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Detection of Sm gene resistance to gray leaf spot in (Stemphylium Spp.) tomato cultivars Iraq

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Abstract

Gray leaf spot, occurs due to *Stemphylium spp.*, is a foliar disease in tomato. The Resistance against gray leaf spot disease is conferred by a single incompletely dominant gene Sm located on chromosome 11. This study aimed to identify cultivar resistant alleles or susceptible alleles by molecular marker tightly linked to the Sm gene and the use of marker-assisted selection inbreeding. In this study, we used eight tomato cultivars Farmed in Iraq. The analysis demonstrated that the co-dominant marker Sm-InDel, which produced 122-bp fragment for resistance in seven genotypes and a 140-bp fragment for susceptible alleles in one genotype, respectively could be utilized in Marker-assisted selection (MAS)for gray leaf spot resistance.

Keywords: Gray leaf spot, Sm gene, tomato, Indel Markers

Introduction

Gray leaf spot is a foliar disease considered a common and devastating has a wide range of plant hosts including important crops such as cucumber (Vakalounakis and Markakis 2013), tomato (Nasehi et al. 2012a), sweet potato (Chai et al. 2015), garlic (Zheng et al. 2008), eggplant(Yang et al. 2017) (Nasehi et al. 2013) And pepper(Cho et al.2001). The pathogen causing in tomato mainly includes four species of Stemphyllium spp: Stemphylium solani Weber, Stemphylium vesicarum (Wallr.), and Stemphylium floridanum Hannon and Weber and Stemphylium botryosum Wallr. (Blancard et al. 1986)(Miranda et al.2010) . gray leaf spot is considered a major disease of cultivated tomatoes and has threatened tomato-growing areas worldwide(Simmons,2001). Tomato gray leaf spot occurs and develops under high humidity and warm air temperature (Kim et al. 1999). It mainly impacts the leaves of tomatoes, beginning with the oldest leaves. In the early stage, the disease symptoms appear as small and brownish-black spots on both two surfaces the leaves, and then, it expands and turns a grayish brown. As the disease progresses the centers of the specks crack and fall out, and affected leaves areas will turn yellow ultimately causing the leaves will dry and drop. this disease does not directly influence fruit, but damage to leaves reduces fruit quality and yield. An outbreak of Tomato gray leaf spot in China was first . in the early 2000s. Since then, the Spread of disease has caused Intense yield losses throughout China (Li et al. 2010), especially in greenhouses in the spring season. 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 favorable environmental conditions, it can the compatible host is devastated within several days (Li et al. 2010). It is hard to control the disease to utilize agrochemicals and traditional

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agricultural practices. Thus, the utilize of resistant cultivars is the economically feasible method to control the disease and environmentally-friendly In a Modern study mapped Sm gene in an interval of0.26-Mb region on chromosom11 based on the bulked segregant analysis in combination with genome resequencing(Yang, et al. 2017). The most noteworthy is the Sm gene gives resistance to four species of Stemphyllium spp, and there are no studies of new virulent races overcoming the resistance (Behare et al. 1991). Permanence disease resistance is the final goal of all breeding programs. There are some major resistance genes, such as Lr34 and Sr2 in wheat, which have proved efficiency over many years of agricultural utilize Therefore, the determinate of the Sm resistance gene will not only enable the design of exemplary markers for marker-assisted selection inbreeding but also supply us a valuable tool to understand the molecular mechanisms of the host resistance against pathogen. Notably, this is the first time that screening of candidate genes for the tomato Stemphylium resistance Sm gene in Iraq. This study will be valuable and facilitate the selection of Genotypes with high levels for leaf spot disease resistance.

Materials and methods

Plant materials

Eight tomato genotypes were used in this Search (Super marimon, Helam, Bushra, GSN, Shady lady, Imperial, Castle Rock, And Shahad). These tomato genotypes were grown in a growth chamber (26-28C, 14h light/ 10h dark, 63.03 µmol·m-2·s-1light intensity)

Genomic DNA extraction and PCR amplification

DNA was extracted from apical fresh leaves after 3-4 weeks fro agriculture using DNA Mini Kit protocol (Geneaid Biotech. Ltd; Taiwan Company) Availability an easy and quick method for purifying total DNA. Extracted DNA was quantified using a Nanodrop. PCR amplification utilize the developed marker Sm-InDel on chromosome 11 (F: 5'-CTA CAC TTT CTC GTT CCC AATG-3', R: 5'-ATC GCC AAA CCA ATC AAA TC-3'). (Lin et al. 2014). The PCR was conducted with an initial denaturation step of 3min at 94°C followed by 32 cycles of the 30s at 94°C, the 30s at 55°C and a30s extension at 72°C. Final reactions were extended for 5min at 72°C. The PCR products were separated from 3% agarose gel, stained with ethidium bromide(Eb), and photographed.

