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Screening and validation of three molecular markers for disease resistance in eggplant

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Abstract: *Fusarium oxysporum* Schlecht. f.sp. *melongenae* (FOM), *Verticillium dahliae* (Ve) and *Ralstonia solanacearum* are major limiting pathogens affecting eggplant cultivation and their yield in the world. Natural resistance genes are the most environmentally friendly method to control the disease. Thus, marker-assisted selection (MAS) is preferred as a tool for screening resistance genes (*R*-genes) in eggplant resistance breeding. In this study, markers that are specifically linked to major disease resistance genes conferring resistance to *F. melongenae*, *V. dahliae* and *R. solanacearum* were tested in a population containing breeding materials to validate the resistance. Resistant *Solanum melongena* accessions LS1934 and LS2436 and their reciprocal crosses were used as the resistance resource for this validation study. Moreover, classical resistance tests to FOM and Ve were performed with the root-dip inoculation method for classification of all the accessions based on their resistance/susceptibility responses. The $SCAR_{426}$, CAPs_903 and SIVR844 markers were highly informative for the determination of resistance genes (*Fomg*, *Ve and ERs1*). Therefore, in areas with high susceptibility to diseases, a highly efficient combination of the relevant *R*-genes and their pyramiding into commercial eggplant varieties are proposed to be implemented as a pragmatic approach.

Keywords: breeding; *ERs1* gene; *Fomg* gene; MAS; *Solanum melongena*; *Ve* gene

Eggplant (aubergine) (*Solanum melongena* L.) is a nutritious vegetable that contains vitamins and minerals (Kumari et al. 2018). The total eggplant yield, as a widely grown vegetable, was nearly 54 077 210 tonnes in 2018. Turkey ranked fourth with 836 234 tonnes among the world's highest eggplant producers following China, India and Egypt (FAO 2020). On the other hand, eggplant cultivation has been affected by several factors, among which, diseases are caused by many different important pathogenic agents (Singh et al. 2014; Nahar et al. 2019). *Fusarium* spp. and *Verticillium* spp. have been identified as destructive pathogens that are widespread in many countries where eggplants are produced (Barchi et al. 2018; Chapman 2020). Likewise, *R. solanacearum* is another serious disease agent that restricts the worldwide eggplant cultivation (Kumbar et al. 2021). These soilborne pathogens can cause heavy yield losses up to 50–75% (Panth et al. 2020).

Verticillium wilt is a soilborne disease caused by *V. dahliae* that greatly reduces the yield and quality of eggplants, especially in greenhouse production (Liu et al. 2014). It can survive in the soil for more

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than six years. Moreover, it cannot be sufficiently controlled by many fungicides, with only a few fungicides protecting infected eggplants from disease (Liu et al. 2014; Nahar et al. 2019). Likewise, fusarium wilt caused by *F. melongenae* can spread widely and can survive for many years in the soil. Therefore, cultural or chemical management methods are not sufficient to eradicate the pathogens (Nahar et al. 2019). Furthermore, *R. solanacearum* is a European and Mediterranean Plant Protection Organization (EPPO) A2 quarantine organism (OEPP/EPPO 1978), and also has quarantine significance for Asia and Pacific Plant Protection Commission (APPPC) and Inter-African Phytosanitary Council (IAPSC).

Grafting is an alternative method to chemical management. However, grafting is expensive. Current breeding studies are aimed to develop F_1 hybrids that have a higher fruit yield, quality and disease resistance (Liu et al. 2014; Miyatake et al. 2016; Rakha et al. 2020, 2021). During the breeding process, genotyping and selection based on DNA markers provide substantial advantages over traditional phenotypic screening alone (Nevamea et al. 2020). Studies on the identification of genes that provide tolerance to biotic and abiotic stresses and their usage in eggplant breeding programmes have been discussed for many years (Chapman 2020; Toppino et al. 2021). The release of public genomic eggplant databases has facilitated the development of various molecular markers for the identification of *R*-genes that confer resistance to Fusarium wilt (Mutlu et al. 2008), Verticillium wilt (Liu et al. 2014) and bacterial wilt (Salgon et al. 2017) diseases for use in marker-assisted selection (MAS) conducted using polymerase chain reaction (PCR)-based techniques.

