

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

GORKEM SULU¹, ILKNUR POLAT^{2*}, HATICE FILİZ BOYACI¹,
AYTUL YILDIRIM³, EMINE GÜMRÜKCÜ¹

¹Bati Akdeniz Agricultural Research Institute (BATEM), Antalya, Turkey

²Akdeniz University, Elmalı Vocational School, Antalya, Turkey

³Pendik Veterinary Control Institute, İstanbul, Turkey

*Corresponding author: ilknurpolat@akdeniz.edu.tr

Citation: Sulu G., Polat I., Boyaci H.F., Yildirim A., Gümrükcü E. (2022): Screening and validation of three molecular markers for disease resistance in eggplant. Czech J. Genet. Plant Breed., 58: 83–92.

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 SCAR₄₂₆, 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

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 F₁ 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. dahliae* (Sakata et al. 1996) and *F. melongenae*] obtained from Malaysia

and their reciprocal crosses, three susceptible parents *S. melongene* pure lines from BATEM (Bati Akdeniz Agricultural Research Institute, Antalya, Turkey), and 46 inbred lines (F₆) 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 m²) 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. dahliae* 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 Cyclers, 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 a 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 ddH₂O, and 25 ng of eggplant DNA. The PCR amplifications were carried out in a gradient thermal cycler (Biorad DNA-Engine Gradient 84 Cyclers, 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

<https://doi.org/10.17221/105/2021-CJGPB>

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

| Genotypes | | <i>Fusarium oxysporum melongenae</i> | | <i>Verticillium dahliae</i> | | | <i>Ralstonia solanacearum</i> |
|-----------|-------------------------------|--------------------------------------|----------------|-----------------------------|-----|----------------|-------------------------------|
| 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 |

Table 1 to be continued

| Genotypes | | <i>Fusarium oxysporum melongenae</i> | | <i>Verticillium dahliae</i> | | <i>Ralstonia solanacearum</i> | |
|-----------|-------------------------------|--------------------------------------|----------------|-----------------------------|-----|-------------------------------|----------------|
| 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 | <i>S. melongena</i> pure line | S | S | S | 3.0 | S | R |
| V52 | <i>S. melongena</i> pure line | S | S | S | 2.8 | S | R |
| V53 | <i>S. melongena</i> 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 dahliae*. 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 10⁶ 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-

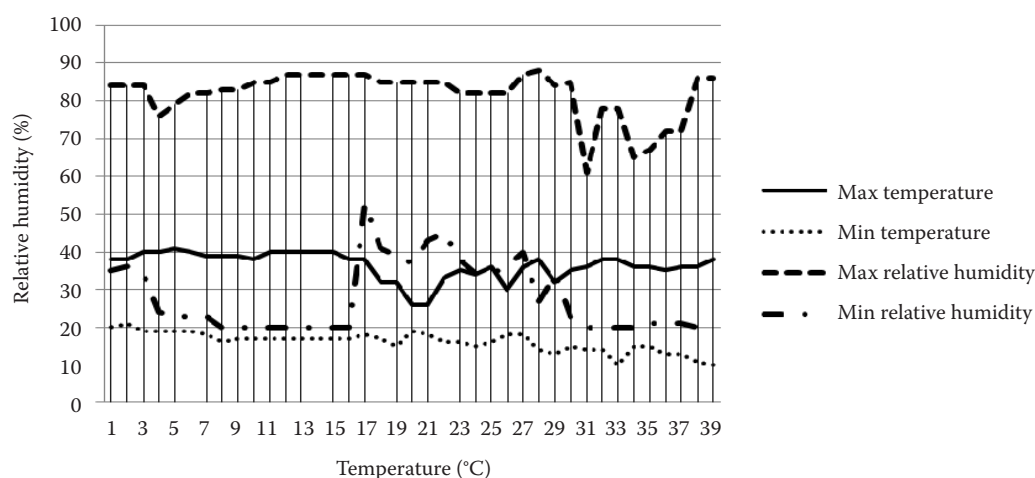


Figure 1. The climatic conditions of the compartment

<https://doi.org/10.17221/105/2021-CJGPB>

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 |
|--------------------------------------|-------------|----------------------|--------------------------------------|------------------------------------|-------------------------|
| <i>Fusarium oxysporum melongenae</i> | <i>Fomg</i> | SCAR ₄₂₆ | TGA GTC CAA ACC GGA CTA CAA G | GAC TGC GTA CGA ATT AAC TCT ACG | Mutlu et al. (2008) |
| <i>Verticillium dahliae</i> | <i>Ve</i> | SIVR844 | GAA TCT TCA CAA GCT CCA GCT CCA T | CTT TCC TTC AAT CAT CTC GT | Liu et al. (2014) |
| <i>Ralstonia solanacearum</i> | <i>ERs1</i> | 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 $MgSO_4 \cdot 7H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 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:

