

Research Article

Differential physiological and molecular responses of susceptible and resistant tomato genotypes to *Alternaria solani* infection

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Abstract: Early blight caused by *Alternaria solani* is a prominent tomato, *Solanum lycopersicum*, disease that destroys a significant part of tomato production worldwide. Cultivating resistant cultivars is notably important in reducing damage caused by early blight disease. Therefore, comprehending the response of different genetic backgrounds to pathogen infection could enhance understanding of the mechanisms involved in plant defense systems against pathogen invasion. In the present study, the differential response of susceptible and resistant tomato genotypes to *A. solani* was investigated from molecular and physiological aspects. The results showed that soluble sugar content in the resistant genotype increased after pathogen inoculation. Although photosynthetic pigments such as carotenoid, chlorophyll a, and chlorophyll b content decreased in susceptible and resistant genotypes, fluorescence chlorophyll indices differed in resistant and susceptible genotypes. Also, transcription analysis revealed that in the resistant genotype, the expression of *SIWRKY1* was 2.58 times more than the control at 48 hpi (hours post inoculation). However, in the susceptible genotype, the expression of the *SINAC1* was 69.12 times more than in control at 24 hpi. The findings of this research provide an improved understanding of tomato plant defense mechanisms against early blight disease.

Keywords: Chlorophyll *a* fluorescence, Early blight disease, qRT-PCR, *SINAC1*, *SIWRKY1*

Introduction

Tomato *Solanum lycopersicum* is an important horticultural plant due to its nutritional and commercial value (Li *et al.*, 2020) and is cultivated in temperate regions (Gong *et al.*, 2017). Plant diseases, especially fungal pathogens, can severely reduce tomato production by infecting plant tissues. One of the

most lethal fungal diseases of tomatoes is early blight, caused by *Alternaria solani* Sorauer (1896), which can reduce tomato production by up to 80% (Nafisa *et al.*, 2020). The main strategies to control early blight disease include the use of fungicides, cultural methods, and resistant genotypes. Early blight is primarily controlled by foliar spraying of certain fungicides applied at 7-10-day intervals

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(Adhikari *et al.*, 2017). Considering the harmful effects of fungicides on the environment and agricultural production, the most suitable and sustainable way to control early blight is the development of resistant cultivars (Adhikari *et al.*, 2017; Akhtar *et al.*, 2019).

Necrotrophic pathogens like *Alternaria* spp. destroy plant tissue (Shao *et al.*, 2021) and cause physiological disorders such as reduced photosynthetic efficiency (Clark, 2022). Studies show that changes in some physiological features are important for plant resistance to pathogens, such as the synthesis of sugars (Morkunas and Ratajczak, 2014), chlorophyll fluorescence indexes (Suárez *et al.*, 2022), and photosynthetic pigments (Parveen and Siddiqui, 2022). Also, concerning the molecular response of plant-pathogen interactions, some transcription factors, such as *NAC1* and *WRKY1*, are considered the most important components of resistance (Rabiei *et al.*, 2022; Shinde *et al.*, 2018).

Comparing the response of susceptible and resistant genotypes can increase our knowledge of the mechanisms involved in plant resistance to pathogen infection. Therefore, it is essential to investigate the responses of susceptible and resistant genotypes under stress conditions (Ray *et al.*, 2015). Although numerous studies have been published on plant-pathogen interactions, few have addressed the comparative response of resistant and susceptible genotypes to pathogen infection (Henriquez *et al.*, 2012; Ray *et al.*, 2015; Yao *et al.*, 2011).

Previous research suggests that the plant's genetic background influences the tomato genotype responding to *A. solani* stress (Nafisa *et al.*, 2020; Ray *et al.*, 2015). A study by Ray *et al.* (2015) found that after pathogen inoculation, defense enzyme activity, H_2O_2 accumulation, and other defense compounds are significantly higher in the resistant than in the susceptible genotype (Ray *et al.*, 2015). In another study, morphological, biochemical, and physiological responses of 29 tomato genotypes were assessed after inoculation with *A. solani*. During the disease stress, the activity of antioxidant enzymes significantly

differed in resistant genotypes compared with sensitive genotypes (Nafisa *et al.*, 2020). However, comparative studies about plant-pathogen interactions regarding Iranian tomato genotypes are more limited.

The main goal and novelty of the present study were the comparative physiological and molecular defense reactions of two common Iranian tomato cultivars, as resistant and susceptible genotypes, under *Alternaria solani* stress. Some physiological parameters were employed to determine differential reactions of resistant and susceptible genotypes under early blight stress. Also, transcription profile of *SINAC1* and *SIWRKY1* was obtained across time, post-infection, with quantitative real-time PCR (qRT-PCR) technique for both resistant and susceptible tomato genotypes.