The goals of this study were to determine the efficiency of molecular markers for the determination of the resistance genes that confer resistance to *F. melongenae*, *V. dahliae* and *R. solanacearum* and combining the relevant resistance genes (*R*-genes) for pyramiding into commercial eggplant varieties for future studies.

MATERIAL AND METHODS

A total of 53 genotypes (Table 1) were used in the present study: two resistance parents LS1934 [resistance to *R. solanacearum* (Sakata et al. 1996) and *F. melongenae* (Sakata et al. 1996; Boyaci et al. 2011)] and LS2436 [resistance to *V. dahlia* (Sakata et al. 1996) and *F. melongenae*] obtained from Malaysia and their reciprocal crosses, three susceptible parents *S. melongeae* pure lines from BATEM (Bati Akdeniz Agricultural Research Institute, Antalya, Turkey), and 46 inbred lines (F_6) developed by classical disease screening methods by crossing two resistant eggplant genotypes. In total, seeds of 53 accessions belonging to *Solanum melongena* were sown (120 seeds/accession) mid-August 2020 in pots containing peat + perlite (1 : 1) with 150 holes (15 × 10) and vermiculite was used to cover them. The pots were kept under glasshouse compartment (100 m^2) conditions. They were watered with a Hoagland nutrient solution and maintenance procedures were carried out until 2–4 leaf uniform seedlings were obtained. After the disease inoculation when the seedlings were at this stage, the plants planted in the pots were kept in the same compartment. The recorded climatic conditions of the compartment are given in Figure 1. The experiments were placed in a randomised block design with three replications using 30 seedlings for all the disease resistance tests. Five plants were planted per plot.

DNA extraction and polymerase chain reaction. The total genomic DNA was isolated from the young leaves, as described by Doyle and Doyle (1990). The molecular marker-assisted selections to *F. melongenae*, *V. dahlia* and *R. solanacearum* diseases were performed according to Mutlu et al. (2008), Liu et al. (2014), and Salgon et al. (2017), respectively (Table 2). The PCR amplifications were carried out in a gradient thermal cycler (Biorad DNA-Engine Gradient Cycler, Hercules, CA, USA). All the PCR amplifications were performed in an 18 μL volume containing 2× PCR Mastermix (Fermentas K0171), 2 μL (0.3 μM of each primer) of forward and reverse primers [the SCAR426 primer is a sequence-characterised amplified region (SCAR) marker to the *Fomg* gene; the SIVR844 primer is an single nucleotide polymorphism (SNP) marker to the *Ve* gene; the CAPs_903 primer is a cleaved amplified polymorphic sequence (CAPS) marker to the *ERs1* gene], 2.5 μL of ddH_2O, and 25 ng of eggplant DNA. The PCR amplifications were carried out in a gradient thermal cycler (Biorad DNA-Engine Gradient 84 Cycler, Hercules, CA, USA) using a programme consisting of an initial denaturation step of 2 min at 94 °C followed by 35 cycles of 2 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C; the programme ended with a 10 min elongation step at 72 °C. All of the PCR products were separated on 2% high-resolution agarose gel (Amresco SFR, OH, USA) in a 1× tris-acetate-EDTA (TAE) buffer

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Table 1. Results of the classical and molecular testing to *F. melongenae*, *V. dahlia* and *R. solanacearum* diseases

