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Research Article

MARKER ASSISTED BACKCROSS BREEDING FOR FUSARIUM WILT (*Fusarium Oxysporum Schlecht. F. Sp. Melongenae*) IN EGGPLANT

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ABSTRACT. Eggplants are produced in both greenhouses and open fields. Plant diseases and pests, and disease causes significant yield, thus economic losses loss. Fusarium wilt (*Fusarium oxysporum Schlecht.f. sp. melongenae*, FOM) is a major soil-borne pathogen, causing vascular wilt disease in eggplant. A molecular marker tightly linked to single dominant gene (FOM) was developed for use in marker assisted selection (MAS). The aim of the study is to develop eggplant lines resistant against Fusarium wilt using a marker assisted backcross breeding approach. Donor parents were carrying the *Fusarium wilt* resistance gene that six commercial hybrids claimed to have fusarium wilt resistance. The eggplant breeding materials (F1 to F8) was first screened with molecular markers linked to the FOM gene. Then, the 533 young seedlings were root-dip inoculated with FOM isolate. The seedlings identified to be resistant using the markers all survived in the inoculation. Although, the six hybrids that did not possess the marker locus for resistance against FOM were all resistant according to classical test. BC1F1, BC2F1 and BC3F1 population were developed from sensitive female and resistance male(commercial hybrids) crossing and all populations tested as classical and molecular. From resistance BC3F1 progenies 25 DH plants were obtained from each combination and resistance status of these plants was determined through initially molecular then classical testings. Results indicate that the marker was reliable to develop eggplant lines resistant against fusarium wilt, and there may can be another source of resistance that is independent from the known resistance gene originating from LS2436.

Keywords: *Disease, Hybrid Eggplant, Marker Assisted Selection, Resistance*

INTRODUCTION

Eggplant (*Solanum melongena* L.) is the third most economically important *Solanaceous* crop after potato and tomato [1]. Eggplant is one of the most cultivated fruit plants worldwide with an 1.858.253 ha harvested area and worldwide annual production is, production is more than 50 million tons in the world [2].

Eggplant cultivated lands are mostly located production area are mainly within subtropical zones and productions are performed either in for both greenhouses or and open fields. , worldwide production area and total yield is condensed in Asia, Africa, Mediterranean Basin and South America are the major eggplant producer continents [3].

In European countries, eggplant is an outlandish vegetable but in Asia and the Mediterranean, it is an important and valuable source of nutrient ingredient, thus it is called as the “king of vegetables” [4].

Eggplant offers the possibility of improvement through heterosis breeding and continues to be a choice of breeders for exploitation of heterosis due to the hardy nature of the plant, comparatively large size of flowers, and large numbers of seeds produced by a single act of pollination. Increased productivity can be achieved in the shortest time can be achieved through heterosis breeding [5]. Highly varied consumer preferences have acceptance directed researchers to demands development of high-yielding F1 hybrids. Exploitation of hybrid vigor has become a potential tool for improvement in eggplant [6], [7]; [8]. The estimation of heterosis for yield and its component characters would be useful in determining the best hybrid combination. Knowledge on genetics of resistance helps in determining the most appropriate breeding method.

Eggplant is susceptible to various diseases especially *fusarium*, *verticillum* and *bacterial wilt* [9]. Soil-borne diseases (e.g. bacterial and fungal wilts, nematodes) and insects are the most serious diseases causing of great losses reduces in yield and quality of eggplants both in greenhouses and in open fields cultivations. Fungal wilts caused by *Verticillium dahliae* (Vd) Kleb. and *Fusarium oxysporum f. sp. melongenae* (FOM) are two of the main diseases in eggplant.

Fusarium wilt, is one of the most devastating and widespread outspreaded diseases of eggplant. Matsuo and Ishigami [10] was published the first study for *Fusarium Wilt* and then, fundamental researches have been conducted printed with the aim of identify resistant eggplant allies.

