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IMPROVEMENT OF INDUSTRIAL PHYTOPHTHORA (*PHYTOPHTHORA CAPSİCİ* LEON.) TOLERANT PEPPER LINES USING MOLECULAR MARKERS AND ANTHER CULTURE

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Abstract
Althougt Turkey has big potential with regard to production area and quality pepper production, equated yield is low because of plant diseases. One of the plant diseases is Phytophthora capsici Leon. Tolerance of this disease in pepper species is controlled by more than one gene (QTL). Until now, there are some reports on QTL mapping for disease tolerance in pepper species and one of them is Phyto.5.2 which is linked to Phytophthora capsici Leon. disease tolerance. In this study, we aimed transfer disease tolerance to pepper genotypes which are grown in Central Anatolia region. Crossing was carried out between Cırgalan pepper and P1(tolerant to Phytophthora capsici Leon) and backcrossed to Cırgalan pepper genotype to obtain BC1F1. BC1F1 plants were tested using molecular marker which linked with Phyto.5.2 QTL. Tolerant plants were backcrossed with Cırgalan pepper genotypes to obtain BC2F1 plants. BC2F1 plants were tested again using molecular markers and tolerant plants selfed to obtain BC2F2. After testing, anthers of tolerant plants were inoculated to get double haploid plants. From the results obtained, 25 pure industrial pepper lines were obtained which had Phytophthora capsici Leon. disease tolerance. These lines will be useful for Phytophthora capsici Leon. disease tolerance breeding programs.

Keywords: Pepper, breeding, Phytophthora capsici Leon

1. INTRODUCTION

Pepper is the most common type of vegetable produced after tomatoes in the world. Pepper a very important in Turkey and world. World pepper production reached 34 million tons in 2016. Turkey is important producer with 2.45 million tons of production with a share of 7% of world production (FAO, 2017). Turkey is one of the important countries in terms of plant genetic resources and genetic diversity in the world. It is known that Central Anatolia is one of the micro gene centers of *Capsicum annuum* L. Pepper production is often made for industrial and fresh consumption purposes. Besides, pepper; after dried for deepening, salting, drying, thickening and separating, it is evaluated as flaked pepper or powdered pepper. If fresh pepper cannot be stored for a long time and is not consumed together after harvest, the majority is thrown away. Central Anatolia food industry needs high amount dried pepper for seasoning spices to use sausage and bacon production. But there is no enough suitable pepper materials for production. Food industries in Central Anatolia import dried pepper from other provinces and countries. Quality and yield cannot be sustained in industrial products especially sausage and bacon. The solution of this problem is development of the local genotypes, improvement of the yield and quality, improvement of the easily harvested varieties.

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Some pathogenic factors such as fungi, viruses, bacteria and nematodes, which are the main limiting biotic factors in pepper cultivation, cause yield losses. It needs new varieties which tolerant to some diseases. But classical methods take long time for improvement of new varieties Molecular markers have used to development disease-tolerant varieties. For example, SCAR and CAPS markers were developed linked to some disease and pest resistance, such as *Phytophthora capsici*, Root Knot Nematodes (*M. incognita, M.arenaria, M. javanica*), Tomato Spotted Wilt Virus (TSWV) (Wang et al., 2009; Quirin et al., 2005; Moury et al., 2000) and have used extensively in breeding trials.

Among these, *Phytophthora capsici* is one of the most important diseases of pepper plant which is a fungal disease which causes significant damage in pepper cultivation, especially in open field. Heavy rainfall, over-irrigation and poor drainage cause Phytophthora root and root rot more easily. Soil temperatures above 18 ° C and long wet periods with hot air between 24-29 ° C are suitable conditions for *Phytophthora*. There is no single method for fighting against *Phytophthora* root and root rot. It is especially true that the local pepper genotypes in Turkey are sensitive to the disease. One of these genotypes is Cirgalan pepper, which is produced for use in the powdered pepper industry in Central Anatolia. It is very important in terms of transferring to *Phytophthora* disease resistance to prevention of yield and quality losses due to the disease in production areas. Incorporation of biotechnological techniques, such as molecular markers and anther cultures, into the breeding program is an important step toward developing a tolerant variety, since the transfer of resistance to this disease using classical breeding methods will take a long time. Quirin et. al. (2005) developed a SCAR marker which linked *Phytophthora capsici* tolerance and have used at pepper breeding programs.

In present study, it was aimed to develop pepper genotypes resistant to *Phytophthora* root and root rot disease, which is one of the problems in the production of local Cırgalan pepper, which used in powdered pepper industry. As in most plant species, plant breeding for transferring new features to pepper varieties and macro and micro yield experiments take long time. It can be shortened breeding programs using biotechnogical methods and knowledge and some results of some breeding projects.