Genotypes		*Fusarium oxysporum melongenae*		*Verticillium dahliae*			*Ralstonia solanacearum*
Code	names	classical test	molecular test	classical test	DSI	molecular test	molecular test
V1	inbred line	R	S	HR	1.0	R	S
V2	inbred line	R	R	HR	1.3	R	S
V3	inbred line	R	R	MR	2.3	R	R
V4	inbred line	R	HtR	HR	1.3	R	S
V5	inbred line	R	S	MR	1.8	R	S
V6	inbred line	R	S	HR	1.8	R	S
V7	inbred line	R	HtR	MR	2.3	R	S
V8	inbred line (F_6)	R	R	HR	1.8	R	S
V9	inbred line (F_6)	R	R	HR	1.8	R	R
V10	inbred line (F_6)	R	HtR	MR	2.3	R	R
V11	inbred line (F_6)	R	HtR	MR	2.3	R	S
V12	inbred line (F_6)	R	HtR	HR	1.0	R	R
V13	inbred line (F_6)	R	HtR	HR	1.5	R	R
V14	inbred line (F_6)	R	HtR	HR	1.3	R	S
V15	inbred line (F_6)	R	R	HR	1.5	R	S
V16	inbred line (F_6)	R	R	HR	1.3	R	S
V17	inbred line (F_6)	R	R	HR	1.5	R	R
V18	inbred line (F_6)	R	R	HR	1.5	R	S
V19	inbred line (F_6)	R	R	HR	1.5	R	S
V20	inbred line (F_6)	R	R	MR	2.0	R	S
V21	inbred line (F_6)	R	R	HR	1.8	R	R
V22	inbred line (F_6)	R	HtR	HR	1.3	R	S
V23	inbred line (F_6)	R	S	HR	1.8	R	S
V24	inbred line (F_6)	R	S	HR	1.5	R	S
V25	inbred line (F_6)	R	HtR	HR	1.0	R	S
V26	inbred line (F_6)	R	S	HR	1.3	R	S
V27	inbred line (F_6)	R	S	HR	1.3	R	S
V28	inbred line (F_6)	R	HtR	HR	1.3	R	R
V29	inbred line (F_6)	R	HtR	MR	2.0	R	R
V30	inbred line (F_6)	R	HtR	HR	1.8	R	R
V31	inbred line (F_6)	R	R	HR	1.5	R	R
V32	inbred line (F_6)	R	HtR	MR	2.3	R	S
V33	inbred line (F_6)	R	HtR	MR	2.3	R	R
V34	inbred line (F_6)	R	R	HR	1.5	R	R
V35	inbred line (F_6)	R	HtR	HR	1.8	R	R
V36	inbred line (F_6)	R	R	HR	1.0	R	S
V37	inbred line (F_6)	R	R	HR	1.3	R	R
V38	inbred line (F_6)	R	R	HR	1.5	R	S
V39	inbred line (F_6)	R	R	HR	1.5	R	S
V40	inbred line (F_6)	R	R	HR	1.0	R	S
V41	inbred line (F_6)	R	S	HR	1.5	R	S
V42	inbred line (F_6)	R	R	HR	1.8	R	S

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Table 1 to be continued

Genotypes		*Fusarium oxysporum melongenae*		*Verticillium dahliae*			*Ralstonia solanacearum*
Code	names	classical test	molecular test	classical test	DSI	molecular test	molecular test
V43	inbred line (F_6)	R	S	HR	1.3	R	S
V44	LS1934	R	HtR	S	2.8	R	R
V45	LS2436	R	HtR	HR	1.0	R	S
V46	LS1934 × LS2436	R	HtR	S	2.7	R	R
V47	LS2436 × LS1934	R	HtR	MR	2.2	R	R
V48	inbred line (F_6)	R	R	S	3.0	R	R
V49	inbred line (F_6)	R	R	S	2.8	R	R
V50	inbred line (F_6)	R	S	S	3.0	S	S
V51	*S. melongena* pure line	S	S	S	3.0	S	R
V52	*S. melongena* pure line	S	S	S	2.8	S	R
V53	*S. melongena* pure line	S	S	S	3.0	S	S

R – homozygous resistant; HtR – heterozygous resistant; S – susceptible; HR – high resistance; MR – moderate resistance; DSI – disease severity index

at 110 V for 3.5 h, and photographed under UV light (ENDURO GDS Gel Documentation System) in dye (EZ-ONE N472-KIT, Amresco) for further analysis. A 100-bp DNA ladder was used as a molecular standard to confirm the marker.

