mediterranean basin, and Southeast Europe. From the 1970s onward, traditional summer open-field eggplant production progressively shifted toward year-round intensive cultivation systems. In Turkiye, a total of 115 eggplant varieties were officially registered as of 2025. These registered varieties include both open-pollinated and hybrid types; however, the majority are hybrids, while only 8% are open-pollinated varieties. Additionally, 13% of the registered varieties are rootstock types, which are mainly interspecific hybrids or hybrids involving *Solanum torvum* (Gökseven and Akbudak, 2022; TTSM, 2026).

Eggplant is susceptible to various biotic stresses, including nematodes, wilt diseases, eggplant fruit and shoot borer, two-spotted spider mites, whiteflies, and aphids. These factors cause significant reductions in yield, fruit quality, shelf life, and nutritional content (Arafa et al., 2022). One of the most important factors limiting eggplant production is its susceptibility to soil-borne diseases, particularly Fusarium and Verticillium wilts. Fusarium wilt of eggplant was first reported and described in Japan by Japanese Plant Pathologist Matuo and Ishigami during 1958. It is caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *melongenae*, which induces wilt disease and leads to severe yield losses, especially in Asian, European, and Mediterranean countries.

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Soil pathogenic eggplant fungus described for the first time in Japan, and found in abundance in Asia: China, Korea (Cho and Shin, 2004; Altınok, 2005; Zhuang, 2005; Dong et al., 2017). Also, *Fusarium oxysporum* f. sp. *melongenae* has been reported in several European countries, including the Netherlands (Van Steekelenburg, 1976), Greece, Spain, and Italy, as well as in Türkiye where it was first documented in 2005 (Van Steekelenburg, 1976; Stravato and Cappelli, 1990; Holevas et al., 2000; Urrutia et al., 2004; Altınok, 2005; Cappelli et al., 2013).

Three eggplant germplasms, *LS1934*, *LS174*, and *LS2436*, have been found to be highly resistant to *Fusarium oxysporum* f. sp. *melongenae* in multiple studies based on genetic mapping and inoculation tests (Sakata et al., 1996; Mochizuki et al., 1997; Monma et al., 1997; Miyatake et al., 2016) mapped a major resistance locus (*FM1*) for Fusarium wilt at the end of chromosome 2, with two allelic forms Fm1(L) from LS1934 and Fm1(E) from LS174.

Mutlu et al. (2008) developed tightly linked SRAP (Me8/Em5) and SRAPRGA markers that flank the Fusarium wilt resistance gene at approximately 1.2 cM, along with a RAPD marker at 2.6 cM. These markers were converted to SCAR markers that were confirmed across F_2, BC_1, and BC_3 generations, demonstrating high reliability for marker-assisted selection of resistant eggplant genotypes. Boyacı et al. (2011) confirmed by classical genetics that resistance in LS1934 and LS2436 follows a single dominant gene inheritance pattern. Sulu et al. (2022), evaluated three molecular markers (SCAR426, CAPs_903, and SIVR844) that are known to be linked with major disease resistance genes in eggplant. The study used breeding materials, including the resistant accessions *Solanum melongena* LS1934 and LS2436, and assessed resistance both with markers and classical inoculation tests (rootdip) against *Fusarium oxysporum* f. sp. *melongenae* (FOM) and other pathogens. Especially SCAR426 marker for Fusarium resistance is useful tools for marker-assisted selection in disease resistance breeding of eggplant.

Molecular testing was conducted using the SCAR426 marker on 77 eggplant genotypes selected for yield and quality traits developed by the Alata Horticulture Research Institute (AL/Mersin/Türkiye). LS2434, LS1934, and Köksal were used as resistant controls, while Kemer, Yula, and NSFB-99 served as susceptible controls. PCR analyses revealed that four eggplant genotypes (P11, P29, P49, and P52) were resistant to *Fusarium oxysporum* f. sp. *melongenae* (FOM), producing the expected 426 bp band, whereas the remaining 73 genotypes were identified as susceptible (Çolak-Ateş et al., 2018).

At the Aegean Agricultural Research Institute, vegetable genetic resources are extensively utilized in both open-pollinated and hybrid eggplant breeding programs. Currently, eight eggplant varieties with diverse fruit characteristics-including reddish-purple and black-purple skin color, fruit lengths ranging from very long to short, and cylindrical to pear-shaped forms-have been registered (Haytaoğlu et al., 2023; TTSM 2026).