Materials and Methods

This research was conducted in research facilities and laboratories of Genetics and Agricultural Biotechnology Institute of Tabarestan, Sari, Iran. Susceptible (Karoon) and resistant (*CH* falat) tomato genotypes seeds used throughout this study were provided by Falat Iranian Zamin Co., Karaj, Iran. The seeds were sown and grown in 17 cm pots filled with a sterile soil mix (equal volumes of peat, perlite, and coconut peat). The pots were incubated in a growth chamber with a photoperiod of 12 h of light (98.02 $\mu\text{mol/s/m}^2$), 70% humidity, and 24-27 °C and were fertilized regularly with Hoagland's nutrient solution (Hoagland and Arnon, 1950). The *A. solani* isolate was kindly provided by the culture collection of the Genetics and Agricultural Biotechnology Institute of Tabarestan (Culture number GTCC0073) at Sari Agricultural Sciences and Natural Resources University, Sari, Iran. 35-day-old seedlings were inoculated with *Alternaria Solani* spores. A spore suspension of *Alternaria Solani* at 1.6×10^6 spores per ml was used for plant inoculation. For physiological analysis, samples were collected seven days after inoculation. However, tomato

leaves were collected for molecular analysis at 12, 24, 48, and 96 h post-inoculation (hpi).

Soluble sugar measurement

The anthrone colorimetric method was used to measure the soluble sugar content of tomato leaves (McCready *et al.*, 1950). For this purpose, 0.2 g of the fresh leaf was powdered in liquid nitrogen. Then 10 ml of 80% methanol was added to each sample, placed on a shaker (150 rpm) in the dark box for 24 hours, and centrifuged at 5000 rcf. Three ml of 10 mM anthrone (dissolved in 70% sulfuric acid) was added to 100 μ l of the methanolic extract and placed at 100°C (Bain-marie) for 20 minutes. Then, the samples were transferred to room temperature and vortexed after 10 minutes. The absorbance of the samples was measured at a wavelength of 620 nm. The soluble sugar content was calculated using the glucose standard curve ($R^2=0.992$, $Y = 0.229 \times 0.0438$).

Measurements of photosynthetic pigments and chlorophyll contents

According to Lichtenthaler and Buschmann (2001) method, the photosynthetic pigments, including chlorophyll a (Chl a), chlorophyll b (Chl b), as well as carotenoids were analyzed by a spectrophotometry technique (T92+, PG instrument limited). Hence, 1.0 cm² of fresh leaf tissue was extracted with 80% methanol at room temperature for 24 h in the dark and measured at 665.2 ($A_{665.2}$), 652.4 ($A_{652.4}$), and 470 (A_{470}) nm. Equations 1 to 3 were used for calculating the contents of Chl a, Chl b, and carotenoid, respectively:

$$\text{Chl a } (\mu\text{g/mL}) = 16.72 A_{665.2} - 9.16 A_{652.4} \quad (1)$$

$$\text{Chl b } (\mu\text{g/mL}) = 34.09 A_{652.4} - 15.28 A_{665.2} \quad (2)$$

$$\text{Carotenoid } (\mu\text{g/mL}) = (1000 A_{470} - 1.63 \text{ Chla} - 104.6 \text{ Chlb}) / 221 \quad (3)$$

Measurements of chlorophyll a fluorescence

Using a portable fluorometer (PAM3000, Walz, Germany), the Chl fluorescence was measured (Genty *et al.*, 1989). The plants were left in the dark for 30 minutes, and then the minimum fluorescence intensity (F_0) and maximum

fluorescence intensity (F_m) were measured in dark-adapted leaves. F_v (The variable fluorescence) and F_v/F_m (maximum photochemical quantum yield of PSII) were calculated as shown in equations 4 and 5, respectively. Moreover, minimum (F'_0), maximum (F'_m), and steady-state fluorescence (F_t) were measured according to the light-adapted leaf in actinic light and 6, 7, and 8 equations, the effective photochemical quantum yield of PSII [$Y(II)$], the quantum yield of regulated energy dissipation [$Y(NPQ)$], the quantum yield of non-regulated energy dissipation [$Y(NO)$], non-photochemical quenching (NPQ) were calculated, respectively:

$$F_v = F_m - F_0 \quad (4)$$

$$F_v/F_m = (F_m - F_0)/F_m \quad (5)$$

$$Y(II) = (F'_m - F_t)/F_m \quad (6)$$

$$Y(NPQ) = (F_t / F'_m) - (F_t / F_m) \quad (7)$$

$$Y(NO) = F_t / F_m \quad (8)$$

$$NPQ = (F_m - F'_m) / F'_m \quad (9)$$

qRT-PCR assay

Total RNA of the plant leaf was extracted with Threezol reagent (Riragene, Iran) according to manufacturer instructions and then treated with DNaseI (Fermentase, Germany) to remove DNA contamination. Based on the manufacturer's protocol, the RevertAid™ Reverse Transcriptase kit (Fermentase, Germany) was used for cDNA synthesis. *Actin* gene was used as an internal reference and the list of primers is available in Table 1. The Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) was used for qRT-PCR reactions. The 15 μ l reaction mixture contained 1.0 μ l of diluted cDNA sample, 0.3 μ M of each forward and reverse primers and 1 $\times$ real-time SYBR Green master mix. The cycling temperature conditions were the first denaturation at 95 °C for 8 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 30 s. Each sample was quantified in three biological and two technical replications. The relative gene expression was quantified by Livak and Schmittgen (2001) method.

Table 1 List of primers used for qRT-PCR.