Classical testing for *Fusarium* ox. f.sp. melongenae and *Verticillium dahlia*. The *F. melongenae* isolate was cultivated in a potato dextrose agar (PDA) medium and inoculated at 24 °C for 10 days. The highly pathogenic isolate AF of *F. melongenae*, isolated from the vascular tissues of a diseased eggplant plant, was used. The AF isolate proved to be more aggressive than other tested isolates in previous work (Mutlu et al. 2008; Boyaci et al. 2013). Discs taken from the fungal culture were inoculated to a liquid synthetic medium prepared according to Pitrat et al. (1991) in Erlenmeyer flasks and grown at 23 ± 1 °C for 8 days on a circular shaker. At the end of this period, the liquid culture was filtered to obtain a spore suspension and the spore density was adjusted to 10^6 conidia/mL using a haemocytometer (Boyaci 2007; Mutlu et al. 2008). The seedling root-dip method was used as the inoculation method in the assays (Gordon et al. 1989). According to the method, the plants were removed from the pots with their roots when they had 2–4 leaves and washed under running tap water. Then, the root tips of the seedlings were shaved and immersed in the suspen-

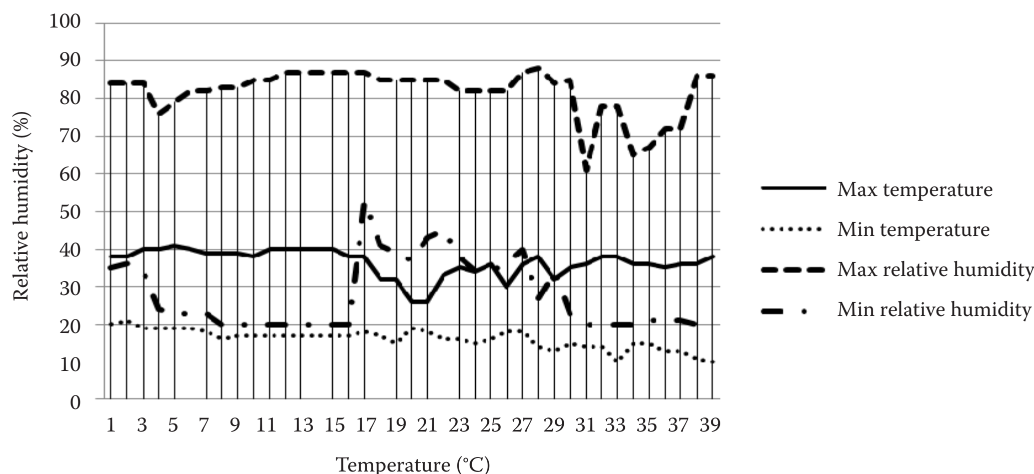

Figure 1. The climatic conditions of the compartment

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Table 2. Gene, marker name and primer sequences for the molecular markers related to the eggplant diseases

Disease	Gene	Marker	Forward primer	Reverse primer	Reference
Fusarium oxysporum melongenae	*Fomg*	$SCAR_{426}$	TGA GTC CAA ACC GGA CTA CAA G	GAC TGC GTA CGA ATT AAC TCT ACG	Mutlu et al. (2008)
Verticillium dahliae	*Ve*	SIVR844	GAA TCT TCA CAA GCT CCA GCT CCA T	CTT TCC TTC AAT CAT CTC GT	Liu et al. (2014)
Ralstonia solanacearum	*ERs1*	CAP s_903	CCC ATT TCA CAC ACA AGC AA	CTC TAT TGC CAC CCC AAG TG	Salgon et al. (2017)

sion prepared at a concentration of 10^6 conidia per mL for about 5 min. After inoculation, the seedlings were immediately transplanted into 180 × 165 mm pots containing a mixture of sterile peat and perlite in a ratio of 1 : 1 (v/v), and maintained in the same glasshouse compartment. The susceptible control, the resistant control, and the uninoculated negative control were used in the assays as the control groups. Stunted or dead plants were evaluated as susceptible, live plants were evaluated as resistant (Cappelli et al. 1993).