In the present study, F_4 eggplant breeding lines and germplasm, including landraces from the institute's gene pool, were screened for resistance to Fusarium wilt using the PCR-based SCAR426 molecular marker. This is the first study to evaluate Turkish local eggplant landraces for Fusarium wilt resistance.

Materials and Methods

The genetic material of eggplant used in this study was obtained from advanced breeding lines maintained in the eggplant gene pool of the Aegean Agricultural Research Institute (AARI), Vegetable Department, Türkiye. A total of 48 genotypes were tested. 21 eggplant landraces (Manisa, Muğla, Kayseri and Niğde provinces) 24 F_4 stage inbred lines and one registered local variety (Yamula). The 'Köksal' cultivar was used as the resistant control, whereas 'Aydın Siyahı-55' was used as the susceptible control. Detailed information regarding the genotypes used in the study is provided in Table 1.

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 breeding lines were screened for the presence of the *FOM* resistance gene using the dominant Sequence Characterized Amplified Region marker SCAR426 (Mutlu et al., 2008). Sequence of SCAR426 was shown in Table 2.

PCR amplifications were carried out in a total reaction volume of 20 µL, containing 5 µL PCR master mix (DreamTaq Green Master Mix, Thermo Fisher Scientific), 1 µL each of forward and reverse primers (10 pmol), 2 µL genomic DNA (15-20 ng), and 11 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 of 57°C for 60 s, and extension at 72°C for 60 s, with a final extension at 72°C for 7 min. The PCR products were separated on a 2% agarose gel and visualized using a GelCapture MiniBIS UV imaging system. The Gene

Ruler 100 bp Plus DNA Ladder (Thermo Scientific) was used as a molecular standard to confirm the marker SCAR426. The SCAR426 dominant primer provided a single 426 bp band for the homozygote resistant (R) genotypes, a single 800 bp band for the susceptible (S) genotypes. The SCAR426 marker screening was repeated twice to confirm the reliability of the results.

Results and Discussion

All 48 eggplant genotypes, including landraces, advanced inbred lines, and control varieties, were successfully amplified using the SCAR426 molecular marker linked to Fusarium wilt resistance. The marker produced clear and reproducible banding patterns, confirming the reliability of the PCR amplification and gel electrophoresis procedures. The SCAR426 marker differentiated the genotypes into two distinct classes based on banding patterns: homozygous resistant genotypes exhibiting a single 426 bp band, susceptible genotypes showing a single 800 bp band. The susceptible control variety Aydın Siyahı-55 produced the expected 800 bp band, while the resistant control Köksal showed the resistant allele, validating the effectiveness of the marker system used in this study (Figure 1).

Among the tested materials, resistance-associated bands were detected in only one inbred line, indicating the presence of the Fusarium wilt resistance gene in the AARI eggplant gene pool. In contrast, a considerable proportion of landraces and breeding lines lacked the resistance-associated allele and were classified as susceptible based on marker analysis. The resistance allele was detected exclusively in the inbred line ETA5.

The SCAR426 marker was originally developed from a cross between the resistant parent 'LS2436' and the susceptible parent 'NSFB99' (Mutlu et al., 2008). However, unlike the mapping population used for marker development, the present study evaluated genetically diverse eggplant materials, including open pollinated registered cultivar, landraces from different geographical regions, and F_4 stage inbred lines (derived from cultivated x wild crosses). The heterogeneous genetic backgrounds of these materials, particularly the landraces, may have reduced the predictive accuracy of the marker. Furthermore, as SCAR426 is a dominant marker, it cannot discriminate heterozygous individuals from homozygous resistant genotypes, which may have contributed to the inconsistencies between marker genotypes and resistance phenotypes.

Çolak-Ateş et al. (2018) evaluated the resistance of 77 eggplant genotypes to Fusarium wilt (*Fusarium oxysporum* f. sp. *melongenae*, FOM), Potato virus Y (PVY), and root-knot nematode (*Meloidogyne incognita*, RN) using marker-assisted selection and classical inoculation test. The SCAR426 marker was used to screen for FOM resistance, with 'Köksal' and 'LS2436' serving as resistant controls and 'NSFB99' as the susceptible control. Molecular marker analysis identified four genotypes (P11, P29, P49, and P52) as heterozygous resistant to FOM. The resistance of these genotypes was subsequently confirmed through classical inoculation testing evaluation. Furthermore, conventional screening for PVY and RN resistance showed that all genotypes were susceptible to PVY, whereas only P29 and P52 exhibited resistance to both FOM and RN.