2. MATERIALS AND METHODS

Two Cırgalan pepper genotypes and one P. capsici resistance genotype were used as plant materials. Breeding program was given at Figure 1. OPD04 717 primer pairs were used for molecular marker analysis to determine P. capsici resistance which developed by Quirin et al. (1999) was identified as a locus of the pepper chromosome tightly bound to Phyto.5.2 on chromosome 5, one of the six loci (QTLs) reported to have contributed to *P. capsici* tolerance. Anther culture process, will be evaluated as described by Buyukalaca et. al. (2004).

DNA was isolated from fresh leaves of pepper genotypes using CTAB methods (Doyle and Doyle, 1990). Molecular analysis was done for Phyto.5.2 region which is linked to Phytophthora capsici Leon. disease tolerance using OPD04 717 primer pairs.. The PCR products were loaded with 3 µl of loading buffer (20 ml glycerol (40%), 30 ml sterile water, 0.05 g bromophenol blue) and the resulting mixture was run under a 115 V for 3 hours in 2.5% agarose gel. To prepare agarose gel, 1X TAE buffer was used and 25 µl (0.5 mg / ml) of ethidium bromide solution was added. A 100 bp DNA ladder was loaded as standard in electrophoresis. After electrophoresis, the gel was placed on a gel imaging device (KODAK Gel Logic 100 System). Gels were scored as 714 bp resistance band for F1(10 plant), GM1F1, GM2 F1 and GM2F2 individuals. Anthers of GM2F2 plants were cultured after molecular marker test according to Buyukalaca et. al. (2004).

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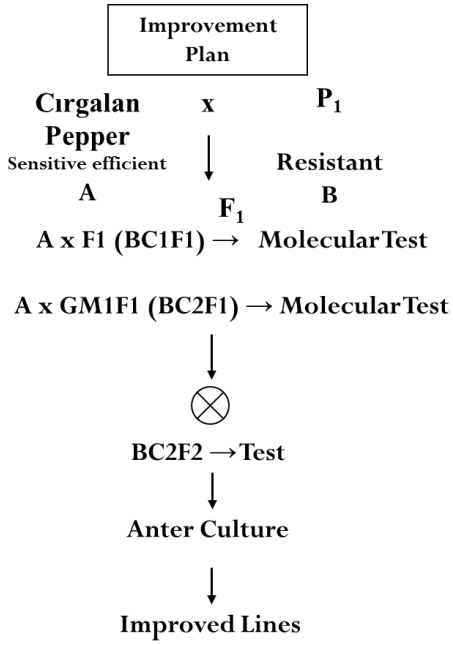

Figure 1. Schematic view of the plant breeding method used in the study

3. RESULTS AND DISCUSSION

In this study, after first flowering 925 and P1 was crossed to obtain F1 progeny. F1 progeny backcrossed to obtain BC1F1. Total 40 seeds of BC1F1 population were sowed to get seedlings. 40 BC1F1 seedlings screened for Phytphthora capsici Leon. disease tolerance using OPD04 717 primer pairs. 23 of 40 plants had 717 bp band for Phytphthora capsici Leon. Tolerance (Figure 1 and Table 1). BC1F1-6 genotype was selected for BC2F1 which looks like famale parent (934). 934 X BC1F1-6 was crossed to get BC2F1 population. Twenty seeds of BC2F1 were sowed and screened using OPD04 717 primer pairs. 9 of 20 plants was carriying 717 bp band for Phytphthora capsici Leon. tolerance (Figure 1 and Table 1). BC2F1-14 was selected for BC2F2 population and BC2F1-14 was selfed to obtain BC2F2. Selfed seeds were harvested and 20 seeds were sowed again to screen 717 bp band for Phytphthora capsici Leon. tolerance. Twelve of them had tolerance band (Figure 1 and Table 1). Bud of 12 genotypes which BC2F2 were collected and anther culture was carried out to obtain pure pepper lines. Total 25 double haploid lines obtained and all of them screened using OPD04 717 primer pairsfor Phytphthora capsici Leon. tolerance. 13 of them had Phyto.5.2 allel (Figure 1 and Table 1). 4 (BC2F2-6, BC2F2-9, BC2F2-14 and BC2F2-18) of 13 pure lines were close to female parent with regard to plant and fruit characteristics.

Phytphthora capsici Leon. disease was controlled by poligene. It was determined Phyto.4.1, Phyto5.1 and Phyto.5.2, Phyto.6.1, Phyto.11.1, and Phyto.12.1 genes regions for Phytphthora capsici tolerance at 4, 5, 6, 11 and 12. chromosomes. Phyto.5..2 region explains 60% of Phytphthora capsici tolerance. Quirin et al.(2009) have identified a locus for the pepper chromosome firmly linked to Phyto.5.2 on chromosome 5 which one of the six loci (QTL).