The *V. dahliae* isolate was cultivated in a *Verticillium* specific medium and incubated for 10 days at 23 ± 1 °C. The Verticillium isolate (accession no OM212817) was provided by Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection collection. To determine the reactions of the eggplant plants against *Verticillium*, discs taken from fungus colonies developed in the *Verticillium* specific medium with the help of cork borer were placed in a liquid synthetic medium (7.5 g of sucrose, 0.5 g of KCl, 1.0 g of K_2HPO_4, 2.0 g of $NaNO_3$, 0.5 g of $MgSO_4\,7H_2O$, 0.01 g of $FeSO_4$ $7H_20$, 1 L of water, 5 mL of ethanol, 100 mg of streptomycin and 250 mg of chloramphenicol) and grown in a circular shaker at 23 ± 1 °C for 15 days. The seedling root-dip method was used as the inoculation method in the assays. According to the method, the plants were removed from the pots with their roots when they had 2–4 leaves and washed under running tap water. Then, the root tips of the seedlings were shaved and immersed in the suspension prepared at a concentration of 10^6 conidia/mL for 45 min. The plants' symptoms were measured two months after inoculation in a pot experiment (Khiareddine et al. 2006; Çolak-Ateş 2020). The *V. dahliae* % disease severity rate was calculated for each eggplant genotype according to the Başay et al. (2011) evaluation scale. The modified scale for the genotypes were: highly resistant (0%: no disease symptoms), resistant (0.1–10%), moderate-level resistant (10.1–25%), moderate-level susceptible (25.1–50%), susceptible (50.1–100%), the resistant level of the eggplant genotypes has been demonstrated. The disease severity index (DSI) for the verticillium resistance test was calculated with the formula:

$$\text{DSI} = \Sigma\,\frac{(\text{No. of plants in each scale} \times \text{scale value})}{(\text{total No. of plants})}$$

RESULTS

Forty-six eggplant inbred lines (F_6), two resistant parents (LS 1934, LS 2436), three susceptible and pure lines of *S. melongena* were tested by molecular markers associated with resistance to *F. melongenae*, *V. dahlia*, and *R. solanacearum* diseases. These genotypes were classified by classical testing to evaluate the resistance against *F. melongenae* and *V. dahlia* under controlled greenhouse conditions. The classical testing for *R. solanacearum* bacterial disease was not performed because it is a quarantine organism and has not yet been detected in Turkey (Table 1).

As result of the molecular screening for resistance against *F. melongenae* disease, ten inbred eggplant lines and the *S. melongena* pure lines in the breeding pool were found to be susceptible, 22 inbred eggplant lines were homozygous resistant, and 23 genotypes were heterozygous resistant. The $SCAR_{426}$ co-dominant primer provided a single 416 bp band for the homozygote resistant (R) genotypes, a single 800 bp band for the susceptible (S) genotypes, and two bands together for the heterozygous resistant (HtR) genotypes (Figure 2). However, all the genotypes except the pure lines of *S. melongena* were resistant according to the classical test results (Table 1).

Using the SIVR844 marker for *V. dahlia*, all the genotypes except three susceptible *S. melongena* pure lines were identified as resistant (Table 1). SIVR844 behaved as a dominant gene and provided a single 844 bp band for the resistant (R) genotypes and no band for the susceptible (S) genotypes (Fig-

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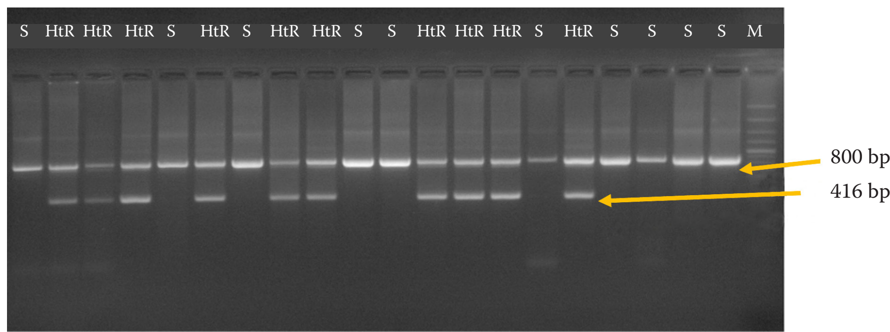