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

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. dahliae*, and *R. solanacearum* diseases. These genotypes were classified by classical testing to evaluate the resistance against *F. melongenae* and *V. dahliae* 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 SCAR₄₂₆ 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. dahliae*, 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-

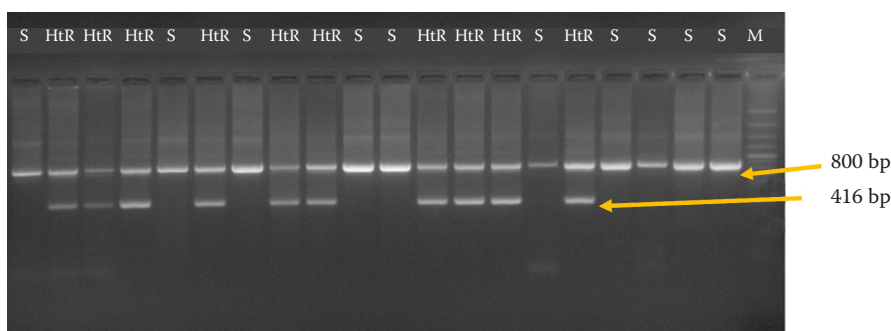


Figure 2. Segregation of the SCAR₄₂₆ marker linked to the *Fomg* locus

M – size marker; HtR – heterozygous resistant; S – susceptible

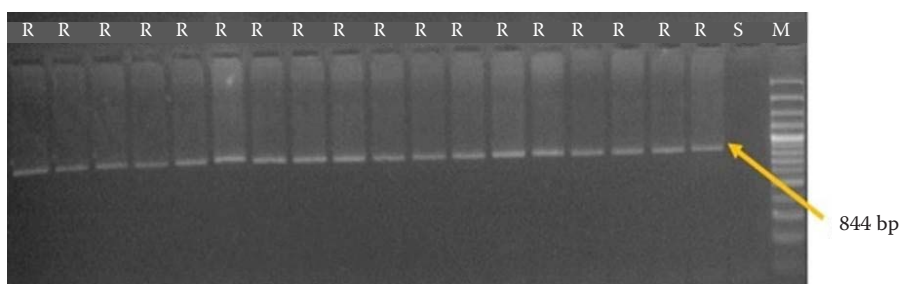


Figure 3. Segregation of the SIVR844 marker linked to the *Ve* locus

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 inbred eggplant lines and LS2436 had high resistance (HT), and LS1934, LS1934 × LS2436, three inbred 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 inbred 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).

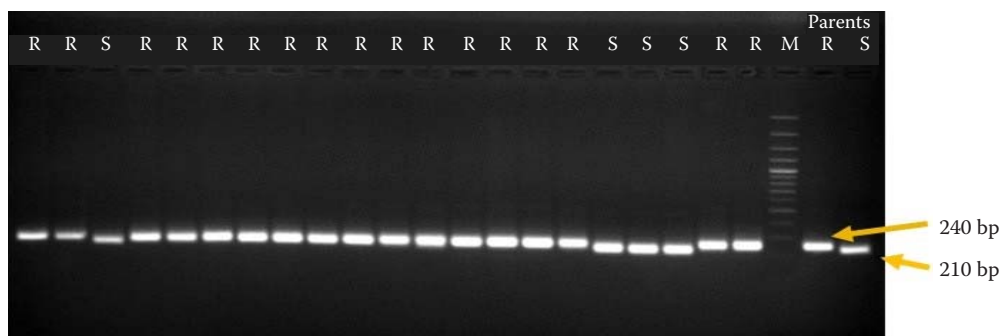


Figure 4. Segregation of the CAP s_903 marker linked to the *ERs1* locus

M – size marker; R – homozygous resistant; S – susceptible

<https://doi.org/10.17221/105/2021-CJGPB>

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 SCAR₄₂₆ 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 SCAR₄₂₆ 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 SCAR₄₂₆ 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 SCAR₄₂₆ marker, we determined the resistance of 42 eggplant genotypes to FOM also phenotyped 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 SCAR₄₂₆ 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. SIVR844, 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

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 phenotypes. *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 *Verticil-*

lium, 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.

