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POLYGENIC RESISTANCE OF IMPROVED RED PEPPER LINES TO PHYTOPHTHORA CAPSICI

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Abstract

Belonging to the Oomycete genus, Phytophthora capsici is responsible of many devastating disease and the most destructive agent of pepper in the World. The most cost-effective method for preventing Phytophthora blight is to use resistant cultivars. In this study, the polygenic resistance of improved pepper lines to Phytophthora Blight were screened by molecular markers placed on three chromosomes in pepper. Sena variety registered as suitable for spice pepper production was used as susceptible female donor and CM 334 and Perennial genotypes were used as male resistance sources. Thirty two genotypes were screened by ASC 035 marker placed on chromosomes P10, ASC 037 on P5 and ASC 031 on P2. Twelve of these improved red pepper lines from different resistance sources were exhibited polygenic resistance according to electrophoretic signals to all of three markers placed on P2, P5 and P10. In order to determine codominance of the resistance genes PCR products of ASC 031 and ASC 037 were restricted by HaeIII and EcoRI respectively. Ten improved lines exhibited codominance related with resistance to Phytophthora Blight concerning ASC 031 and only one line was showed codominance for ASC 037. Three different lines had one resistance gene on P5 and on P10 independently. Only T12 line holds two resistance genes both P2 and P10. DD and 63 lines were most promising genotypes related with their resistance to pathogen. Molecular assisted selection was found useful for improving Phytophthora resistant red pepper lines.

Key words: Phytophthora capsici, polygenic resistance, molecular markers, breeding, pepper

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INTRODUCTION

The most important diseases that limit the pepper cultivation are Phytophthora blight (Phytophthora capsici Leonian). P. capsici was first described as an agent of pepper wilt in New Mexico in 1918 (Leonian, 1922). P. capsici is widespread in Mediterranean and European countries (Italy, France, Spain, Bulgaria, Yugoslavia and the Netherlands) and the Far East countries (Korea and Japan) (Pochard, 1964; Yamakawa et al., 1979; Simon et al., 1979; Abak 1982; Kim et al., 1989).

Phytophthora blight was reported in Turkey (Iren and Maden 1976; Kiran and Ertunc, 1998; Soylu and Kurt, 2001) and in the World (Lee et al., 2001; Choi et al., 1985; Galmarini, 1997; Guerrero-Moreno and Laborde, 1980; Kim and Hwang, 1992) as a fungal disease that was common in pepper cultivated areas and causes significant damage. The use of resistant genotypes is one of the most effective methods to prevent damage of the disease. Generally, it is very difficult to find genotypes that can resist to the pathogen in all circumstances. Durability is formed in the host-pathogen-environment triangle. These three factors affect each other even more in terms of their inherent polygenic resistance (Lefebvre and Palloix, 1996). The development of resistant varieties to resist the pathogen has been studying for many years (Smith et al., 1967; Sotirova and Daskalov, 1983; Milkova et al., 1988). With the development of molecular techniques, the resistance to the Phytophthora blight has been found to have a complex structure. However, the genetically complexity of the resistance is mainly demonstrated by 6 phenotypic observations.

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Three of the resistance mechanism, which were revealed by the stem inoculation test developed by Pochard and Daubeze (1982) based on the lesion length are the receptivity, inducibility, and the stability. The other three are root rot index, leaf and fruit rot. With this study, genes that contribute to the resistance against Phytophthora blight are combined and verified by molecular assisted selection. CM334 Perennial and KM211 genotype selected as resistant to the pathogen from the Kahramanmaraş pepper population was used as resistance sources and Sena variety as a susceptible recipient. As a result of comprehensive breeding program carried out by EMTZARI, DD and 63 lines were developed as carrying the resistance gene in all three chromosomes.

MATERIAL AND METHOD

Plant Materials

In this study, 32 genotypes which were developed by EMTZARI (East Mediterranean Transitional Zone Agricultural Research Institute) were used as plant material. CM334, Perennial and KM211 genotypes were resistance sources of improved lines. The phylogenetic relationship of selected (resistant, partially resistant and sensitive) pepper genotypes with each other was determined by using RAPD molecular marker analysis system.

DNA Extraction and PCR Analysis

DNA isolation for molecular assays was performed using the method proposed by Ruffel et al., (2006) using TRIZOL, Chloroform and isopropanol. PCR content and conditions were reported as Thabuis et al. (2003). The base sizes were determined by using Qiaxcel Advanced System with AM320 method and DNA scanning cartridge at the electrophoretic analysis of PCR products.

