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DETERMINATION OF THE RESISTANCE OF CERTAIN EGGPLANT LINES AGAINST FUSARIUM WILT, *POTATO Y POTYVIRUS* AND ROOT-KNOT NEMATODE USING MOLECULAR AND CLASSIC METHODS

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ABSTRACT

In recent years, the fact that the healthy life ideal has become prominent in the agenda resulted in an increase in eggplant consumption and utilization, similar to other vegetables, promoting an increase in eggplant cultivation. In the present study, resistance of 77 different eggplant genotypes against Fusarium wilt (*Fusarium oxysporum* f.sp. *melongenae*-FOM), *Potato Y potyvirus* (PVY) and Root-knot nematode (*Melodiogyne incognita*-RN) were determined with molecular marker assisted selection and classical testing. To determine FOM resistance, 77 eggplant genotypes were screened with the SCAR426 marker, which was identified to be the closest to the gene at 1.2 cM, and it was determined that the four eggplant genotypes, namely P11, P29, P49 and P52 were heterozygous resistant against FOM. FOM-resistant lines were verified by classical testing. As a result of classical testing conducted to determine PVY and RN resistance of eggplant genotypes, it was determined that all lines were PVY-sensitive and P29 and P52 genotypes were resistant to FOM and RN. Disease-resistant genotypes determined in the present study would contribute to the development of the new F1 hybrid eggplant cultivar.

KEYWORDS:
Eggplant (*Solanum melongena*), *Fusarium oxysporum* f. sp. *melongenae*, *Potato Y potyvirus, Meloidogyne incognita,* disease and pest resistance, molecular marker

INTRODUCTION

In recent years, the knowledge that eggplants (*Solanum melongena* L.) contain significant vitamins and minerals for human health similar to other vegetables, the fact that several dishes could be cooked with eggplants, the increased use of eggplants in salads, jams and pickles promoted an increase in eggplant cultivation. Globally, China is the leading eggplant producer, followed by India, the USA and Turkey. According to 2015 data, the annual Turkish eggplant production was 805, 259 tons and the highest amount was produced in Mediterranean region with 365, 637 tons [28]. Eggplant production in Turkey can be both open field and greenhouse,but they can not be cultivated in every region because of preference for special climate and soil, as well as the preference for maintenance conditions and crop rotation. Fusarium wilt (*Fusarium oxysporum* f. sp. *melongenae*- FOM*), Potato Y potyvirus* (PVY*)* and Root-knot nematode (*Meloidogyne incognita*-RN) are among the most important plant diseases limiting the eggplant production in the regions of production in Turkey [1-8-24].

In areas where eggplants are cultivated in the region, several chemicals are commonly used in the fight against diseases and pests. Among alternative agricultural systems, cultural measures are prominent among the methods used in the fight against plant diseases. Thus, the use of resistant cultivars in the fight against the pathogen is the most effective, most economic, environment-friendly and sustainable method. Today, improvement on studies primarily aim at developing resistant varieties. The morphological determinants used in classical breeding studies could help distinguish genotypes, however these could be affected by environmental factors. If any of these characteristics is recessive, then homozygous and heterozygous individuals cannot be detected. Thus, the development of the use of molecular markers (MAS) in biotechnology in resistance to diseases during recent years became significant in breeding plants. Therefore, hundreds of plants can be selected reliably, and quickly and concurrently, saving time and space in the selection resistant varieties [4].

It was reported that Fusarium wilt in eggplants caused significant yield losses in several countries such as Italy, Israel, Japan and the Netherlands, as well as Turkey, both in outdoor and greenhouse cultivation [1]. In a study conducted by Yücel [30] in Turkey, it was found that the wilt disease was caused by *Fusarium* sp. in Adana, Icel and Antalya

provinces, but mostly induced by *Verticillium* sp. in Hatay province. In a study by Altınok [1] that determined the prevalence of the pathogen that caused FOM disease in Mersin and Adana provinces, it was found that the prevalence of the disease in greenhouses and tunnels in Mersin province was 42.1% and the same rate was 33.5% in outdoor fields. In Adana province, where the cultivation is mainly in open fields, the prevalence rate of the disease was 33.2%. Mutlu et al. [18] demonstrated the use of the MAS breeding program by developing two dominant SCAR markers that determine molecular resistance to the disease, using the Fusarium wilt resistant LS 2436 genotype.