Gene	Accession No.	Sequence 5'-3'	Length (bp)	Reference
<i>SlActin</i>	NM_001308447.1	AACAGACAGGACACTCGCACT TTAGCACCTTCCAGCAGATGT	126	(Issa <i>et al.</i> , 2018)
<i>SINAC1</i>	NM_001247553.2	GGCAACCGGAGCTGATAAAC AGGCGGTACTCGTGCATAATC	127	(Ghorbanpour <i>et al.</i> , 2018)
<i>SlWRKY1</i>	XM_019214874.2	TAGCAGTGAAGTGGATGTAGTC TTGGATTATGGGATGACCTCTC	155	(Moghaddam <i>et al.</i> , 2019)

Statistical analysis

The factorial experiment was based on a completely randomized design (CRD) with three replicates. The plant genetic backgrounds (susceptible and resistant genotypes) were considered the first factor, and the state of plant inoculations (inoculated and non-inoculated samples) the second factor. Also, two technical repetitions were done for molecular tests. The least significant difference test (LSD) was performed at 1% probability for mean comparison. All statistical analyses were carried out using SAS statistical package (SAS Institute, Cary, NC).

Results

Sugar content

The results showed that in the resistant genotype, the soluble sugar level significantly increased (71.74%) after pathogen inoculation. In contrast, in the sensitive genotype, a significant decrease (24%) of the soluble sugar was observed after pathogen inoculation (Fig. 1A).

Photosynthetic pigments

In this study, carotenoid content significantly decreased (49%) in susceptible genotype following the pathogen infection. While no significant change was observed in the resistant one after pathogen inoculation (Fig. 1B). The chlorophyll a content in susceptible and resistant genotype decreased after inoculation by 46.74 and 45%, respectively. Similarly, the chlorophyll b content in susceptible and resistant genotypes were reduced by 50 and 48%, respectively, after *A. solani* inoculation (Fig. 1C, and D).

Chlorophyll a fluorescence

Analysis of variance for chlorophyll a fluorescence parameters, including Fv, Fv/Fm, Y(NPQ), and NPQ showed a significant change after pathogen inoculation (presented in Supplemental Table S1). Means comparison (LSD 99%) showed that the pathogen infection did not significantly affect the Fv and Fv/Fm indices in the resistant genotype. While in the sensitive genotype, the Fv and Fv/Fm indices were significantly reduced by 72.9 and 53%, respectively, after pathogen inoculation (Table 2). Also, Y(NPQ) and NPQ increased in resistant genotype after pathogen inoculation by 2.73 and 3.2 times, respectively. In contrast, Y(NPQ) and NPQ were significantly decreased by 60% and 53.33%, respectively, in the susceptible genotype (Table 2).

Molecular analysis

In the present study, differential expression of two prominent genes related to stress was investigated by the qRT-PCR technique. The results show that *SlWRKY1* was up-regulated at 12hpi, 24hpi, and 48hpi in the resistant genotype by 1.49, 2.29, and 2.58 times more than the control, respectively. Also, in the susceptible genotype, the gene expression up-regulated at 12hpi and 42hpi by 1.3 and 1.4 times more than the control, respectively (Fig. 2A).

Expression of *SINAC1* expression gene in the resistant genotype was up-regulated (27.45 times more than control) at early steps of pathogen penetration (12hpi). However, at 24hpi, 48hpi, and 96 hpi the transcription levels were 4.3, 3.8, and 1.13 times more than the control, respectively. While in the susceptible genotype,

expression of *NAC1* gene increased by 62.73 and 96.12 times more than the control at 12hpi and 24hpi, respectively. Also, at 48hpi and 96hpi the transcription levels were 6.5 and 1.5 times more than the control (Fig. 2B).

Discussion

Understanding the differential response of resistant and sensitive genotypes to pathogen infection is fundamental in basic knowledge and applied sciences, such as developing resistant cultivars (Ray et al., 2015).

Sugar content

Inoculation of tomato plants using *Alternaria solani* spore suspension indicated the disease process was faster and more destructive in the susceptible genotype than the resistant genotype. In this study, a significant increase of soluble sugar was observed in the resistant

genotype. In contrast, in sensitive cases, soluble sugar significantly decreased after pathogen infection (Fig. 1A). Generally, reducing sugar content indicates that the pathogen uses plant sugar for energy and structural purposes, as well as the inhibition of photosynthesis due to the destruction of plant tissue by the penetration of the pathogen. In previous studies, a decrease in sugar content after *Alternaria* sp. attack has been reported. For example, The results of Garg et al. (2020) on tomatoes infected by *A. solani* showed that the total sugar content of leaves decreased sharply after pathogen inoculation. Similar results were found when the tomato was inoculated with *A. alternata* (Meena et al., 2017). Moreover, Bhale et al. (2010) observed that the total sugar of leaves decreased after *A. spinaciae* inoculation in the susceptible spinach.