REFERENCES

- Barchi L., Toppino L., Valentino D., Bassolion L., Portis E., Lanteri S., Rotino G.L. (2018): QTL analysis reveals new eggplant loci involved in resistance to fungal wilts. *Euphytica*, 214: 20.
- Başay S., Şeniz V., Tezcan H. (2011): Reactions of selected eggplant cultivars and lines to Verticillium wilt caused by *Verticillium dahliae* Kleb. *African Journal of Biotechnology*, 10: 3571–3573.
- Boyacı H.F. (2007): Resistance resources and its inheritance against to Fusarium wilt in eggplants. [Ph.D. Thesis.] Adana, Cukurova University.
- Boyacı H.F., Abak K. (2008): Resistance resources and its inheritance against to Fusarium wilt in eggplants. [Ph.D. Thesis.] Adana, Institute of Natural and Applied Sciences, University of Cukurova.
- Boyacı H.F., Unlu A., Abak K. (2011): Genetic analysis of resistance to wilt caused by Fusarium (*Fusarium oxysporum melongenae*) in eggplant (*Solanum melongena*). *Indian Journal of Agricultural Sciences*, 81: 812–815.

<https://doi.org/10.17221/105/2021-CJGPB>

- Boyacı F., Gumrukcu E., Topçu V., Abak K. (2013): Reaction of some resistant and susceptible eggplant genotypes to different isolates of Fusarium wilt. In: Breakthroughs in the Genetics and Breeding of Capsicum and Eggplant: Proc. XV EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant, Torino, Sept 2–4, 2013.
- Capelli C., Polverari A., Stravato V.M. (1993): *Fusarium oxysporum* f.sp. *melongenae* on the eggplant. *Informatore-Fitopatologica*, 43: 51–54.
- Chapman M.A. (2020): Eggplant breeding and improvement for future climates. In: Kole C. (ed.): *Genomic Designing of Climate-Smart Vegetable Crops*. Cham, Springer: 257–276.
- Çolak-Ateş A. (2020): Investigation of resistance to Verticillium wilt disease (*Verticillium dahlia* Kleb.) in eggplant genotypes. *Plant Protection Bulletin*, 60: 5–11.
- Colak-Ates A., Fidan H., Ozarslandan A., Ata A. (2018): Determination of the resistance of certain eggplant lines against fusarium wilt, potato Y potyvirus and root-knot nematode using molecular and classic methods. *Fresenius Environmental Bulletin*, 27: 7446–7453.
- Doyle J.J., Doyle J.L. (1990): Isolation of plant DNA from fresh tissue. *Focus*, 12: 13–15.
- FAO (2020): FAOSTAT. Available at http://faostat3.fao.org/browse/rankings/commodities_by_regions/E. (accessed Jan 22, 2020).
- Gordon T.R., Okamoto D., Jacobson D.J. (1989): Colonization of muskmelon and nonsusceptible crops *Fusarium oxysporum* f.sp. *melonis* and other species of *Fusarium*. *Phytopathology*, 79: 1095–1100.
- Khiareddine H.J., Remadi M.D., Hibar K., Ayed F., Mahjoub M.E. (2006): Pathogenicity of Tunisian isolates of three *Verticillium* species on tomato and eggplant. *Plant Pathology Journal*, 5: 199–207.
- Kumari A., Chawla N., Dhatt A.S. (2018): Genotypic differences for anthocyanins in different parts of eggplant (*Solanum melongena* L.). *International Journal of Advanced Research in Biological Sciences*, 5: 12–18.
- Kumawat G., Kumawat C.K., Chandra K., Pandey S., Chand S., Mishra U.N., Lenka D., Sharma R. (2020): Insights into marker assisted selection and its applications in plant breeding. In: Abdurakhmonov Y. (ed.): *Plant Breeding – Current and Future Views*. London, IntechOpen.
- Kumbar S., Narayanankutty C., Kurian P.S., Sreelatha U., Barik S. (2021): Evaluation of eggplant rootstocks for grafting eggplant to improve fruit yield and control bacterial wilt disease. *European Journal of Plant Pathology*, 161: 73–90.
- Lewis Ivey M.L., McSpadden Gardner B.B., Opina N., Miller S.A. (2007): Diversity and geographic distribution of *Ralstonia solanacearum* from eggplant in the Philippines. *Phytopathology*, 97: 1467–1475.
- Liu J., Zheng Z., Zhou X., Feng C., Zhuang Y. (2014): Improving the resistance of eggplant (*Solanum melongena*) to Verticillium wilt using wild species *Solanum linnaeanum*. *Euphytica*, 201: 463–469.
- Miyatake K., Saito T., Negoro S., Yamaguchi H., Nunome T., Ohyama A., Fukuoka H. (2016): Detailed mapping of a resistance locus against Fusarium wilt in cultivated eggplant (*Solanum melongena*). *Theoretical and Applied Genetics*, 129: 357–367.
- Mutlu N., Boyacı F.H., Göçmen M., Abak K. (2008): Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a Fusarium wilt resistance gene in eggplant. *Theoretical and Applied Genetics*, 117: 1303–1312.
- Nahar N., Islam R., Uddin M.M., de Jong P., Struik P.C., Stomph T.-J. (2019): Disease management in eggplant (*Solanum melongena* L.) nurseries also reduces wilt and fruit rot in subsequent plantings: A participatory testing in Bangladesh. *Crop Protection*, 120: 113–124.
- Nevamea A.Y.M., Xia L., Wenting Z., Nchongbohc C.G., Wenhua L., Hasand M.M., Alame A., Longtinga S. (2020): Validation of some disease-resistance molecular markers associated with multiple diseases in tomato for marker-assisted selection program. *ScienceAsia*, 46: 19–29.
- OEPP/EPPO (1978): *Pseudomonas solanacearum* (E.F. Smith) E.F. Smith. Data sheets on quarantine organisms. *Bulletin OEPP/EPPO Bulletin*, 8: 53–57.
- Osei M.K., Prempeh R., Adjepong-Danquah J., Opoku J.A., Danquah A., Danquah E., Blay E., Adu-Dapaah H. (2018): Marker-assisted selection (MAS): A fast-track tool in tomato breeding. Chapter 6. In: *Recent Advances in Tomato Breeding and Production*. London, IntechOpen.
- Panth M., Hassler S.C., Baysal-Gurel F. (2020): Methods for management of soilborne diseases in crop production. *Agriculture*, 10: 16.
- Pitrat M., Risser G., Epinat C., Ferrière C., Ricard M., Olivier C., RuYnato A., Lecoq H., Blancard D., Bertrand F., Nicot A., Glandard A., Molot P.M., Mas P. (1991): Techniques d'inoculation artificielle du melon avec différents agents pathogènes pour la sélection de variétés résistantes. *Informal Technical Bulletin Edited by INRA, Montfavet, Station d'Amélioration des Plantes maraîchères and Station de Pathologie végétale*.
- Rakha M., Namisy A., Chen J.R., El-Mahrouk M.E., Metwally E., Taha N., Prohens J., Plazas M., Taher D. (2020): Development of interspecific hybrids between a cultivated eggplant resistant to bacterial wilt (*Ralstonia solanacearum*) and wild eggplant relatives for the development of rootstocks. *Plants*, 9: 1405.
- Rakha M., Prohens J., Taher D., Wu T.H., Solberg S.Ø. (2021): Eggplant (*Solanum melongena*, *S. aethiopicum* and *S. mac-*