Studies on viral diseases that limit the production of eggplants, which are less popular than tomatoes, peppers and potatoes, have increased in recent years. The first paper on prevalent viral diseases on eggplant was published in the 1950s [10]. Symptoms such as mosaic stunting, small and amorphous fruit formation were reported from every climatic and geographical region where eggplants were cultivated [16]. Although there are limited number of studies on eggplant viruses, viruses such as Potato virus Y (PVY), Tomato spotted wild virus (TSWV) and eggplant mottled dwarf virus (EMDV) were reported in Turkey [24]. PVY resistant pepper and tomato lines were developed for PVY control, which is very common in tomato and pepper fields, but similar studies were not conducted for eggplants. In recent years, efforts to improve eggplant breeding and resistant varieties increased [5].

The main goal in eggplant breeding is to develop hybrid varieties with high quality that are resistant to diseases - pests. In countries where eggplant cultivation is intensive and in Turkey, it is necessary to develop varieties that are resistant to soil-borne pathogens and nematode damage [25]. It was reported that root-knot nematodes cause significant damage to vegetable and eggplant cultivation fields in the Mediterranean region in Turkey [8]. It was reported that root-knot nematodes cause significant yield losses in vegetables amounting to 42-54% in tomatoes and 30-60% in eggplants [22]. It was determined that 53% of the global root-knot nematode population were *M. incognita* [23].

The present project aimed to determine the resistance of eggplant genotypes that are significant with respect to yield and certain quality cirteria against Fusarium wilt, Potato Y potyvirus, and root-knot nematode which economically limit eggplant cultivation, with molecular marker assisted selection and classical testing.

MATERIAL AND METHODS

A total of 77 eggplant genotypes were used as material in the experiment that were prominent in eggplant breeding programs conducted at the Alata Horticulture Research Institute (AL/Mersin/Turkey) based on yield and certain quality criteria.

Determination of FOM resistant eggplant lines using molecular marker

The LS 1934 and LS 2436 genotype DNAs were used as resistant controls; Kemer, Yula and NSFB-99 were used as sensitive controls in this PCR study [2].

Genomic DNA isolation

DNA Purification Mini Kit (Thermo Sc. G.JT Plant-K0792) was used for total genomic DNA isolation on eggplant lines. For this purpose, 200 mg was obtained from the young eggplant leaves. The obtained DNAs were adjusted to 40 ng with the a spectrophotometer, 0.8% agarose gel controls were conducted and stored at -20 °C.

PCR conditions

SCAR426 primer, which were found to be at the closest distance with 1.2 cM were used in the study to determine the FOM resistance [18] (Figure 1). PCR Reaction (25 µL); 22 µL DreamTaq Green PCR Master Mix (Thermo Sc.) (0.5 U Taq polymerase, 2X DreamTaq Green buffer, 0.4 mM dNTPs and 4 mM $MgCl_2$), 1 µL primer and 2 µL DNA (10 ng) were used. PCR conditions; 3 min pre-denaturation at 94 ° C followed by 35 cycles including 1 min at 94 ° C, 50 sec at 57 ° C, 70 sec at 72 ° C, and 1 cycle of 10 min final extension at 72 °C were programmed on a GeneAmp 9700 thermocyler. The PCR products were electrophoresed in 2% agarose gel and stained in a Ethidium Bromide solution, were imaged under UV light and the results were recorded.