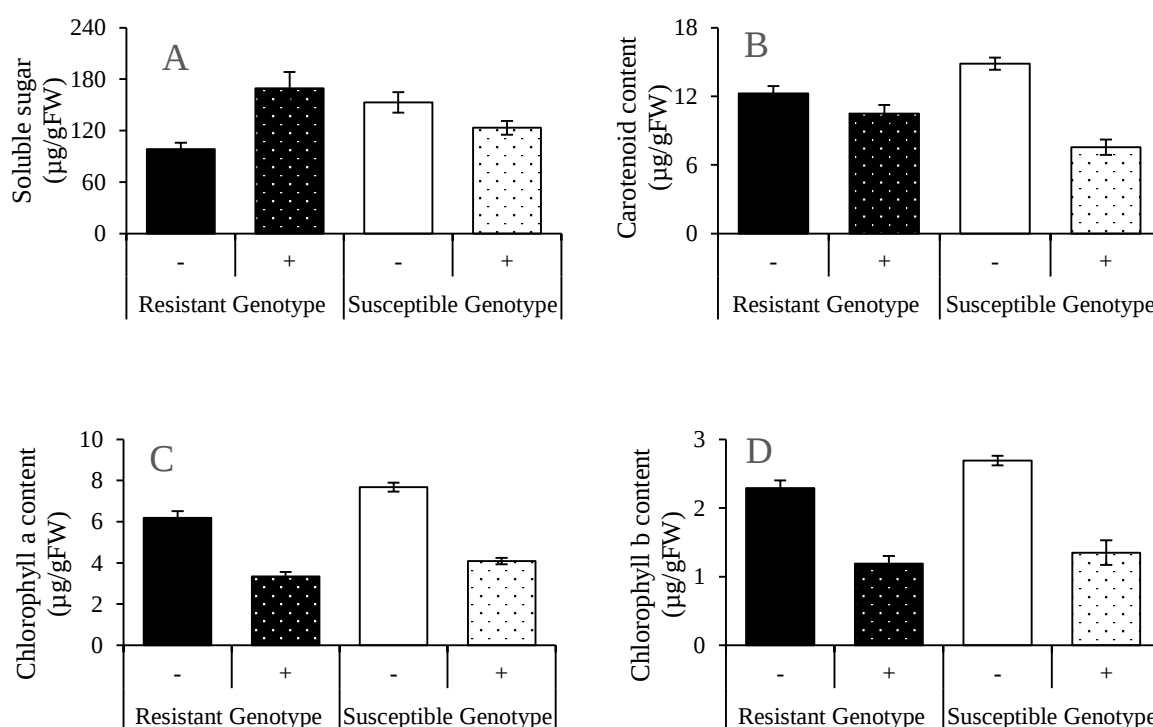


Figure 1 Effect of the *Alternaria solani* on sugar contents and leaf pigments of resistant and susceptible tomato genotypes. A) Sugar, B) Carotenoid, C) Chlorophyll a, and D) Chlorophyll b contents. +) as pathogen inoculation and -) as no pathogen inoculation.

Table 2 Mean comparison of the effect of *Alternaria solani* stress on Chlorophyll fluorescence parameters of two tomato genotypes.

Genotypes	Inoculation	Fv	Fv/Fm	NPQ	Y(NPQ)
CH	no	432.67 ± 24.33b	0.84 ± 0.05a	0.003 ± 0.0006c	0.002 ± 0.0001b
	yes	422.44 ± 24.11b	0.84 ± 0.04a	0.008 ± 0.0005b	0.006 ± 0.0002a
K	no	493.67 ± 14.15a	0.83 ± 0.01a	0.015 ± 0.0015a	0.005 ± 0.0011a
	yes	133.67 ± 6.66c	0.39 ± 0.07b	0.007 ± 0.0009b	0.002 ± 0.0003b

In each column, means with the same letter(s) are not significantly different according to LSD test at P < 0.01.