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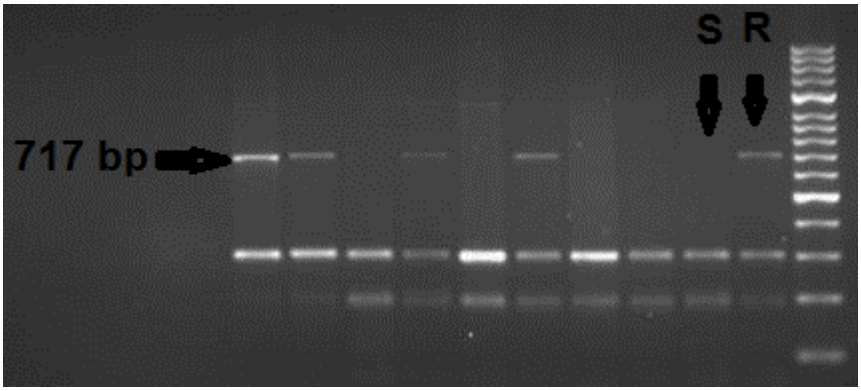

Figure 2. PCR image of parents and progenies used in present study

Table 1. Phytophthora capsici Leon. tolerances of said pepper genotypes

No	*G*enotypes	*Phy*	*G*enotypes	*Phy*	*G*enotypes	*Phy*	*G*enotypes	*Phy*
1	P1	+	BC1F1-21	+	BC2F1-1	+	BC2F2-1	-
2	934	-	BC1F1-22	+	BC2F1-2	+	BC2F2-2	-
3	934xP1	+	BC1F1-23	+	BC2F1-3		BC2F2-3	-
4	BC1F1-1	-	BC1F1-24	+	BC2F1-4		BC2F2-4	+
5	BC1F1-2	-	BC1F1-25	-	BC2F1-5	-	BC2F2-5	+
6	BC1F1-3	-	BC1F1-26	+	BC2F1-6	+	**BC2F2-6**	+
7	BC1F1-4	-	BC1F1-27	-	BC2F1-7	+	BC2F2-7	+
8	BC1F1-5	+	BC1F1-28	-	BC2F1-8	+	BC2F2-8	+
9	**BC1F1-6**	+	BC1F1-29	+	BC2F1-9	-	**BC2F2-9**	+
10	BC1F1-7	-	BC1F1-30	+	BC2F1-10	-	BC2F2-10	-
11	BC1F1-8	+	BC1F1-31	-	BC2F1-11	-	BC2F2-11	-
12	BC1F1-9	+	BC1F1-32	+	BC2F1-12	+	BC2F2-12	+
13	BC1F1-10	+	BC1F1-33	+	BC2F1-13	-	BC2F2-13	-
14	BC1F1-11	-	BC1F1-34	-	**BC2F1-14**	+	**BC2F2-14**	+
15	BC1F1-12	-	BC1F1-35	+	BC2F1-15	+	BC2F2-15	+
16	BC1F1-13	+	BC1F1-36	+	BC2F1-16	-	BC2F2-16	-
17	BC1F1-14	+	BC1F1-37	-	BC2F1-17	-	BC2F2-17	+
18	BC1F1-15	+	BC1F1-38	-	BC2F1-18	+	**BC2F2-18**	+
19	BC1F1-16	-	BC1F1-39	-	BC2F1-19	-	BC2F2-19	+
20	BC1F1-17	+	BC1F1-40	+	BC2F1-20	-	BC2F2-20	-
21	BC1F1-18	+						
22	BC1F1-19	+						
23	BC1F1-20	+						

Phytophthora capsici pepper causes very large losses in many plant species that are included in pepper (*Capsicum annuum*) which is highly complex as genetic and physiological resistance. One

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(OpD04) RAPD marker showed a single band in resistant genotypes of C. annuum and C. chinense species, which showed high level of resistance. The PCR product of the obtained band was cloned and converted to the SCAR marker. This marker has been identified as one of the six QTLs associated with the Phyto.5.2 locus on chromosome 5 of the pepper (Quirin et al., 2009).
Xu et al. (2014) were used a joint analysis of six-generations (P1, P2, F1, F2, BC1 and BC2) was performed from cross between CM334 and a highly susceptible pepper line 949. F2 population was analyzed with SRAP molecular markers. They reported that a single pair of nuclear and dominant gene controlled the resistance in CM334. Among 64 pairs of SRAP primers, 21 pairs of primers produced polymorphic bands in parents. They obtained a SRAP marker linked with Phytophthora blight resistance, designated SRAP- Me6/Em15 (Xu et al. 2014).

4. CONCLUSIONS

The anther culture, molecular and phenotypic evaluation of the breeding scheme for Phythophtora capsici tolerance in pepper showed that most of the objectives were achieved, but also suggested some improvements of the strategy. In this study, molecular marker which was developed for Phythophtora capsici tolerance and anther culture which was optimized for pepper varieties were used and new pepper variety candidates were developed. But new breeding lines needs classical testing after molecular studies. Our results showed that molecular markers and anther culture technique have accelerated breeding programs. Also improved new pepper genotypes can be used for industrial pepper production and breeding programs.

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