- rocarpon) breeding. In: Advances in Plant Breeding Strategies: Vegetable Crops. Cham, Springer: 163–203.
- Sakata Y., Monma S., Narikawa T., Komochi S. (1996): Evaluation of resistance to bacterial wilt and Verticillium wilt in eggplants (*Solanum melongena* L.) collected in Malaysia. Journal of the Japanese Society for Horticultural Science, 65: 81–88.
- Salgon S., Jourda C., Sauvage C., Daunay M.-C., Reynaud B., Wicker E., Dintinger J. (2017): Eggplant resistance to the *Ralstonia solanacearum* species complex involves both broad-spectrum and strain-specific quantitative trait loci. Frontiers in Plant Science, 8: 828.
- Simsek D., Samur D., Mutlu N., Pinar H. (2020): Marker assisted backcross breeding for fusarium wilt (*Fusarium oxysporum* Schlecht. f.sp. *melongenae*) in eggplant. International Journal of Agricultural and Natural Sciences, 13: 91–100.
- Singh B.K., Singh S.S.B.K., Yadav S.M. (2014): Some important plant pathogenic diseases of brinjal (*Solanum melongena* L.) and their management. Plant Pathology Journal, 13: 208–213.
- Song R., Li J., Xie C., Jian W., Yang X. (2020): An overview of the molecular genetics of plant resistance to the verticillium wilt pathogen *Verticillium dahlia*. International Journal of Molecular Sciences, 21: 1120.
- Syfert M.M., Castañeda-Álvarez N.P., Khoury C.K., Särkinen T., Sosa C.C., Achicanoy H.A., Bernau V., Prohens J., Daunay M.-C. Knapp S. (2016): Crop wild relatives of the brinjal eggplant (*Solanum melongena*): Poorly represented in genebanks and many species at risk of extinction 1. American Journal of Botany, 103: 635–651.
- Tanksley S.D. (1983): Molecular markers in plant breeding. Plant Molecular Biology Reports, 1: 3–8.
- Toppino L., Prohens J., Rotino G.L., Plazas M., Parisi M., Carrizo García C., Tripodi P. (2021): Pepper and eggplant genetic resources. In: The Wild Solanums Genomes. Cham, Springer: 119–154.
- Wechter W.P., Whitehead M.P., Thomas C.E., Dean R.A. (1995): Identification of a randomly amplified polymorphic DNA marker linked to the Fom-2 Fusarium wilt resistance gene in muskmelon MR-1. Phytopathology, 85: 1245–1249.

Received: November 2, 2021

Accepted: February 1, 2022

Published online: February 17, 2022