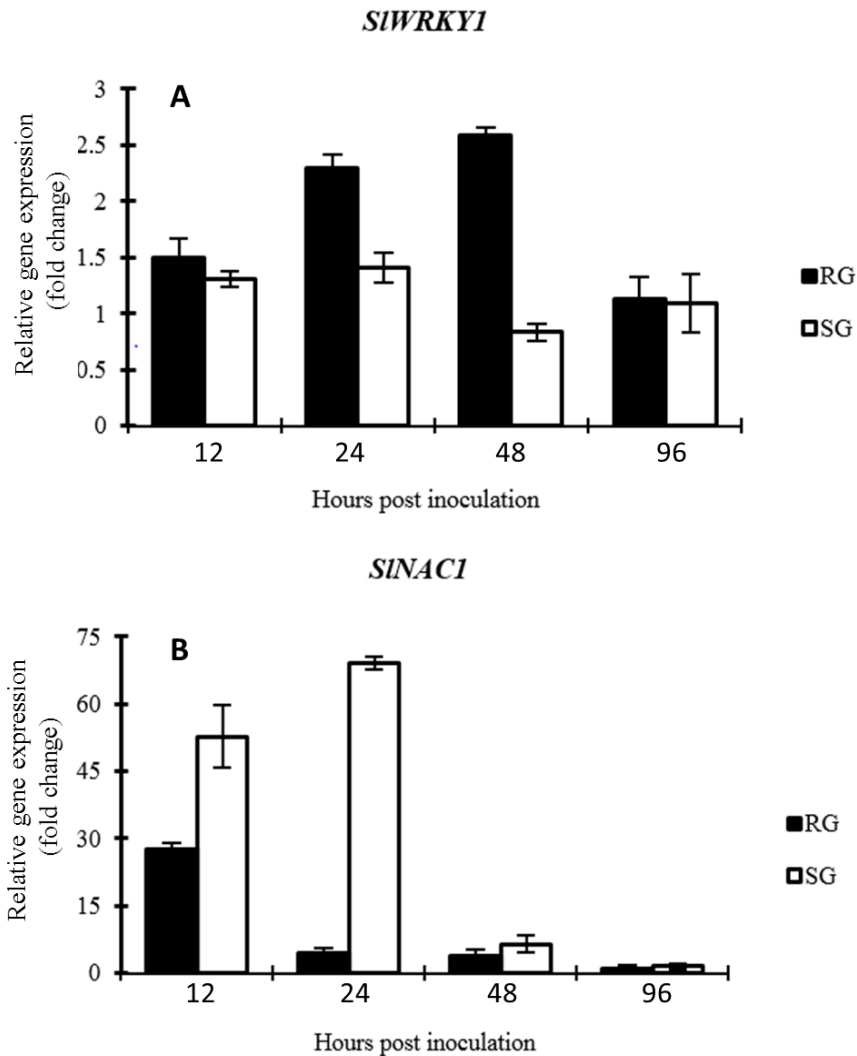


Figure 2 Relative gene expression profile of *SIWRKY1* and *SINAC1* over a time course from 12 to 96 hours post inoculation in resistant (RG) and susceptible (SG) tomato genotypes following *Alternaria solani* inoculation.

However, sugar can act as a signalling molecule in plant cells (Morkunas and Ratajczak, 2014) and cause plant defense systems to be

stimulated (Herbers *et al.*, 1996). Therefore, the increase in sugar content that was observed in this study can be part of the ability of the defense

system of the resistant genotype. Also, Formela-Luboińska *et al.* (2020) stated that an increase in sugar content enhanced the level of salicylic acid and stimulated the ethylene production in tomato leaves under pathogen stress (Formela-Luboińska *et al.*, 2020). The rapid hydrolysis of plant sugar content by pathogenic enzymes is the main reason for reducing sugar content during plant-pathogen interaction. On the other hand, soluble sugars such as sucrose, glucose, and fructose are involved in plant-pathogen interactions in multiple ways (Morkunas and Ratajczak, 2014; Trouvelot *et al.*, 2014) and improved the plant-related defense systems to overcome biotic stress (Formela-Luboińska *et al.*, 2020).

Photosynthetic pigments

Carotenoid plays an antioxidant role during oxidative stress in plants (Kasote *et al.*, 2015) and is considered a stress index. In this study, although carotenoid content significantly decreased in the resistant genotype, no significant change was observed in the sensitive genotype after pathogen inoculation. Awan *et al.* (2018) classified different tomato cultivars based on *A. solani* resistance levels. They acknowledged that although carotenoid content decreased in susceptible genotypes, no remarkable changes were observed in the resistant group. Also, investigating tomato-*A. alternata* interactions showed that carotenoid content decreased at 96 hours after pathogen inoculation (Tiwari and Upadhyay, 2016). In addition, it was observed in Agamy *et al.* (2013) study that *A. tenuissima* causes the reduction of carotenoids in tomato leaves. The reduction of carotenoids in tomato leaves under *Alternaria* stress may be due to the release of fungal toxins that induce oxidative stress and damage the plant (Howlett, 2006). However, no significant change of carotenoids in resistant genotypes may be due to insignificant effects of oxidative stress. In contrast, in susceptible genotypes, the reduction of carotenoids indicates the destruction of the plant tissue in the face of the pathogen.

The destruction of plant cells by the pathogen appears as specific symptoms, including wilting, growth suppression, chlorosis, necrosis, and

spotting. In the present study, the pattern of the chlorophyll contents response was almost the same in both cases and significantly decreased after the pathogen inoculation (Fig. 1C, and D). Similar results were observed in several studies (Attia *et al.*, 2017; Meena *et al.*, 2016). Attia *et al.* (2017) revealed that after *A. solani* infection, the chlorophyll contents significantly decreased. In another study, Meena *et al.* (2016) showed that chlorophyll contents decreased in plants inoculated with *A. alternata* by 85.8% compared with control plants. Following the pathogen infection, the rate of ROS (reactive oxygen species) increases on the surface of plant leaves, and this oxidative stress can damage the chlorophyll contents (Ali *et al.*, 2006; Kyseláková *et al.*, 2011). In total, it seems that, although *A. solani* could damage the leaf tissue of resistant tomatoes, it could not cause widespread destruction, unlike the susceptible genotype.

