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DEVELOPMENT OF *FUSARIUM OXYSPORUM F. SP. LYCOPERSICI* (FOL) AND *FUSARIUM OXYSPORUM F.SP. RADICIS LYCOPERSICI* (FORL) RESISTANT TOMATO LINES WITH THE AID OF MARKER ASSISTED SELECTION

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Abstract
Molecular markers have extensively been used along with the classical methods in tomato breeding. Many molecular markers developed for the resistance to biotic stresses, especially the ones controlled by a single gene or major Quantitative Trait Loci (QTL). The objective of this study was to present potential use of the molecular markers to develop resistant lines against Fusarium spp. Hence, the molecular markers, linked to Fusarium oxysporum f. sp. Lycopersici (FOL) and Fusarium oxysporum f.sp. radicis lycopersici (FORL) were tested for FOL I-2, and I-3 genes for Fusarium oxysporum f. sp. Lycopersici, and FORL Frl gene for Fusarium oxysporum f.sp. radicis lycopersici. At the end of the breeding program I-2, I-3, and Frl genes were pyramided at the same tomato lines. Results showed that these markers can aid development of tomato lines resistant against multiple races of Fusarium spp in a MAS program.

Keywords: Tomato, molecular markers, MAS.

1. INTRODUCTION

Tomato with an annual production of 129 million tons is the leading vegetable worldwide (FAO, 2016). Viruses, bacteria, nematodes and fungi are the greatest restrictors in tomato production activities and such agents result in serious yield losses. There are three strategies adapted to control pests and diseases. These are chemical treatments, cultural practices and use of resistance genotypes. Although chemical treatments may prevent the spread of some pests and diseases, they may exert serious health risks for farmers, increase input production costs and leave residues over the vegetables. Pests and disease control with chemical and cultural practices are not always possible. Then, use of resistant genotypes come into prominence as an economic and environment-friendly practice for the control of pests and diseases.

More than one pests and disease agent can exist simultaneously in plant species cultivated over large fields, like tomatoes and such agents result in significant yield and quality losses. Several plant species and cultivars are resistant to one or more pests and diseases. Especially the hybrid vegetable cultivars have multiple resistances against different pathogens and/or races. In tomatoes, pests and disease resistance are mainly under the control of a single gene with a dominant characteristic. Up to now, 15 resistance genes from different sources were transferred to tomatoes

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(Barone, 2004). Since the beginning of 20th century, resistance to pests and diseases have been transferred to cultivated species through classical breeding methods. However, resistance to a single agent is not sufficient in intensively cultivated species like tomatoes because number of pests and diseases cause economic damage. Because of rapid spread of disease and pest to production areas, multiple resistance is required for a healthy production. Development of multi resistant lines/hybrids and pyramiding of resistance genes via classical breeding take many years and complex backcrossing, selfing and progeny testing steps to complete.

Molecular markers have intensively been used in several plant species since 1980s. Markers were especially developed for the genes related to pests and diseases in tomatoes and they were used in various breeding programs. Up to now, more than 40 genes (single gene and QLT) for resistance against pests/pathogens were mapped. The mapped characteristics are used in breeding programs through marker assisted selection (MAS) method. MAS method has been an integral part of plant breeding programs since 1990s. Because it eases the transfer and pyramiding of resistance genes through backcrossings, facilitates the break out of the linkage between the resistance gene and undesirable characters, make it possible to screen for resistance against quarantine pests, and thus allows breeders early eliminataion of unwanted plants, saving time and labor.

Type of marker is a significant issue in using molecular markers in breeding programs. Therefore, especially co-dominant markers are used in MAS. The co-dominant markers can distinguish heterozygote and homozygote individuals. MAS programs dominantly focus on co-dominant SCAR (Sequence Characterized Amplified Regions) and CAPS (Cleaved Amplified Polymorphic Sequence) markers (Collard and Mackill, 2008). These marker systems do not require much labor, tools and equipment, thus have a cost advantage over the other marker systems (Kumar, 2009). Tomato is among the earliest species in which use of molecular markers is recommended for indirect selections of breeding programs (Tanksley, 1983; Tanksley et al., 1992; Foolad, 2007). For instance, about 30 years ago, iso-enzyme marker was used in indirect selection for breeding of a cultivar resistant to acid phosphatase (Aps-1 locus) against root-knot nematode (Medina-Filho and Stevens 1980). MAS has started to be routinely used by private sector in identification of resistance to some diseases (Panthe and Foolad, 2011). Various researchers developed SCAR and CAPS markers against tomato nematodes, tomato mosaic virus, Verticillium wilt, Fusarium wilt, tomato spotted wilt virus, and tomato yellow leaf roll virus and they are intensively used in breeding programs (Barone, 2004).