Phylogenetic Analysis

The genotypes were compared with Principal Component Analysis, which were generated by using internet-based software that was available at https://biit.cs.ut.ee/clustvis/ (Metsalu and Vilo, 2015).

RESULTS

P28, PR5, PR2, 63, DD, P29, T13, 67, K2, P36, P22, T14 and P32 lines amplified DNA close to the expected size with all three markers. T12 and K1 lines were amplified with ASC 035 on 2^{nd} chromosome and ASC 031 on 10^{th} chromosome. P37, P23, P19 and P30 lines has only one of the resistance components by matching with ASC 031 marker in the 2^{nd} chromosome. The P33 and T5 lines are the genotypes that have the only resistance gene indicated by the marking of ASC 037 on the 5^{th} chromosome.

The lines P21, DG and 42 are found to have the resistance gene on the 10^{th} chromosome. P27 line has the resistance genes on the 2^{nd} and 5^{th} chromosomes coexist. C.B, T18, P20, T6, C.C. and 62 genotypes did not possess any resistance genes with molecular markers (Table 1).

Table 1. Band size of improved pepper lines according to molecular markers placed $2^{nd,}$ 5^{th} and 10^{th} chromosome (bp)

	RAPD MARKER				RAPD MARKER		
Lines	**ASC 031 (P2)**	**ASC 037 (P5)**	**ASC 035 (P10)**	**Genotip**	**ASC 031 (P2)**	**ASC 037 (P5)**	**ASC 035 (P10)**
P28	1200	1737	603	67	1228	1831	621
P33	0	1830	0	K2	1224	1735	631
T18	0	0	0	P36	1209	1781	615
DG	0	**1916**	623	P20	0	0	0
PR5	**1256**	1778	625	P22	1277	1337	639
PR2	1280	1769	618	C.B	0	0	0
63	**1251**	**1853**	**647**	T6	0	0	0
DD	**1258**	**1880**	**648**	P30	**1241**	0	0
P19	1297	0	0	T12	1201	0	637
P21	0	0	615	C.C.	0	0	0
62	0	0	0	T14	**1269**	1785	618
T5	0	**1902**	0	P32	0	**1861**	632
P29	1196	**1872**	592	42	0	0	630
T13	1296	**1862**	625	K1	1226	0	631
67	1228	1831	621	P23	1	0	0
K2	1224	1735	631	P27	1178	**1884**	0
P36	1209	1781	615	P37	1296	0	0

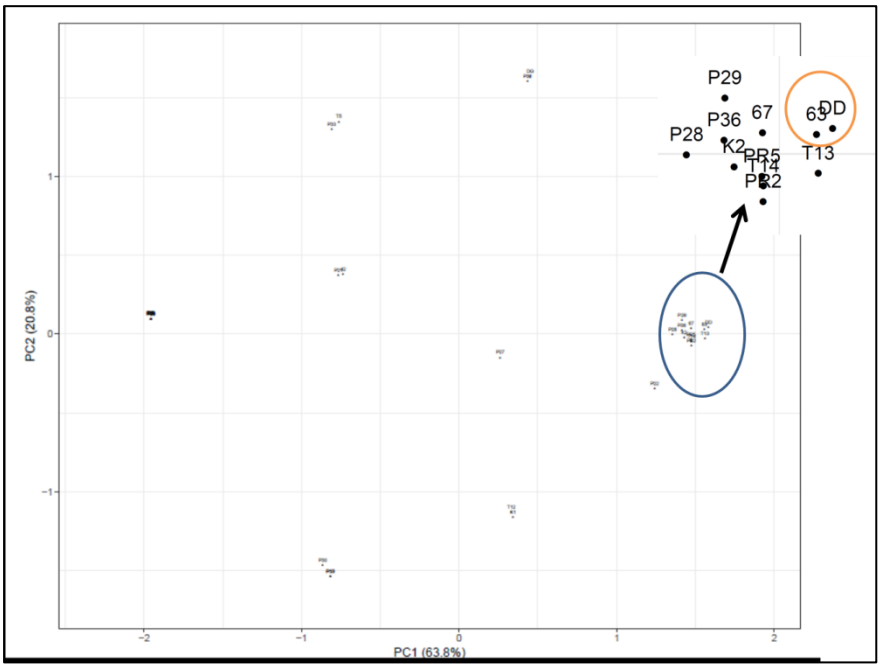

Figure 1. Principal component analysis of genotypes related with amplified base size 2nd, 5th and 10th chromosomes