Figure 2. Segregation of the $SCAR_{426}$ marker linked to the *Fomg* locus
M – size marker; HtR – heterozygous resistant; S – susceptible

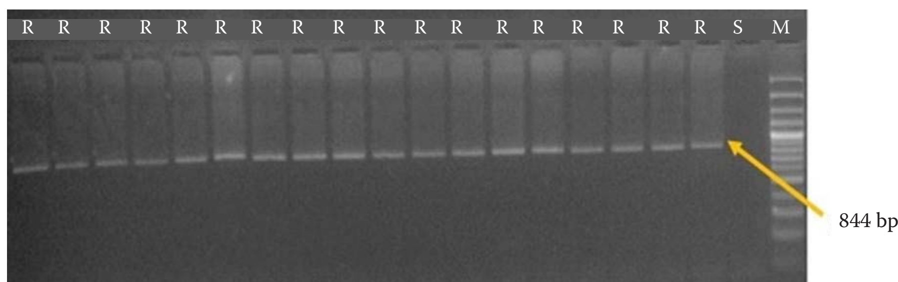

Figure 3. Segregation of the SIVR844 marker linked to the *Ve* locus
M – size marker; R – homozygous resistant; S – susceptible

ure 3). In classical test results, the disease severity of nine inbred eggplant lines and the LS2436 × LS1934 genotype varied from 10.4 to 24.53% and they were considered to be moderately resistant (MR). Moreover, 34 inbreed eggplant lines and LS2436 had high resistance (HT), and LS1934, LS1934 × LS2436, three inbreed eggplant lines, three *S. melongena* pure lines showed 50.1% disease severity and were considered susceptible (S). The DSI values for Verticillium wilt resistance ranged from 1.0 to 3.0. As seen in Table 1, the DSI values varied between 1.0 and 1.8 in the high resistance genotypes, between 2.0 and 2.3 in the moderately resistant genotypes and between 2.7 and 3.0 in the susceptible genotypes.

The eggplant genotypes were tested for *R. solanacearum* resistance with molecular markers by Salgon et al. (2017), 17 inbreed eggplant lines, LS1934, two interspecific genotypes and two *S. melongena* pure lines were found to be homozygote resistant, and the other 31 genotypes were susceptible (Table 1). The CAPs_903 co-dominant marker produced a single 240 bp band for the homozygous resistant (R) genotypes and a single 210 bp band for the susceptible (S) genotypes (Figure 4).

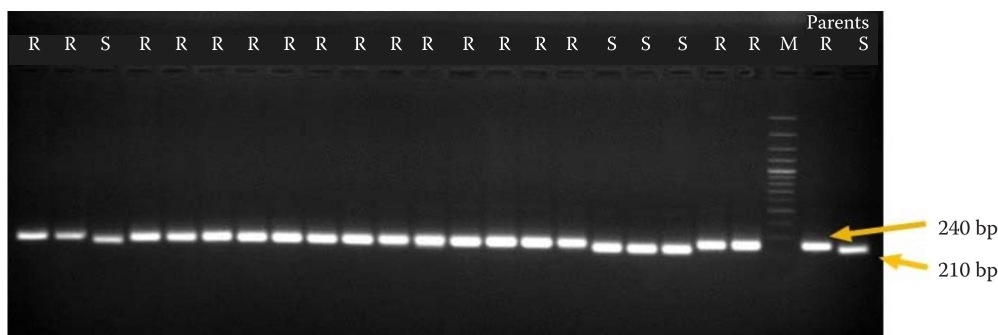

Figure 4. Segregation of the CAP s_903 marker linked to the *ERs1* locus
M – size marker; R – homozygous resistant; S – susceptible

