

Research Article

Detection of resistance gene to gray leaf spot caused by *Stemphylium* spp. in tomato cultivars of Iraq

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Abstract

The resistance against disease is granted by one incompletely dominant gene existing on chromosome eleventh. Gray leaf spot is caused by *Stemphylium* sp.. This study aimed to distinguish the cultivar resistant alleles or susceptible alleles by molecular marker linked to Resistance Gene (Sm gene) using Marker Assisted Selection (MAS) inbreeding. In this study, we used eight tomato cultivars farmed in Iraq. The results showed that the codominant marker InDe, after PCR amplification, produced a 122bp fragment for resistance in seven genotypes and a 140bp fragment of susceptible alleles in one genotype, respectively could be utilized in MAS for resistance disease of gray leaf spot.

Keywords: Gray leaf spot, Sm gene, Tomato, Indel markers.

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Introduction

Leaf spot is a common disease devastating a broad range of plants, including important crops like sweet potato (Chai et al. 2015), tomato (Nasehi et al. 2012a), eggplant (Nasehi et al. 2013; Yang et al. 2017), and pepper (Cho et al. 2001). The pathogen source in tomatoes mainly includes four species of *S. vesicarium* (Wallr.), *S. solani* Weber and *S. floridanum* Hannon and Weber and *S. botryosum* Wallr. (Blancard et al. 1986; Miranda et al. 2010) that contaminated all tomato-growing areas worldwide (Simmons 2001). Kim et al. (1999) showed that tomato spot develops in high humidity and warm temperature. It impacts the leaves, firstly the oldest one. This disease symptom appears as small and brownish-black spots on both surfaces of the leaves, and expand as greyish brown. As the disease progresses, the centers of the specks are damaged and drop and the affected leaves areas will convert yellow causing them dry and fallout. It does not influence fruit but damages their leaves decreasing fruit quality

and production rate. In the early 2000s,

The fast spread of foliar disease in China was observed. Since then, the spread of the foliar disease has caused Intense yield losses throughout China, particularly in greenhouses in spring. *Stemphylium solani* was diagnosed as an epidemic species. The fungal spores are prevalent by water and wind and can stay for a long time on weeds and plant debris or in soil. Under appropriate environmental conditions, it can the appropriate host be ruined within a few days (Li et al. 2010).

The disease is difficult to control by utilizing agrochemicals and classic agricultural habits. Thus, the utilization of resistant varieties is an economically practical method to control. In a study Sm gene in an interval of 0.26Mb region on chromosome 11 were revealed using the sergeant analysis and genome resequencing (Yang et al. 2017), showing that it gives resistance to all four species of *Stemphyllium* genus (Behare et al. 1991). Development of a disease resistance variety is the

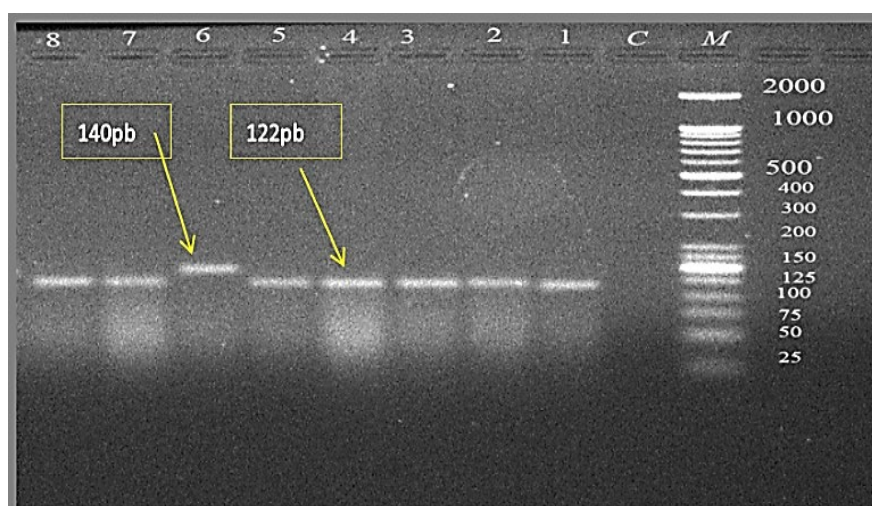


Fig.1. PCR fragments amplified from tomato cultivars by marker Sm InDel to distinguish leaf spot susceptible cultivars and resistance in a 3% agarose gel. Lane1: Super marimon, Lane2: Helam, Lane3: Bushra, Lane4: Shady lady, Lane5: Imperial, Lane6: GSN, Lane7: Castle Rock, Lane8: Shahad. M, 25-2000bp DNA ladder.

final goal of all breeding programs (Zeina et al. 2018). Some main resistance genes in wheat such as Lr34 and Sr2 were found (Krattinger 2009).

Therefore, the determinate of the Sm resistance gene is not only enables to design of exemplary markers for Marker Assisted Selection (MAS) inbreeding but also supplies a tool to understand the molecular mechanisms of the host resistance against the pathogen. Notably, this is the first screening of the candidate genes for *Stemphyllium* resistance in Iraq. This study helps to facilitate the selection of genotypes with high levels of leaf spot disease resistance.

Materials and methods

Plant materials: Eight genotypes of the tomato, including Super marimon, Helam, Bushra, GSN, Shady lady, Imperial, Castle Rock and Shahad were used in this work. These genotypes were grown in a growth chamber (26-28°C, 14h light/ 0h dark, m-2 s-1 light intensity).

DNA extraction and PCR amplification: DNA was extracted from the apical fresh leaves after 3-4 weeks using DNA Mini Kit protocol (Geneaid Biotech. Ltd; Taiwan Company). An easy and quick method was used for purifying total DNA. The extracted DNA was quantified using a Nanodrop. In PCR

amplification was used a developed marker for Sm-InDel on chromosome 11 as: F (5'-CTACACTTTCTCGTTCCTCAATG-3') and R (5'-ATCGCCAAACCAATCAAATC-3') (Lin et al. 2014). PCR was conducted as follows: initial denaturation in 3min at 95°C, the number of cycles was 35 cycles in 30s at 95°C, the 30s at 56°C and a 30s extension at 73°C. The Final reaction was extended for 5min at 72°C. Using 3% agarose gel, the PCR products were examined after staining with ethidium bromide (Eb), and then photographed.

Results and Discussion

MAS is an effective and important tool in plant breeding since it links with the target gene. In this work, Sm has been characterized as a dominant gene to find the resistance to the gray leaf spot (Frazier & Hendrix 1949). For this purpose, disease screening was conducted on eight cultivars using Sm-InDeler marker. A total of 122bp fragment of this gene was observed in the gel showing resistance in seven genotypes (Super marimon, Helam, Bushra, Shady lady, Imperial, Castle Rock and Shahad). The other fragment with 140bp was susceptible alleles in GSN genotype (Fig. 1) as reported by Xiaomei et al. (2015). In addition, an 18bp deletion was found 15kilopair from the Sm candidate gene.

Using Sm InDel marker that produced 140bp or 122 bp fragments as resistant alleles showed gray leaf spot resistance or susceptibility in germplasm with around 95.5% in this study. We conclude SmInDel marker (SmInDel) a close gene of Sm responsible for the resistance and could be utilized in MAS breeding for examining resistance tomato cultivars (Xiaomei et al. 2015). The utilization of molecular markers related to the resistance is an important of the superb strategies for breeding programs and multiple biotic stresses (Rani et al. 2020).

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