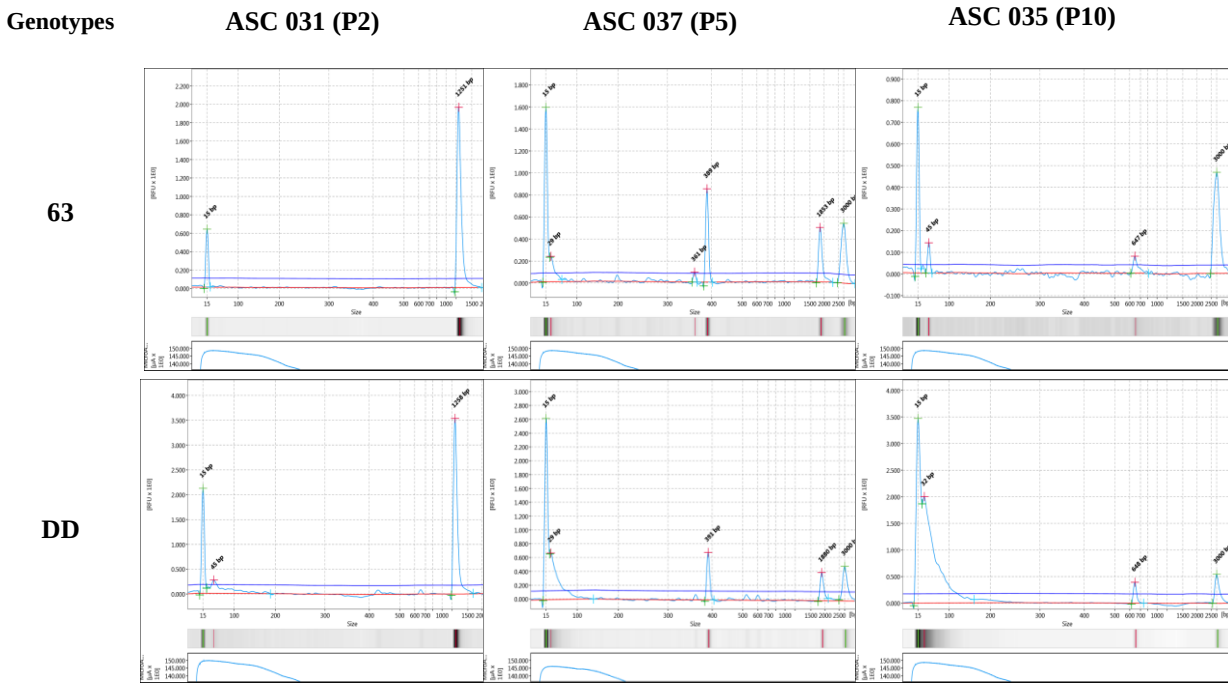

Figure 2. Electrophoretic chromatograms of DD and 63 genotypes related with amplified base size 2nd, 5th and 10th chromosomes

Base sizes derived by the molecular markers in the three chromosomes were subjected to Principal Component Analysis. Most of genotypes were clustered together and DD and 63 lines are very close to each other (Fig. 1). These two lines were found to be quite close to the base size of the amplified markers (Fig 2). The differentiation of the base size expected can be explained by the possible variations such as deletion and mutation in the resistance genes.

DISCUSSION AND CONCLUSION

Thabuis et al. (2003) stated that susceptible parents has resistance gene and resistance can be transferred from 3rd 5th and 11th chromosomes. Therefore, it is necessary to verify the resistance of genotypes have markers different sizes by stem inoculation and root rot index tests.

Thabuis et al. (2004 a) determined the QTL (Phyto 6.1.) on 6th chromosome in the populations improved from Yolo Wonder and CM334 genotypes by recurrent selection method using the markers ASC 012 and ASC 014. Bonnet et al. (2007) have identified 8 QTL in 1, 4, 5, 6, and 11. Chromosomes of 5th generation of Yolo Wonder and CM334 crosses and observed 4 chromosomes affect many of the resistance component. Thabuis et al. (2003) reported that the genes on the fifth chromosome were specific to genotypes such as Vania and Perennial and other QTLs developed specifically for hybrids. They stated that the additive gene effects are formed by the action of five genes in Perennial and the resistance in CM334 becomes more complicated.

Marker-assisted selection provides many advantages for plant breeding especially determining polygenic characters. It is accepted as a promising tool for breeding quantitative resistance (Thabuis et al. 2004). During breeding program three resistance genes could be transferred to red pepper genotypes suitable for powdered or grinded chili pepper.

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