Chlorophyll a fluorescence

In this study the level of Fv and Fv/Fm decreased in susceptible genotype after pathogen inoculation. Variable fluorescence (Fv) indicates the state of electron flow from the photosystem to QA (Baker and Rosenqvist, 2004), and a reduction of this parameter indicates a remarkable decrease in electrons flow rate (Ramezani *et al.*, 2017; Zhou, 1999). However, The Fv/Fm ratio indicates the maximum photochemical quantum efficiency of PSII and can be used to estimate the amount of plant infection by pathogens (Hou *et al.*, 2020). Similar to the reaction of the sensitive genotype in this research, a decrease in Fv/Fm has been reported in banana and corn plants infected with *Fusarium* (Kuckenberg *et al.*, 2007) and also in melon plants infected with *Dickeya dadantii* (Pineda *et al.*, 2018). Also, Moradi *et al.* (2018) observed that Fv and Fv/Fm parameters in susceptible cucumber genotypes significantly decreased after pathogen (*Podospaera* sp.) inoculation. Similar results were obtained in Ramezani *et al.* (2017) study. They stated that stress conditions such as pathogen infection and inducer treatment could cause a decrease in Fv and Fv/Fm through damage to the complex of photosystem II.

Also, in the present study, Y(NPQ) and NPQ enhanced in resistant genotype after pathogen inoculation. Plants have developed numerous photoprotection mechanisms to mitigate the harmful effects of reactive oxygen species (ROS) accumulation, such as producing various antioxidants, the hypersensitive response at the infected site, and NPQ operation (Xing *et al.*, 2013). NPQ systems can dissipate extra energy captured by Light-harvesting complex II (Liu *et al.*, 2012) and protect plant photosystems. Increasing NPQ value indicates the initiation of photoprotection mechanisms related to the xanthophyll cycle and the formation of ΔpH through the thylakoid membranes (Zhang *et al.*, 2014). Instead, the reduction of NPQ can imply the breakdown of light protection systems under stressful conditions, which can cause significant damage to photo complexes and plant photosynthetic systems. Several studies have reported a significant increase in NPQ under biotic stress (Rajendran *et al.*, 2016; Rodríguez-Moreno *et al.*, 2008; Zou *et al.*, 2005).

On other hand Y(NPQ) and NPQ were significantly decreased in the susceptible genotype. García-Villaraco *et al.* (2021) observed that NPQ value of tomato plant declined after inoculation with *Pseudomonas fluorescens*. Also, Bonfig *et al.* (2006) reported that NPQ of Arabidopsis plant decreased when infected with *P. syringae* pv. *tomato*. They attributed this reduction to the hypersensitivity defense reaction that prevents the penetration and spread of the pathogen in the plant tissue by the death of cells around the infection site. It should be considered that a hypersensitive reaction is suitable for biotrophic pathogens response, while in the face to necrotrophic pathogens like *A. solani*, it causes the expansion and spread of the disease.

Molecular analysis

Previous studies about the genetic concept of plant-pathogen interactions illustrated that transcription factors play a remarkable role in resistance to early blight (Moghaddam *et al.*, 2019; Rabiei *et al.*, 2022; Shinde *et al.*, 2018). In the present study, *SIWRKY1* was up-regulated in both resistant and susceptible

genotypes after pathogen inoculation. Shinde *et al.* (2018) showed that over-expression of *SIWRKY1* significantly enhanced the resistance level to *A. Solani* penetration while early blight disease was very aggressive in RNAi lines. Also, a molecular study of some transcription factors and PR genes in 35 different tomato genotypes under early blight stress demonstrated that in resistant genotypes, there is a positive correlation between *SIWRKY1* expression and the two most important defense genes, *PR7* and *PDF1.2* (Moghaddam *et al.*, 2019). However, Saleh *et al.* (2015) state that *WRKY1* plays a critical role in regulating the SA signalling pathway and controls this related plant defense system through the cytosolic form of *NPR1*.

Abiotic stress such as high salinity, drought, and pathogens infection could induce *NAC1* gene expression (Wu *et al.*, 2009). In the case of biotic stress, this transcription factor plays a dual role. Some reports mentioned the role of *NAC1* (*ATAF1*) in increasing resistance to pathogens (Wang *et al.*, 2009), while some other studies have discussed the role of this gene in causing sensitivity (He *et al.*, 2016; Wang *et al.*, 2015). Our results showed that in the resistant genotype, *SINAC1* expression was remarkably up-regulated only at early steps of pathogen penetration (12hpi). While in the susceptible genotype, expression of *NAC1* gene strongly increased at 12, 24, and 96 hpi. Wu *et al.* (2009) observed the negative function of *NAC1* (*ATAF1*) in necrotrophic disease processes. Also, the over-expression of *ATAF1* increased the generation of reactive oxygen species (Wu *et al.*, 2009). Considering the prominent effect of ROS in the development of necrotrophic disease, it can be concluded that *NAC1* up-regulation may sensitize the reaction of plant cells to ROS accumulation and, consequently, help to increase plant sensitivity after infection with necrotrophic pathogens.

Conclusion

Molecular and physiological investigation of plants response to pathogen infection leads to a

deep understanding of plant-pathogen interactions. Our research shows that susceptible and resistant genotypes react differently to *A. solani* infection. The reaction of the resistant genotype against the pathogen was noteworthy in terms of soluble sugar accumulation, photosynthetic efficiency, and *SIWRKY1* gene expression. Soluble sugar accumulation was the main key to the physiological reaction, which is dominant in a different pathway. The soluble sugar is an energy source for plant growth development and metabolic reactions; on the other hand, it plays the role of a signalling molecule during plant-pathogen interactions. Also, maintaining photosynthetic efficiency while battling the pathogen infection is another influential physiological point in resistance to early blight disease. In addition, up-regulation of *SIWRKY1* gene at the primary steps can improve the resistance level during tomato- *A. solani* interactions.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Statements and Declarations

The authors declare that they have no competing interests.