Of these diseases, *Fusarium oxysporum f. sp. lycopersici* and *Fusarium oxysporum f.sp. radicis lycopersici* result in serious yield losses in tomatoes in Turkey. As it was in the other pests and diseases, breeding programs have been initiated for resistance against the both diseases, and molecular markers linked to the resistance genes were used in the breeding program.

Fusarium crown and root rot (caused by *Fusarium oxysporum f. sp. radicis-lycopersici*, FORL) and Fusarium wilt (caused by *Fusarium oxysporum f. sp. lycopersici*, FOL) are the most important diseases to affect tomatoes in protected growing conditions in the eastern Mediterranean region of Turkey, causing significant yield losses.

Fusarium oxysporum f. sp. lycopersici (Sacc.) Snyder and Hansen are soil-born fungus and result in wilts in tomatoes (*Lycopersicon esculentum* Mill.). The fungus infects vascular system of the roots, hinders water transport and result in rapid plant die outs (McGrath et al.1987, Malhotra and Vashistha 1993). Therefore, using resistant cultivar is considered as a better alternative than the chemical treatments. There are three races of FOL identified as race 1, 2 and 3 (Stevens and Rick 1986, Beckman 1987). The resistance genes were genetically mapped and transferred from the wild species to commercial cultivars (Huang and Lindhout, 1997; Frary and Tanksley, 2001). Of these

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genes, I-1 and I-3 are located on 7th chromosome and I and I-2 are located on 11th chromosome (Stall and Walter 1965). A CAPS marker linked to I-2 gene (TAO1902) has been used in breeding programs (Simons et al., 1998; Staniaszek et. al., 2007; Scott et al., 2004).
This study was conducted to develop tomato lines resistant to Fusarium oxysporum f. sp. lycopersici and Fusarium oxysporum f.sp. radicis lycopersici with the aid of molecular markers.

2. MATERIALS AND METHODS

Plant materials include 10 tomato lines with superior agronomic characteristics, but susceptible to FOL and FORL, and 14 resistant tomato cultivars. Initially, susceptible lines and resistant cultivars were crossed, obtaining 140 hybrids. In seedling stage, these hybrids were screened with the molecular markers linked to resistance genes I-2, I-3 and Frl. DNA of tomato genotypes were extracted with the aid of CTAB method (Doyle and Doyle 1990). Resultant DNAs were tested with molecular markers linked to I-2 (Staniaszek et al., 2007) and I-3 (Hemming et al. 2004), and to Frl (Mutlu et al., 2015) genes as described in literature. The primer sequences for these genes were as follows: for I2 gene -forward ATTTGAAAGCGTGGTATTGC and reverse CTTAAACTCACCATTAAATC; for I3 gene – forward GGATTTTGGTGCTGTATTTGAAG and reverse TAGCCTGATGTTCCTCTCATTGTTC; for Frl gene - forward AAGTATGCCGTGCCACGTCAGC and reverse TCAACTCCTGGTCCCACTCCTCC. Through the marker screening, the lines bearing I-2, I-3 and Frl genes with superior characteristics were determined.