Results and Discussion

Marker-assisted selection MAS is an effective tool in plant breeding. The development of molecular markers tightly linked with the target gene is a prerequisite for efficient MAS. Until now, there have been few reports of molecular research concerning tomato resistance to gray leaf spot. the Inheritance of gray leaf spot resistance in tomato cultivars formerly, Sm has described as an incompletely the dominant gene for resistance to the gray leaf spot (Hendrix and Frazier 1949). To prove this conclusion, we performed the disease assay on eight tomato cultivars. through from use Sm-InDeler mark .it was produced a 122-bp fragment for resistance in seven genotypes(Super marimon, Helam, Bushra, Shady lady, Imperial, Castle Rock, And Shahad and a 140-bp fragment for susceptible alleles in GSN genotype (**Figure 1**).Agree with (Xiaomei et al. 2015). An 18-bp deletion

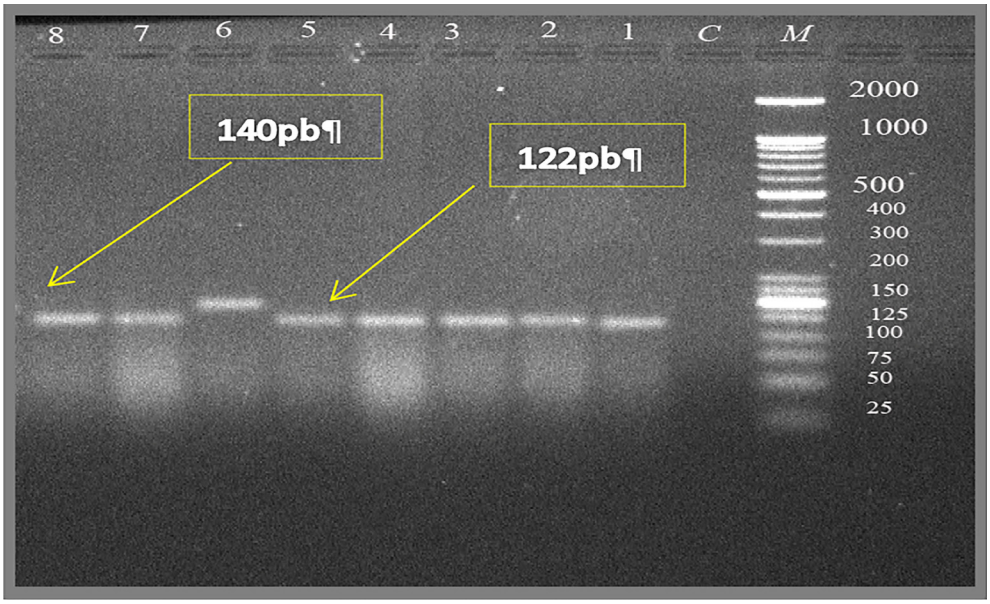

Figure. 1 PCR fragments amplified use marker Sm-InDel from tomato cultivars gray leaf spot susceptible and resistant resolved in a3% agarose gel. Lane 1: Super marimon, Lane 2: Helam, Lane 3: Bushra , Lane 4: Shady lady, Lane 5: Imperial, Lane 6: GSN, Lane 7: Castle Rock, Lane 8: Shahad. M, 25-2000bp DNA ladder

was found downstream 15 kb from the Sm candidate gene

In recent research, the Sm gene was mapped and screened based on the bulked segregant analysis in combination with genome resequencing, accompanied by a SCAR marker D5 designed (Yang et al. 2017). However, this marker hardly distinguishes resistant and susceptible tomato germplasm in our verification experiment, which indicated that the marker showed poor applicability in tomato breeding. In the present study, use a codominant marker Sm-InDel was developed that produced a 122-bp or a 140-bp fragment for resistant or susceptible alleles, respectively. It can detect gray leaf spot resistance in germplasm with 96.5% accuracy and easily resolved in a standard agarose gel. This result indicates that the co-segregating marker Sm-InDel is very close to the gene Sm and could be used in MAS breeding for gray leaf spot resistance. (Xiaomei et al 2015). the utilize of linked molecular markers related to resistance characteristic is one of the superb strategies for pyramiding of gene for future breeding programs. and multiple biotic stresses(Rani et al. 2020).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.