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DISCUSSION

Especially over the last 20 years, molecular biology has revolutionised conventional breeding techniques in all areas. Many breeders started using markers to increase the effectiveness of the selection in breeding and to significantly shorten the development time of varieties with the desired genotypes and the selection of hundreds of plants in a single day (Boyaci 2007). The introgression of a disease resistance gene into cultivated eggplants would allow the development of disease-resistant eggplants (Miyatake et al. 2016; Simsek et al. 2020). Most of the available studies regarding biotic stress resistance in eggplants have, so far, generally focused on resistance for one or two diseases. Testing with different markers in the same genotype for multiple resistance was initially the subject in this article.

While LS1934 was identified as resistant to bacterial wilt, LS2436 was highly tolerant to Verticillium wilt (Sakata et al. 1996). Both genotypes demonstrated resistance to Fusarium wilt in previous studies (Sakata et al. 1996; Boyaci et al. 2011). However, there is no report of the existence of FOM pathogen races or race specificity of resistance genes in eggplant. LS1934, LS174 and LS2436 accessions are known to be highly resistant to the pathogen and their resistance loci have been mapped (Mutlu et al. 2008; Miyatake et al. 2016). These accessions to FOM are controlled by a single dominant gene (Boyaci & Abak 2008). Identification of DNA markers that are tightly linked to a resistance gene is a powerful and useful tool to avoid the previously mentioned drawbacks in breeding programmes such as time-consuming process and reliability (Osei et al. 2018; Kumawat et al. 2020).

The application of MAS requires a tight linkage (less than 2 or 3 cM) between the marker and the gene of interest or using closely linked markers flanking the targeted locus (Tanksley 1983). Although many linkages between the resistance gene and several markers have been established (Miyatake et al. 2016; Barchi et al. 2018), the $SCAR_{426}$ marker, which was found to be linked to the resistance gene at 1.2 cM, and developed by Mutlu et al. (2008), was useful for the determination of resistance against FOM from the resistance source LS2436 in eggplant breeding programmes. Moreover, the use of SCAR markers is quite reliable, accurate, and easy detectable on an agarose gel for the gene in the homozygous or heterozygous resistance of inheritance in breeding populations (Simsek et al. 2020). However, disease resistance should be verified with classical tests; although the marker is very close to the gene and the recombination rate is quite low, there is still a chance of crossovers (Mutlu et al. 2008). To determine the FOM resistance, 77 eggplant genotypes were screened with the $SCAR_{426}$ marker, and it was determined that four eggplant genotypes were heterozygous resistant. Additionally, FOM-resistant lines were verified by classical testing (Colak-Ates et al. 2018). In another study, 533 eggplant breeding materials were screened with $SCAR_{426}$ molecular markers and classical testing. Among the genotypes, although six hybrids did not have the marker locus for resistance against FOM, they were all resistant according to the classical tests (Simsek et al. 2020). Using the $SCAR_{426}$ marker, we determined the resistance of 42 eggplant genotypes to FOM also pheno-typed by classical testing. However, ten genotypes that were resistant according to the classical test results were susceptible according to the molecular results. The most important reason why susceptible genotypes cannot be distinguished in classical testing is the inoculum and symptomatic problems affected by the temperature and humidity (Wechter et al. 1995). Furthermore, the $SCAR_{426}$ molecular marker only displayed a band when it was used in *S. melongena* L. as a resistance source (Mutlu et al. 2008).