Additional information

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References

Adhikari, P., Oh, Y. and Panthee, D. R. 2017. Current status of early blight resistance in tomato: an update. International Journal of

Molecular Sciences, 18(10): 2019. Available from: doi10.3390/ijms18102019.

Agamy, R., Alamri, S., Moustafa, M. F. M. and Hashem, M. 2013. Management of tomato leaf spot caused by *Alternaria tenuissima* Wiltshire using salicylic acid and Agrileen. International Journal of Agriculture & Biology, 15(2): 1560-8530.

Akhtar, K. P., Ullah, N., Saleem, M. Y., Iqbal, Q., Asghar, M. and Khan, A. R. 2019. Evaluation of tomato genotypes for early blight disease resistance caused by *Alternaria solani* in Pakistan. Journal of Plant Pathology, 101(4): 1159-1170. Available from: doi: 10.1007/s42161-019-00304-8.

Ali, S. H., Eisa, S. S. and El-Dougdoug, K. 2006. Role of reactive oxygen species and antioxidants in hypersensitive local virus-infected plants. Journal of Soil Sciences and Agricultural Engineering, 31(11): 7465-7480.

Attia, M. S., Sharaf, A. and Zayed, A. S. 2017. Protective action of some bio-pesticides against early blight disease caused by *Alternaria solani* in tomato plant. International Journal of Innovative Science, Engineering & Technology, 4(11): 67-94.

Awan, Z. A., Shoaib, A. and Khan, K. A. 2018. Variations in total phenolics and antioxidant enzymes cause phenotypic variability and differential resistant response in tomato genotypes against early blight disease. Scientia Horticulturae, 239: 216-223. Available from: doi: 10.1016/j.scienta.2018.05.044.

Baker, N. R. and Rosenqvist, E. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. Journal of Experimental Botany, 55(403): 1607-1621. Available from: doi:10.1093/jxb/erh196.

Bhale, U. N., Kamble, S. S. and Gangawane, L. V. 2010. Biochemical changes in spinach infected with carbendazim resistant *Alternaria spinaciae*. Indian Phytopath, 63 (2): 230-231.

Bonfig, K. B., Schreiber, U., Gabler, A., Roitsch, T. and Berger, S. 2006. Infection with virulent and avirulent *P. syringae* strains

- differentially affects photosynthesis and sink metabolism in Arabidopsis leaves. *Planta*, 225: 1-12. Available from: doi:10.1007/s00425-006-0303-3.
- Clark, M. M. 2022. Diseases diagnosed on commercial crop samples submitted to the pei analytical laboratories plant disease diagnostic service (pdds) in 2021. *Canadian Journal of Plant Pathology*, 102: 55-58.
- Formela-Luboińska, M., Chadzinikolau, T., Drzewiecka, K., Jeleń, H., Bocianowski, J., Kęsy, J., Labudda, M., Jeandet, P. and Morkunas, I. 2020. The role of sugars in the regulation of the level of endogenous signaling molecules during defense response of yellow lupine to *Fusarium oxysporum*. *International Journal of Molecular Sciences*, 21(11): 4133. Available from: doi: 10.3390/ijms21114133.
- García-Villaraco, A., Boukema, L., Lucas, J. A., Gutierrez-Mañero, F. J. and Ramos-Solano, B. 2021. Tomato Bio-Protection Induced by *Pseudomonas fluorescens* N21. 4 Involves ROS Scavenging Enzymes and PRs, without Compromising Plant Growth. *Plants*, 10(2): 331. Available from: 10.3390/plants10020331.
- Garg, S., Kumhar, D. R., Verma, R. K. and Chaudhary, K. 2020. Evaluation of biochemical changes in infected and non-infected plants of tomato with *Alternaria solani*. *International Journal of Chemical Studies*, 8(1): 1232-1235. Available from: doi: 10.22271/chemi.2020.v8.i1q.8419.
- Genty, B., Briantais, J.-M. and Baker, N. R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 990(1): 87-92. Available from: doi: 10.1016/S0304-4165(89)80016-9.
- Ghorbanpour, A., Salimi, A., Ghanbary, M. A. T., Pirdashti, H. and Dehestani, A. 2018. The effect of *Trichoderma harzianum* in mitigating low temperature stress in tomato (*Solanum lycopersicum* L.) plants. *Scientia Horticulturae*, 230: 134-141. Available from: doi:10.1016/j.scienta.2017.11.028.
- Gong, C., Liu, Y., Liu, S., Cheng, M., Zhang, Y., Wang, R., Chen, H., Li, J., Chen, X. and Wang, A. 2017. Analysis of *Clonostachys rosea*-induced resistance to grey mould disease and identification of the key proteins induced in tomato fruit. *Postharvest Biology and Technology*, 123: 83-93. Available from: doi: 10.1016/j.postharvbio.2016.08.004.
- He, X., Zhu, L., Xu, L., Guo, W. and Zhang, X. 2016. GhATAF1, a NAC transcription factor, confers abiotic and biotic stress responses by regulating phytohormonal signaling networks. *Plant Cell Reports*, 35(10): 2167-2179. Available from: doi: 10.1007/s00299-016-2027-6.
- Henriquez, M. A., Adam, L. R. and Daayf, F. 2012. Alteration of secondary metabolites' profiles in potato leaves in response to weakly and highly aggressive isolates of *Phytophthora infestans*. *Plant Physiology & Biochemistry*, 57: 8-14. Available from: doi: 10.1016/j.plaphy.2012.04.013.
- Herbers, K., Meuwly, P., Métraux, J.-P. and Sonnewald, U. 1996. Salicylic acid-independent induction of pathogenesis-related protein transcripts by sugars is dependent on leaf developmental stage. *Febs Letters*, 397(2-3): 239-244. Available from: doi: 10.1016/S0014-5793(96)01183-0.
- Hoagland, D. R. and Arnon, D. I. 1950. The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station*, 347, 32.
- Hou, R., Shi, J., Ma, X., Wei, H., Hu, J., Tsang, Y. F. and Gao, M.-T. 2020. Effect of phenolic acids derived from rice straw on *Botrytis cinerea* and infection on tomato. *Waste and Biomass Valorization*, 11(12): 6555-6563. Available from: doi: 10.1007/s12649-020-00938-1.
- Howlett, B. 2006. Secondary metabolite toxins and nutrition of plant pathogenic fungi. *Current Opinion in Plant Biology*, 9: 371-375. Available from: doi: 10.1016/j.pbi.2006.05.004.
- Issa, A., Esmaeel, Q., Sanchez, L., Courteaux, B., Guise, J.-F., Gibon, Y., Ballias, P., Clément, C., Jacquard, C. and Vaillant-Gaveau, N. 2018. Impacts of *Paraburkholderia*