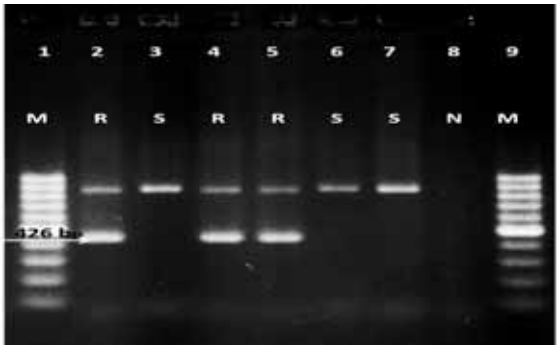


FIGURE 1
Agarose gel image obtained with SCAR426 primer of resistant and sensitive controls. (2,4,5; Resistant, LS2434, LS 1934, Köksal; 3,6,7; Sensitive NSFB99, Yula, Kemer; N; negative control, M:100bp)

Classical testing. Classical testing for FOM resistance. In the present study, classical tests on 4 heterozygous FOM-resistant eggplant lines identified with SCAR426 primers on 77 eggplant genotypes were conducted with seedling root dip treat-

ment. For this purpose, high virulence FOM isolate obtained from greenhouse eggplant fields was grown in PDA for 7 days, mycelial discs that included microconidia were obtained and the pathogen was inoculated in 250 ml Erlenmeyer flasks that contained 100 ml FOM liquid synthetic medium. After the inoculation, the Erlenmeyer flasks were shaken in the climate chamber at 24°C and for 16 hours under light and 8 hours in the darkness in 100 rpm mixer for 5-8 days to obtain fungus liquid culture. Microconidia suspension was obtained by passing the micella that developed during incubation through double-layered cheesecloth. For the FOM inoculation test ($1x10^6$ spores/ml) was carried out according to Çolak and Biçici [7] in the 2-4 leaf eggplant seedling period. Each three inoculated eggplant seedlings were planted in one 15x15 cm pot that contained (1: 1) peat and perlite. The experiment was conducted with 5 pots for each line with completely randomized design and 3 plants were planted in each pot. The pots were kept in the climate chamber located at the Directorate of Biological Control Research Institute (BC) at a temperature of 26 ± 2 °C, under 16 hours of light and 8 hours of darkness and a relative humidity of 60-70%. After 25-30 days into the experiment, plants were identified as resistant (0: symptom-free plants: no disease) and sensitive (≥ 1: plants with symptoms: disease is present) plants on a 5-point scale based on the symptoms exhibited by the plants and the browning in the plant root vascular system [27].

Classical testing for PVY resistance. The characterized PVY isolate, which is the most common virus observed in the eggplants and could be transmitted by mechanically or by seeds, was obtained as a lyophilized isolate from Spain (Murcia), which is used in European Union Vegetable Breeding resistance tests. PVY was inoculated by mechanical inoculation during the cotyledon leaf period of the plants obtained from eggplant seeds. For mechanical inoculation, extraction of leaves from infected plants with PVY was obtained according to Fidan et al. [11]. The obtained extract was inoculated into eggplant genotypes after filtering with 2 layers of cheesecloth. The plants that were inoculated with PVY were kept in the dark for one day in a climate chamber at 22-24 °C and then transferred to the greenhouse and the formation of PVY symptoms was observed periodically. Two weeks after the first inoculation, young leaves reaching half of full size were re-inoculated as described above. Four weeks after the second inoculation, virus indications were recorded as present or absent [11].