Fungus penetrates into the roots and proliferates in the vascular tissue. Wilting progresses from lower to upper leaves, followed by collapse of the plant. When the stem and roots are cut diagonally, reddish-brown streaks are visible in the vascular tissues. [11]. Pathogen can live in the soil for many years [12].

Fungicides cannot control Fusarium wilt effectively, other solutions, such as soil fumigation or grafting might work well but they constitute are either additional cost items and may exert threats on hazardous to the environment and human healthy [13]. For economic and safety reasons, resistant crop breeding is a most efficient way to avoid from this disease [14].

A source of resistance against Fusarium wilt was resistance source has been identified in *S. aethiopicum* Gilo Group and *S. aethiopicum* Aculeatum Group both are relatives of which are *Solanum melongena*'s relatives [15]. LS1934, LS174, and LS2436, have resistance and these eggplants were been defined as to be completely source of resistance source [16]; [17]. *Rf1* is a single dominant identifed as a resistance locus with Cleaved Amplified Polymorphic Sequences (CAPS) tightly linked to the gene of interest[18]. SCAR markers linked to a *Fusarium* Resistance locus in eggplant line, LS2436 with bulked segregant analyses were published by Mutlu in [15].

Conventional breeding and molecular marker analysis can be used to increase disease resistance and, improve yield traits of and use cultivated eggplant. A molecular marker tightly linked to single dominant gene (FOM) was developed for use in marker assisted selection (MAS).

The primary objective of the present study aim of the study is to develop eggplant lines resistant against Fusarium wilt with the use of using a marker- assisted backcross breeding approach. This makes available economic damage to producers can be prevented. And breeding with molecular marker assisted will make time consuming for breeders.

MATERIALS AND METHODS

Plant Materials

120 eggplant lines, 4 commercial cultivars, pGM1F1, pGM2F1 and pGM3F1 progenies were used as plant materials.

Molecular Marker Screening

Molecular marker assisted selections were performed in accordance with Mutlu et al. (2010). Parent (F1, BC1F1, BC2F1, BC3F1 and DH plants) DNA was extracted from young leaves using a modified CTAB extraction protocol (Doyle and Doyle 1990). PCR reactions were performed in 15µL volumes in Akdeniz University, Agricultural Biotechnology Laboratory (MJ RESEARCH PTC-225 Peltier Thermal Cycler). All PCR products were separated on a 1.5% agarose gel (Thermo scientific Gel Tank), visualized with ethidium bromide staining under ultraviolet light, and photographed with Minilumi, DNR Bio-Imaging Systems. Classical testing for *Fusarium Wilt* Resistance Using *Fusarium Oxysporum f.sp.* Melongeae Isolate The *Fusarium oxysporum f.sp.* Melongeae isolate was supplied from BATEM institute, Antalya, Turkey. The *Fusarium oxysporum* melongenae isolate was grown on the potato dextrose agar (PDA) at 24°C in dark for 10 days. Liquid medium was prepared from this culture. Liquid cultures were shaken at 50 rpm in a rotary shaker for 8 days at 24 - 25°C. The suspensions were filtered through cheesecloth. The spores were re-suspended and spore density was adjusted to 1 × 106 conidia/ml. Seedling roots were washed with clean tap water and freed from the soil. The 1/3 of roots were first trimmed with a sterile scissor to create scar tissue to promote infection. Wounded roots were submerged into the beaker containing 106 concentration of FOM isolate for 5 minutes (Herman and Perl-Treves, 2007; Karimi et al. 2010). For control groups, 12 seedlings from each of parents were submerged either into distilled water or into FOM isolate. The seedlings were planted into small pots and maintained in the nursery. Seedlings were planted into 48-well trays containing sterile torf. After inoculation, seedlings were kept at 27°C/18°C under 12-h photoperiod. Five weeks after inoculation, disease symptoms were recorded as 1 (resistant) no symptoms of disease and 0 (susceptible) dead plant.