In another study, Şimşek et al. (2020) initially screened 120 eggplant inbred lines and four commercial hybrid cultivars previously reported as resistant using the SCAR426 molecular marker. All inbred lines were identified as susceptible, whereas all four commercial hybrid cultivars exhibited the resistance-associated marker profile. Subsequently, eggplant breeding materials (F_1-F_8 generations) were screened using the SCAR426 marker linked to the *FOM* resistance gene, and 533 seedlings were evaluated by root-dip inoculation with a *Fusarium oxysporum* f. sp. *melongenae* (FOM) isolate. All seedlings identified as resistant by the marker survived the inoculation assay. However, six hybrids lacking the SCAR426 resistance-associated marker were also found to be resistant in the classical inoculation test. These findings indicate that SCAR426 is a useful marker for developing eggplant lines resistant to Fusarium wilt, but they also suggest the existence of an additional source of resistance independent of the resistance gene derived from the genotype 'LS2436'. Among the genotypes evaluated in this study, only the inbred line ETA5 carried the resistance-associated allele detected by the SCAR426 marker. ETA5 exhibited pear-shaped fruits, slightly striped green skin, and white fruit flesh characteristics (Figure 2). The remaining breeding lines and all landrace genotypes were classified as susceptible according to the marker analysis.

Conclusions

In this study, 48 eggplant genotypes, including landraces, advanced inbred lines, and registered varieties, were screened for Fusarium wilt resistance using the SCAR426 molecular marker. The marker effectively distinguished resistant and susceptible genotypes, producing clear 426 bp bands in homozygous resistant lines and 800 bp bands in susceptible ones. Several advanced inbred lines and selected landraces were found to carry the resistance allele, while a considerable portion of the tested germplasm remained susceptible. As classical

phenotypic screening was not performed in the present study, the resistance indicated by the SCAR426 marker for ETA5 requires confirmation through classical disease resistance assays. Nevertheless, the resistant control 'Köksal' consistently produced the expected resistant band, in agreement with previous studies, indicating stable marker performance in this genotype. Similarly, the genotypes evaluated in the present study may exhibit resistance when subjected to classical phenotypic screening, even if they are not identified as resistant by the SCAR426 marker. Considering the possibility of alternative resistance sources independent of the LS2436-derived resistance gene, confirmation through classical phenotypic evaluation would provide a more reliable assessment of resistance. If the resistance of ETA5 is confirmed through classical phenotypic screening, this genotype could represent an alternative source of resistance and may be valuable for future eggplant breeding programs. In the next steps, susceptible genotypes identified in this study should be further evaluated through classical resistance assays and screened with newly developed resistance-associated markers to better understand their genetic background.

Acknowledgements

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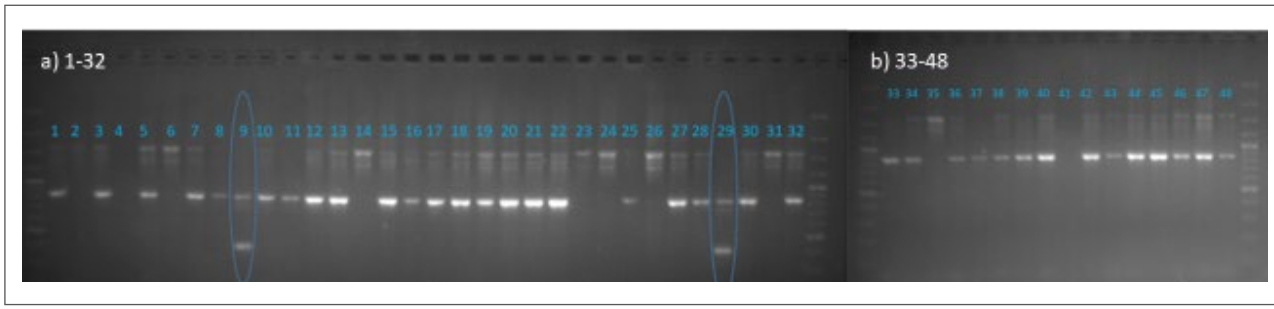

Figure 1. The genotypes used in the study. a) Genotypes 1-32 genotypes 1.Aydın Siyahı-55 (Susceptible Control) 9. Köksal (Resistant Control). 29. ETA 5 Inbred Line b) Genotypes 33-48 homozygous resistant genotype =426 bp band susceptible control variety Aydın Siyahı-55 produced 800 bp band. The GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific).