Verticillium wilt is a destructive disease in eggplant production (Çolak-Ateş 2020), and also cultural and chemical controls are not very effective (Colak-Ates et al. 2018). Therefore, establishing breeding programmes to obtain disease-resistant varieties is the best way to control Verticillium wilt (Liu et al. 2014; Çolak-Ateş 2020). Many resistance-related genes are summarised to provide a theoretical basis for better understanding of the molecular genetic mechanisms of plant resistance to *V. dahlia* (Song et al. 2020). *Ve* genes are responsible for Verticillium wilt resistance in tomatoes and other species. SlVR844, which is the gene-specific marker for the *Ve* homologue in PI388846, was developed to test *V. dahliae* resistance in the backcross population. Moreover, the marker was tested using the BC4 (Back Cross 4) population according to classical test disease evaluation. Furthermore, ten resistant progenies scored as 0 and ten susceptible progenies scored as three were selected. The PCR detection results indicated that the marker could correctly distinguish the resistant/susceptible genotype dominated by the *Ve* gene. However, the marker was tested

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on 140 BC4 plants, where 67 plants were determined as resistant, and seven plants scored as 1 could not be detected although they were resistant (Liu et al. 2014). The present findings indicated that the marker was reliable for selecting eggplant lines resistant to Verticillium wilt, and there may be another source of resistance independent of the known resistance gene originating from LS2436. In the present study, verification of resistance of 45 eggplant genotypes to Verticillium wilt disease was conducted using classical testing and the SIVR844 marker. However, four genotypes that were susceptible according to classical test results were resistant according to the molecular results. This may be caused by the marker not being closely linked to the targeted gene (Tanksley 1983) or inoculum created symptomatic problems affected by temperature and humidity (Colak-Ates et al. 2018; Colak-Ates 2020). There is a need for different marker types to identify resistance genes in alien eggplant species.

Ralstonia solanacearum has been considered as a complex species and five races based on the host range (Lewis Ivey et al. 2007; Salgon et al. 2017). However, eggplants and their close wild relatives also display resistance to a broad range of isolates (Syfert et al. 2016; Salgon et al. 2017). Three resistance (*EBWR9*, *EBWR2* and *EBWR14*) quantitative trait loci (QTLs) control strains belong to one or more phylotypes. *EBWR9* (previously known as *ERs1*) is located at the end of chromosome 9, a region reported to be rich in disease-resistance factors. A major gene (*EBWR9*) was previously mapped in eggplants using an intraspecific population of recombinant inbred lines derived from the cross of MM738 × AG91-25. The CAP s_903 specific marker for the *ERs1* gene was developed to determine the *R. solanacearum* resistance in the recombinant inbred line (RIL) population (Salgon et al. 2017). Although *R. solanacearum* is common in many countries, it has not yet been detected in Turkey. This disease, which is very aggressive, is also a quarantine pest according to EPPO (OEPP/EPPO 1978). Hence, classical testing for the *R. solanacearum* disease was not performed.

Molecular markers, which are very closely linked to the gene, are highly effective in diseases controlled by a single gene, such as FOM. Unfortunately, the marker used for *V. dahliae* is not very close to the related gene, thus, there are deficiencies in distinguishing between susceptible and resistant genotypes. The inconsistency on the validation results among the observed and expected, especially for *Verticillium*, is an important finding in this study. Therefore, the development of a marker system tightly linked to resistance genes for each species may be a greater gain for breeding efforts. Transferring the major *ERs1* gene to susceptible lines by MAS is effective, but more breeder friendly markers must be developed from closely linked single nucleotide polymorphisms (SNPs) sequences. On the other hand, we need to discover other major genes/QTLs that are usable by breeders for the future creation of broad-spectrum resistance by pyramiding. *R. solanacearum* is a quarantine pathogen that is included in the A2 list (high risk) by EPPO. Therefore, molecular testing is significant in determining the resistant genotypes to *R. solanacearum* in plant breeding.

According to both the classical and molecular analysis results, the V3, V9, V10, V12, V13, V17, V21, V28, V29, V30, V31, V33, V34, V35 and V37 inbred lines were resistant to *F. melongenae* (*Fomg* gene), *V. dahliae* (*Ve* gene) and *R. solanacearum* (*ERs1* gene) diseases. As result, genes combined to provide multiple resistance to three diseases caused by soil-borne pathogens were detected for the first-time using MAS in this study in genotypes of *S. melongena*. The opportunity to perform a multiplex PCR for MAS may arise. Within the scope of this study, the genotypes, which were found to be resistant against the pathogen, can be used as a source of resistance in future breeding studies.

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