The best proper stage for anther culture in eggplant is the off-centered position of microspors at the end of mono-nucleus stage. It corresponds to growth stage with 2 mm long and light yellow-yellow petal. Flower buds were disinfected properly, then buds were opened over sterile filter papers with a pens and bistouries, anthers were separated from the filaments and sown into growth media as to dorsal sections touched the media(MS 01. Mg/kinetin and 0.1 mg mg/l 2,4 D +MS + 30 gr sucrose [19]).

The anthers placed into MS were subjected to pre-treatment in an incubator at +35ºC under dark conditions for 8 days, then petri dishes were kept in a climate chamber at 3600 lux light intensity, 25±1°C temperature under 16:8 light/dark photoperiod for 8 days. Proper adaptation producers were followed while taking cultures to outdoor conditions.

For plodiy analysis, very small leaf samples in a petri dish was supplemented with 1.5 ml cystatin solution. About 0.3 ml of resultant mixture was placed into plastic Eppendorf tube and placed into flow cytometry device. Based on device peak ranges, chromosome pattern (haploid or diploid) was determined. The 0.5% colchicine-absorbed cottons were placed over the buds and kept for 2 hours for stratification of haploid plants.

RESULTS AND DISCUSSION

Initially, 120 eggplant lines and 4 commercial cultivars which declared as resistant were tested with molecular markers [15]. While 120 pure eggplant lines in gene pool were found to be susceptible, 4 commercial hybrid cultivars were found to be resistant. These 4 hybrid cultivars found to be resistant in marker analysis were then subjected to classical testing and status of resistance was verified. Later on, following the general combination tests, 4 pure lines with high heterosis ability, but without FOM resistance were selected from the gene pool. These 4 lines were hybridized for BC program with resistant sources of which FOM resistance was proved through molecular markers and classical testing and F1 hybrids were obtained. In hybridizations performed with 4 pure lines as mother and 4 commercial cultivars as father, 100 plants from each hybrid combination were subjected to molecular tests and heterozygote resistant (about 50% resistant) plants were selected and transplanted into a greenhouse. Observations were made since the initial flowering and following the first fruit set, selected 10 plants were backcrossed to main plant to get pGM1F1(Table 1, Figure 1). Following the backcrossing, observations were made until the end of harvest season and seeds of the backcrossed hybrid combination with the best performance throughout the entire season were harvested. In the next season, seeds of hybrid pGM1F1 hybrid combinations were sown into viols and subjected to marker tests. Resistant plants were transplanted into greenhouse. Again, observations were made since the initial flowering and following the first fruit set, selected 10 plants were backcrossed to main plant to get pGM2F1. Following the backcrossing, observations were made until the end of harvest season and seeds of the backcrossed hybrid combination with the best performance throughout the entire season were harvested. Then in the next season, hybrid pGM2F1 hybrid combinations were sown into viols and subjected to marker tests. Resistant plants were transplanted into greenhouse. Again, observations were made since the initial flowering and following the first fruit set, selected 10 plants were backcrossed to main plant to get pGM3F1. Following the backcrossing, observations were made until the end of harvest season and seeds of the backcrossed hybrid combination with the best performance throughout the entire season were harvested. In subsequent season, seed of hybrid pGM3F1 hybrid combinations were sown into viols and subjected to marker tests. The 20 pGM3F1 plants from 4 hybrid combinations (total 80 plants) identified as resistant with molecular tests were transplanted into greenhouse. Buds were taken from these plants and anther culture was performed. Following the anther culture, 25 DH plants were obtained from each combination and resistance status of these plants was determined through initially molecular then classical testings.