3. RESULTS AND DISCUSSIONS

The advanced lines bearing one or more these genes were separated into close groups based on their fruit types (cocktail, beef and cherry). These three genes (I-2, I-3 and Frl) were transferred to the tomato lines with superior agronomic characteristics through backcrossing and MAS in each segregating population. Again initially MAS was applied to BC1F1, BC2F1 and BC3F1 generations and the single plants with superior agronomic characteristics and target resistance gene(s) were used in backcrossings. Following the BC4F1 generation, resistant plants were selfed to get homozygous BC4F6 generation. Then, resultant BC4F6 lines were evaluated for agronomic characteristics.
Pseudo-BC (back-crossing) was performed to transfer resistance genes (FOL I-2, I-3, and FORL Frl) to the ten FOL and FORL susceptible advanced lines with superior agronomic traits, using 14 resistant commercial hybrids as donor parents. Initially the lines in gene pools of tomato breeding programs were subjected to selections in terms of morphological (plant heights, inter-node lengths, plant growth habitus, leaf lengths and etc.), post-harvest quality attributes (fruit shape, taste, aroma, color, size, firmness, pH, citric acid, vitamin C, and etc.). Then, selected 10 tomato genotypes (F6 generation) were hybridized with 14 resistant cultivars and pseudo-back-crosses - BC1F1 were obtained. The individuals obtained from these hybrids were tested with molecular markers for I-2, I-3 and Frl genes. Backcrossing and MAS continued until BC4F1 after every backcrossing. Following the BC4F1 generation, heterozygous resistant plants were selfed to get homozygous BC4F2. MAS continued to get homozygote resistant individuals until BC4F6 generation. The results obtained through molecular markers are provided in Table 1.
As can be inferred from Table 1, the 117 tomato genotypes were identified as bearing Frl, I2 and I3 genes together. Of these genotypes, 15 were advanced up to F6 generation. On the other hand in F6 level, 124 tomato lines only carried Frl gene, 49 only I2 gene, 58 had both Frl and I2 genes, and 34 had I-2 and I-3 genes. The populations subjected to selections in terms of the parents of qualified cultivars and agronomic traits had two and three of *Fusarium oxysporum f. sp. lycopersici* (FOL I-2

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I-3) and *Fusarium oxysporum f.sp. radicis lycopersici* (FORL Frl) alleles. In molecular marker scanning for Frl gene yielding resistance to FORL, the upper band close to 750 bp DNA marker was identified as sensitive allele (r) and lower band was identified as resistant (R) allele. For I-2 gene yielding resistance to FOL (race-2), lower band (about 900 bp) was identified as resistant allele. For I-3 gene, the band close to 500 bp DNA marker indicate resistant (R) allele. This dominant marker was the marker yielding the closest – the most accurate outcome to I-3 gene.

Table 1. Number of resistant genotypes obtained after testing with molecular markers

Genes	BC_4F_1	BC_2F_3	BC_1F_4	F_6	Total
Frl	80	120	63	124	387
I2	45	80	40	49	214
I3	42	70	28	0	140
Frl, I2	25	50	30	58	163
FRL, I3	15	30	18	0	63
Frl, I2, I3	30	60	12	15	117
I2, I3	34	70	8	34	146

Resistance to FOL has already been transferred to several commercial cultivars through classical and MAS breeding programs. However in developing new cultivars resistant to this disease, use of molecular markers efficiently pyramided multiple race-specific resistance genes into a single line. Markers linked to genes providing resistance to FOL races (0, 1, 2) were developed in tomatoes and used in routine breeding programs.

El Mohtar et al. (2007) obtained expected outcomes in 39 of 40 genotypes known as resistant to FOL race-2 with the marker developed for I-2 gene providing resistance to the same race and indicated that only one genotype had I-3 gene and the method was validated with molecular and classical testing carried out in 3 different countries. Arens et al. (2009) used At-2-F3-R3 primer pairs and tested I-1 gene-related resistance in tomatoes and indicated that relevant marker could be used for identification of I-1 gene.

4. CONCLUSIONS

In this study, potential parents of multi-resistant hybrid tomato cultivars were developed via marker assisted backcrossing. In order get competitive advantage in markets, number of tomato lines bearing Frl, I2 and I3 genes with different fruit types and superior agronomic characteristics were developed. Use of molecular markers both accelerated breeding programs and allowed transfer of three resistance genes together into tomato lines. Present findings revealed that molecular markers developed for Frl, I2 and I3 genes could efficiently be used in routine breeding programs aiming to develop Fusarium resistant tomato lines.

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