Testing for root-knot nematode resistance. The root-knot nematode (*M. incognita* Chitwood, 1949) that would be used in nematode resistance tests was cultured in the nematode sensitive "Soray F1" tomato variety. Egg packages were removed under stereo binoculars from the cultivated plants and 2 term infectious larvae were obtained with Baermann funnel method to inoculate the plants (2-4 leaf period). The inoculation was conducted by counting the 2 term larvae in centrifuge tubes so that an average of 1000 larvae would be included in each pot and they were administered at a depth of about 2 cm in the vicinity of the root region. The plants were kept in a climate chamber with 25±1 ºC temperature and 60 ± 10% humidity and 16:8/L:D lighting conditions at the BC. The experiment was set up with five replications, according to completely randomized design [13]. After the inoculation of the root-knot nematode, the plants were grown in the climate chamber for 60 days and at the end of this period, the plants were uprooted and evaluated with root knotting rate. In this case, the assessment was conducted based on the 0-5 scale (0-2: resistant, 3-5: sensitive) according to the method specified by Barker [3].

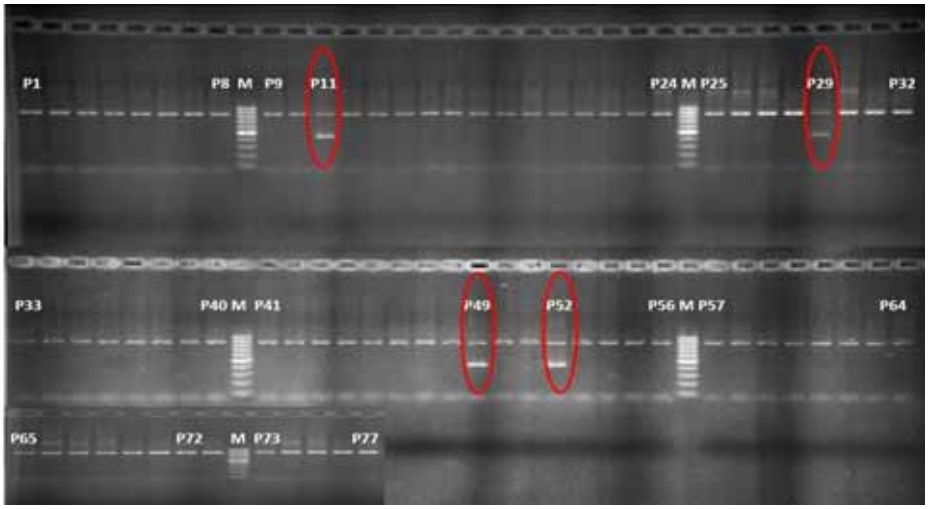


FIGURE 2
Agarose gel of eggplant genotypes tested with SCAR426 primer in determination of resistance to FOM

RESULTS

FOM resistance, one of the most important fungal diseases that limit eggplant cultivation, was identified with molecular studies conducted on 77 eggplant genotypes. For this purpose, eggplant genotypes were tested with the SCAR426 marker, which was determined to be the closest with 1.2 cM, to determine FOM resistance. PCR studies demonstrated that 4 eggplant genotypes (P11, P29, P49 and P52) were FOM resistant (426 bp) and 73 eggplant genotypes were sensitive (347 bp) (Figure 2).