Table 1. *Clasical and molecular testing results of parents and pGM1F1 population for Fusarium oxysporum Schlecht. f sp. Melongenae resistance*

	Inoculated Plants No	Dead Plants No	Healthy Plants No	SCAR Marker resistance band
Resistant (Parent) 1	12	0	12	12
Resistant (Parent) 2	3	0	3	3
Resistant (Parent) 3	3	0	3	3
Susceptible (control) 1	8	8	0	0
Susceptible (Parent) 2	3	3	0	0
Susceptible (Parent) 3	3	3	0	0
Susceptible (Parent) 4	3	3	0	0
Susceptible (Parent) 5	3	3	0	0
Susceptible (Parent) 6	3	3	0	0
PBC 1A-1	24	11	13	12
PBC 1A-2	24	6	18	18
PBC 1A-4	24	19	5	5
PBC 2A-1	24	16	8	8
PBC 2A-2	24	12	12	12
PBC 2A-4	24	6	18	17
PBC 3A-1	24	20	4	4
PBC 3A-2	24	10	14	14
PBC 3A-4	24	12	12	12
PBC 4A-1	24	9	15	15
PBC 4A-2	24	15	9	8
PBC 4A-4	24	15	9	9
PBC 5A -1	24	11	13	12
PBC 5A -2	24	10	14	13
PBC 5A -4	24	15	9	9
PBC 6A-1	24	12	12	12
PBC 6A-2	24	4	20	18
PBC 6A-4	24	7	17	17
Total	**432**	**210**	**222**	**215**

More than 500 seedlings belonging to various genetic background were inoculated and 380 plants derived from 11 different genetic materials were identified as resistant. One month after root dip inoculation, resistant and susceptible plants were able to be identified.

Same genetic materials were tested with molecular markers reported by Mutlu at all. [15] with the use of FOM markers, 3 genotypes were identified as resistant. These genotypes were able to survive with the root-dip inoculation. Six commercial hybrids

that did not possess the marker locus for resistance against FOM were all identified as resistant according to the classical testing.

Present findings indicated that the marker was reliable to develop eggplant lines resistant against fusarium wilt and there may be another source of resistance independent from the known resistance gene originating from LS2436.

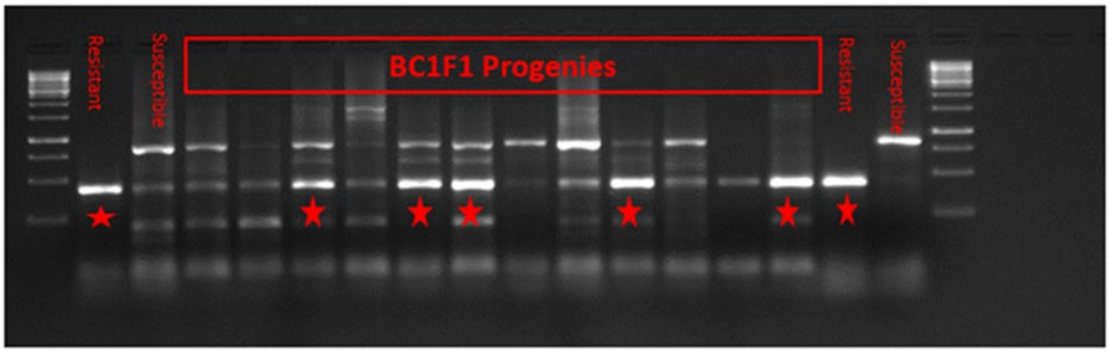

Figure 1. *Gel image for molecular testing using BC1F1 population (L=1kb ladder; Resistant bands have been marked with red color and R=Resistant, S=Susceptible)*

X^2 test results for classical and molecular testing as dominant one gene for Fusarium oxysporum Schlecht. f sp. Melongenae resistance using BC1F1 population were given in Table 2. 432 BC1F1 progenies were used for classical testing and X^2 test results showed to 1:1 mendelian ratio (p 0,99), and 88 BC1F1 progenies were used for molecular testing [15] and also X^2 test results showed to 1:1 mendelian ratio (p 0.96).

