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Faculty of Agriculture
Department of Plant Breeding and Biotechnology
The Thesis Submitted for the Degree of PhD (in the field of Plant Breeding Science)

Candidate gene Identification for *Ascochyta blight* in Chickpea using SNP-based mapping

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Abstract

Ascochyta blight is one of the most destructive plant diseases of chickpea cultivation in the world which causes significant damage to chickpea cultivation every year. Several methods have been identified to control this disease but the most effective of which is the use of cultivars with durable resistance. Identifying sources of *Ascochyta blight* resistance has been considered as a major goal in most chickpea breeding programs. The aim of this study was to investigate the performance of a functional SNP in differentiating sensitive and resistant Iranian chickpea cultivars against *Ascochyta blight* in order to be use in breeding programs. For this purpose, based on the previous studies, a number of SNPs were introduced on a specific region of the chickpea genome, which may play an effective role in the development of *Ascochyta blight* resistance in chickpeas. Based on the bioinformatics analysis, it was found that among the introduced SNPs, one case is located within the exon sequence, which was introduced as a functional SNP, and its presence among Iranian cultivars was tracked using PCR and sequencing. Amplification of the fragment containing the desired SNP was performed using PCR with designed primers. The sequencing results showed that a number of new point mutations at loci SNP18, SNP18-2147, SNP18-2491 and SNP18-2554 belonging to the GSH118 gene among sensitive (ILC263) and resistant (MCC133) cultivars. Among the identified point mutations, the SNP18 and SNP18-2147 position in the aforementioned situations, the amino acid changed from aspartate to asparagine and from alanine to glycine, respectively, causing a change in the peptide sequences which were expressed at the protein level at positions corresponding to amino acids 499 and 554. More bioinformatics studies have shown that the GSH118 gene is a leucine-rich kinase receptor and encodes a membrane protein that can be involved in identifying microorganisms and initiating immune signaling pathways in plants. As a result, it is a suitable defense and signaling gene in the early stages of the pathogen attack to the plant.

Keywords: Molecular marker, *Ascochyta rabiei*, Sequencing, Amino acid