In breeding studies, rapid scanning of disease-resistant lines using molecular markers saves time, space and provide reliability in selection of the material obtained with the desired genotypes and selection of hundreds of plants in a single day [4]. Goth and Webb [12] reported that there were no eggplant varieties that were resistant to *Verticillium* wilt solitude, while certain eggplant varieties were tolerant to Fusarium wilt. Genetic resistance studies on FOM demonstrated that resistance is controlled by a single dominant gene. However, studies that aimed to transfer the resistance in wild forms to the culture plants were not quite successful. Mochizuki et al. [20] reported that the LS174 line was resistant to Fusarium, and found that the resistance in LS174 was managed by a single dominant gene. In a study by Monma et al. [21] it was reported that the LS 1934 and LS 2436 genotypes of the *S. melongena* species were resistant to FOM. Boyaci and Abak [6] investigated the inheritance of resistance between the resistant genotypes LS 1934 and LS 2436 of the *Solanum melongena* species and the susceptible genotype NSFB-99 and found that the resistance was monogenic dominant for both resistant genotypes. Genomic mapping was conducted on the F2 and BC1 populations obtained by cross-breeding the FOM-resistant genotype LS 2436 and sensitive NSFB-99 and the H-12 primary marker with a distance of 2.6 cM to the resistance gene was identified. However, in the conducted RADP marker rehabilitation study, it was demonstrated that the inheritance of resistance could not be determined as homozygous or heterozygous, and the reproducibility was difficult, so it was reported that further studies were needed. Mutlu et al. [18] developed SRAP, SRAP-RGA, RAPD and SCAR markers to determine the resistance to FOM pathogen. It was determined as a result of 2,316 primer combinations that three markers were the closest to gene with 2.6 cM in the study. It was determined that the co-dominant SRAP marker Me8 / Em5 and the dominant SRAP-RGA marker Em12 / GLPL2 were linked to the resistance gene with 1.2 cM, while the RAPD marker H12 was linked with 2.6 cM to the both alleles. In the study, it was determined that the SRAP marker F2 and backward hybrid 3 generations were linked to the resistance gene, and it was transformed to two dominant SCAR markers. It was reported that the two SCAR markers designed in that study might be useful in MAS-selective breeding studies to determine resistance to Fusarium wilt in eggplants. The preference of the closest primer used in breeding studies is important for the reliability of the results. In the present study, eggplant genotypes were tested with the SCAR426 primer, which was found to be linked to the resistance gene at 1.2 cM.

The use of SCAR markers is quite reliable, accurate and easily detectable on agarose gel due to its close proximity to the gene in homozygous or heterozygous determinations of inheritance in F2 and F3 populations in breeding studies when compared to other SRAP RAPD and RGA [19-27]. The findings of the present study once more demonstrated that by selecting adequate genotypes with MAS selection in breeding studies would save money, time and contribute to the rapid commercialization of the lines in cross-breeding programs.



FIGURE 3
Disease symptoms of eggplant Fusarium wilt.
a) yellowings on the leaves b) brown discoloration of the vascular system

Mutlu et al. [18] stated that disease resistance should be verified with classical tests that would be conducted in regular intervals on breeding material that were identified as resistant with markers in breeding programs, although these types of markers, such as SCAR426 and SCAR347, are very close to the gene and their recombination rate is quite low. As a result of molecular work conducted in the present study, verification of the resistance of the 4 eggplant genotypes to FOM pathogen using SCAR426 primer was conducted with classical testing. As a result of the classical testing conducted with this purpose, evaluations of the plant and the root demonstrated that there were no FOM symptoms (Figure 3).

Potato Y potyvirus (PVY) was described initially by Smith [26]. It was determined that PVY was the most common viral disease in eggplant fields, causing the highest damage with mosaic stunting and small and amorphous fruit formation [16]. PVY in the nature is mostly transported by vectors (*Myzus persicae*). It was reported that the eggplant cultivated in the same period with tomato and pepper was effective in high PVY infection [5]. In a study conducted in India, it was determined that the natural inoculation of eggplant with PVY spreads rapidly in the presence of vector aphids, indicating that it was the most harmful viral disease in eggplant fields [5]. Mechanical inoculation is the most preferred method for resistance studies, while vectors are very effective in spreading the disease. For this purpose, the source of PVY resistance in eggplant genotypes was found and classical testing was conducted to provide the possibility of developing new resistant varieties. Mechanical infection of eggplant genotypes with PVY resulted in systemic infections such as mosaic, paling of color, stunning, and leaf deformations (Figure 4). As a result of the observations, 77 eggplant genotypes tested were determined as sensitive to PVY by mechanical inoculation method. Although PVY is widespread throughout the world, it is the most important potyvirus in Europe, characterized by the loss of pepper and tomato crops especially in the Mediterranean basin [17]. Thus, studies on the resistance of tomatoes and peppers against PVY were conducted previously and resistant and sensitive populations were created with mechanical inoculations, and markers were developed for use in resistance [15]. Since eggplants have lower economic significance when compared to tomatoes and peppers, the above-mentioned studies on eggplants are new.