Table 2. *X^2 test results for classical and molecular testing as dominant one gene for Fusarium oxysporum Schlecht. f sp. Melongenae resistance using BC1F1 population*

Population	Resistant plants (no.)	Susceptible plants (no.)	Expected ratio	$\chi 2$	Probability (P)
BC1F1	222	210	1:1	0.82	0.99

Population	Resistant plants (no.)	Susceptible plants (no.)	Expected ratio	$\chi 2$	Probability (P)
BC1F1	40	44	1:1	0.76	0.96

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 [20]. Goth and Webb [21] 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 was 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. [22] 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. [23], it was reported that the LS 1934 and LS 2436 genotypes of the *S. melongena* species were resistant to

FOM. Boyaci and Abak [24] investigated the inheritance of resistance between the resistant genotypes LS 1934 and LS 2436 of the *S. 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 a 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. [15] 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 codominant 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 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 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 the 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. Mutlu et al. [15] 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 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 [25]. Eggplant (*Solanum melongena* L.) has the potential for improvement through heterosis breeding, which can be further utilized for development of desirable recombinants. A Line × Tester mating design was used to determine heterosis over better parent, combining ability, and gene action for 11 characters in eggplant. Crosses showing high specific combining ability (sca) and yield involved parents showing high general combining ability (gca) for fruit weight, fruit diameter, or fruit length. Two hybrids, ‘VNR-218’ × ‘BCB-11’ and ‘Arka Nidhi’ × ‘KS-331’ were selected on the basis of their per se (mean) performance: heterosis and the sca effects. These hybrids could be used commercially due to high yield and low percentage disease index (PDI) values for bacterial wilt disease. The preponderance of nonadditive gene action was evident for control of all characters studied. Parental lines and hybrids were categorized according to disease reaction. Average large vessel area of

stems and roots of the hybrids was negatively correlated with PDI. Larger vessel area in the vascular bundle needs to be considered in the selection of hybrids for resistance to bacterial wilt disease [26]. The introgression of its disease resistance gene into cultivated eggplants would allow for breeding disease resistant eggplants. In this study, interspecific hybridization and subsequent backcrossing between PI388846 and cultivated eggplants were performed. The results showed that *Verticillium wilt* resistance was successfully introduced into the cultivated eggplants, and the agronomic traits of the interspecific hybrid progeny were improved by continuous backcrossing with the cultivated eggplants. In addition, a gene specific marker for the Ve homolog in PI388846 was developed to detect *Verticillium wilt* resistance in the backcross population. The results represent a positive beginning for the genetic enhancement of cultivated eggplants for *Verticillium* wilt resistance (Liu et al., 2015). Sousa et al., (1998) carried out a study to obtain estimates of heterosis in crosses between seven eggplant cultivars (Embu = E; Santa Genebra = SG; Viserba = V; Aubergine de Barbentane = AB; Florida Market 10 = FM; Black Beauty = BB, and Melitino = M) and two breeding lines (B-14-07 = B1 and B-31-06 = B2). The F1 hybrids used were: E x FM; E x BB; E x M; E x B1; E x B2; SG x FM; SG x BB; SG x M; SG x B1; SG x B2; V x FM; V x B1; V x B2; AB x FM; AB x M; AB x B1; AB x B2 and M x FM. Cultivars, lines and hybrids were evaluated at the ESAL experimental field in Lavras, MG, from February to October 1992. A randomized complete block design with three replications was used. Significant heterosis relative to the parental means was detected for all traits studied. Their values ranged from +41.23% to +113.31% for total fruit yield, from -11.45% to +26.17% for average fruit weight, and from +27.98% to +141.81% for early production. Heterosis relative to the superior parent ranged from +13.89% to +92.51% for total fruit yield. Hybrid pairs: SG x FM and AB x B1, V x FM and AB x FM, E x M and AB x B1 were the most heterotic relative to the parental mean for total fruit production, mean fruit weight and early production, respectively. The hybrids displaying highest heterosis relative to the superior parent for total yield were AB x B1 and SG x FM.

Colak Ates et al. [25] conducted a study 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.

CONCLUSION

MAS should be continued up to BC3F2 generation and allelism test should be initiated to understand whether a new and unique gene exists for resistance against FOM. DH is being used at BC1-BC3 generations to develop FOM resistant eggplant lines and to transfer the resistance gene into parental lines of the hybrid.

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