Figure 2. Fruit Shape of Susceptible control Aydin Siyahı-55 variety,Resistant Control Köksal, Resistant inbred line ETA5 and Yamula local variety (Original).

Table 1. The genotypes used in the study. The 'Aydın Siyahı-55' and 'Köksal' cultivars were used as susceptible and resistant controls, respectively.

No	Genotype Name	Material Type / Origin	Source Institution
1	Aydın Siyahı-55	Registered Variety (Susceptible Control)	Aegean Agricultural Research Institute
2	GK1	Landrace, Province Manisa	Aegean Agricultural Research Institute
3	GK2	Landrace, Province Manisa	Aegean Agricultural Research Institute
4	GK3	Landrace, Province Manisa	Aegean Agricultural Research Institute
5	GK4	Landrace, Province Manisa	Aegean Agricultural Research Institute
6	GK5	Landrace, Province Manisa	Aegean Agricultural Research Institute
7	GK6	Landrace, Province Manisa	Aegean Agricultural Research Institute
8	Yamula patlıcanı	Registered Variety	Registered local variety
9	Köksal	Registered hybrid Variety (Resistant Control)	Yüksel Seed Company
10	GK7	Landrace, Province Nigde	Aegean Agricultural Research Institute
11	GK8	Landrace, Province Nigde	Aegean Agricultural Research Institute
12	GK9	Landrace, Province Nigde	Aegean Agricultural Research Institute
13	GK10	Landrace, Province Nigde	Aegean Agricultural Research Institute
14	GK11	Landrace, Province Nigde	Aegean Agricultural Research Institute
15	GK12	Landrace, Province Mugla	Aegean Agricultural Research Institute
16	GK13	Landrace, Province Mugla	Aegean Agricultural Research Institute
17	GK14	Landrace, Province Mugla	Aegean Agricultural Research Institute
18	GK15	Landrace, Province Mugla	Aegean Agricultural Research Institute
19	GK16	Landrace, Province Manisa	Aegean Agricultural Research Institute
20	GK17	Landrace, Province Manisa	Aegean Agricultural Research Institute
21	GK18	Landrace, Province Manisa	Aegean Agricultural Research Institute
22	GK19	Landrace, Province Kayseri	Aegean Agricultural Research Institute
23	GK20	Landrace, Province Kayseri	Aegean Agricultural Research Institute
24	GK21	Landrace, Province Kayseri	Aegean Agricultural Research Institute
25	ETA1	Inbred Line	Aegean Agricultural Research Institute
26	ETA2	Inbred Line	Aegean Agricultural Research Institute
27	ETA3	Inbred Line	Aegean Agricultural Research Institute
28	ETA4	Inbred Line	Aegean Agricultural Research Institute
29	ETA5	Inbred Line	Aegean Agricultural Research Institute
30	ETA6	Inbred Line	Aegean Agricultural Research Institute
31	ETA7	Inbred Line	Aegean Agricultural Research Institute
32	ETA8	Inbred Line	Aegean Agricultural Research Institute
33	ETA9	Inbred Line	Aegean Agricultural Research Institute
34	ETA10	Inbred Line	Aegean Agricultural Research Institute
35	ETA11	Inbred Line	Aegean Agricultural Research Institute
36	ETA12	Inbred Line	Aegean Agricultural Research Institute
37	ETA13	Inbred Line	Aegean Agricultural Research Institute
38	ETA14	Inbred Line	Aegean Agricultural Research Institute
39	ETA15	Inbred Line	Aegean Agricultural Research Institute
40	ETA16	Inbred Line	Aegean Agricultural Research Institute
41	ETA17	Inbred Line	Aegean Agricultural Research Institute
42	ETA18	Inbred Line	Aegean Agricultural Research Institute
43	ETA19	Inbred Line	Aegean Agricultural Research Institute
44	ETA20	Inbred Line	Aegean Agricultural Research Institute
45	ETA21	Inbred Line	Aegean Agricultural Research Institute
46	ETA22	Inbred Line	Aegean Agricultural Research Institute
47	ETA23	Inbred Line	Aegean Agricultural Research Institute
48	ETA24	Inbred Line	Aegean Agricultural Research Institute

Table 2. Sequence of SCAR 426 (Mutlu et al., 2008).

Primer SCAR 426	Sequences 5'- 3'
Forward	TGA GTC CAA ACC GGA CTA CAA G
Reverse	GAC TGC GTA CGA ATT AAC TCT ACG

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