Root-knot nematode resistance: In the study where the resistance of the tested eggplant genotypes against root-knot nematode (*M. incognita*-RN) was determined, it was identified that 14 eggplant genotypes were resistant with 0-2 scale values and 63 eggplant genotypes were sensitive with 3-5 scale values (Figure 5). Knotting rates for eggplant genotypes that are resistant to RN and FOM based on the 5-pont scale are presented in Table (1). As a result, two eggplant genotypes (P29 and P52) resistant to both FOM and root-knot nematode were determined. RN destroy the vascular system tissues in the plants by creating knots of different sizes in plant roots, restricting water and nutrient uptake and leading to slow or ceased plant growth [14]. If the population density is high, the plant may completely fade. Root-knot nematodes also cause indirect damages by facilitating other disease pathogens. It is known that the areas on the roots where the root-knot nematodes feed on are particularly used as gates of entry by several soil-based disease pathogens. Disease pathogens that cannot infect under normal conditions could easily infect the nematode-damaged plants. *M. incognita*, which was determined as the most common root-knot nematode species in studies conducted on the prevalence of root-knot nematodes in Turkey, was found in 93% of the Central Anatolia region soil, 80% of the Aydın province, 42% of Adana, Hatay and Antalya provinces, and 44% of Mersin province [22-23-29]. In several studies conducted on nematode and fungus interactions, it was reported that in the presence of nematodes soil-borne pathogenic fungi were observed earlier periods and their severity was increased [9].



FIGURE 4
Symptoms of mosaic, stunting and foliage deformations in genotypes after infection with PVY by mechanical inoculation



FIGURE 5
Symptoms of root-knot nematode in eggplant a) Root-knot with infected eggplant greenhouse. b) Root-knot in P11 eggplant line

TABLE 1
Galling index rate of *Meloidogyne incognita* at the roots of eggplant genotypes

Eggplant genotypes	Galling index	Resistant (R) Susceptible (S)
P11	4,40 ± 0,25 c	S
P29	0,00 ± 0,00 a	R
P49	5,00 ± 0,00 c	S
P52	0,80 ± 0,37 b	R
Resistant control	1,00 ± 0,44 b	R
Sensitive control	5,00 ± 0,00 c	S

The varieties containing the same letters in the same column are significantly different from each other at $P < 0.05$ according to Duncan's Multiple Range Test

CONCLUSION

The objective of the present study was to identify varieties that contain the resistant gene source against all three diseases (FOM + PVY + RN). As a result of molecular and classical tests, 4 FOM resistant eggplant genotypes were identified. As a result of conventional testing for PVY and RN resistance on these lines, it was determined that all lines were PVY sensitive, while P 29 and P 52 genotypes were resistant to FOM and RN. In order to increase the efficiency of fight against soil-borne pathogens, in addition to the selection of resistant varieties, it is necessary to consider the system as a whole and combine control applications for preservation of environmental resistance. In this context, a variety that is resistant against a single pathogen created with classical breeding methods is not sufficient for an extensively cultivated plant species such as eggplant prone to several diseases and pests in terms of productivity and quality. Under contemporary conditions, it is necessary to provide resistance against at least 3 or more disease-damaging based on cultivation site and time. The number of resistant needed is longer than the breeding period. While a marker was developed in determining the resistance against Fusarium wilt, which caused significant damage during the last few years due to increased cultivation, there were no studies conducted on PVY and root-knot nematode so far. With the present study, we contributed to the infrastructure for the development of these markers and disease-resistant and sensitive eggplant genotypes. The present study results determined the resistance against important biotic factors, thus saving time required for the commercialization of multi-resistant variety candidates. Furthermore, the identification of FOM and root-knot nematode resistant parents would contribute the alternative projects (F1 hybrid) to obtain new varieties based on prominent global fruit quality criteria demands for these parent lines by the line-owner institution.

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