- phytofirmans* strain PsJN on tomato (*Lycopersicon esculentum* L.) under high temperature. *Frontiers in Plant Science*, 9: 1397. Available from: doi:10.3389/fpls.2018.01397.
- Kasote, D. M., Katyare, S. S., Hegde, M. V and Bae, H. 2015. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences*, 11(8): 982. Available from: doi: 10.7150/ijbs.12096.
- Kuckenberger, J., Tartachnyk, I., Schmitz-Eiberger, M. and Noga, G. 2007. Early detection of leaf rust and powdery mildew infections on wheat leaves by PAM fluorescence. *Precision Agriculture*, 8: 515-521.
- Kyseláková, H., Prokopová, J., Nauš, J., Novák, O., Navrátil, M., Šafářová, D., Špundová, M. and Ilík, P. 2011. Photosynthetic alterations of pea leaves infected systemically by pea enation mosaic virus: a coordinated decrease in efficiencies of CO₂ assimilation and photosystem II photochemistry. *Plant Physiology and Biochemistry*, 49(11): 1279-1289. Available from: doi: 10.1016/j.plaphy.2011.08.006.
- Li, R., Sheng, J. and Shen, L. 2020. Nitric oxide plays an important role in β -aminobutyric acid-induced resistance to *botrytis cinerea* in tomato plants. *The Plant Pathology Journal*, 36(2): 121. Available from: doi: 10.3390/ijms18102019.
- Lichtenthaler, H. K. and Buschmann, C. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food Analytical Chemistry*, 1(1): 3-4. Available from: doi: 10.1002/0471142913.faf0403s01.
- Liu, Y. F., Qi, M. F., Li, T. L. 2012. Photosynthesis, photoinhibition, and antioxidant system in tomato leaves stressed by low night temperature and their subsequent recovery. *Plant Science*, 196: 8-17. Available from: doi: 10.1016/j.plantsci.2012.07.005.
- Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative pcr and the 2^{- $\Delta\Delta C_T$} method. *Methods*, 25(4): 402-408.
- McCready, R. M., Guggolz, J., Silveira, V. and Owens, H. S. 1950. Determination of starch and amylose in vegetables. *Analytical Chemistry*, 22(9): 1156-1158.
- Meena, M., Prasad, V. and Upadhyay, R. S. 2017. Evaluation of biochemical changes in leaves of tomato infected with *Alternaria alternata* and its metabolites. *Vegetos*, 30(2): 1-5.
- Meena, M., Zehra, A., Dubey, M. K., Aamir, M., Gupta, V. K. and Upadhyay, R. S. 2016. Comparative Evaluation of Biochemical Changes in Tomato (*Lycopersicon esculentum* Mill.) Infected by *Alternaria alternata* and Its Toxic Metabolites (TeA, AOH, and AME). *Frontiers in Plant Science*, 7: 1408. Available from: doi: 10.3389/fpls.2016.01408.
- Moghaddam, G. A., Rezayatmand, Z., Esfahani, M. N. and Khozaei, M. 2019. Genetic defense analysis of tomatoes in response to early blight disease, *Alternaria alternata*. *Plant Physiology and Biochemistry*, 142: 500-509.
- Moradi, N., Rahimian, H., Dehestan, A., Babaeizad, V. and Yaghoubian, Y. 2018. Screening of cucumber cultivars resistant to powdery mildew and comparative assessment of chlorophyll florescence in resistant and sensitive cultivars. *Iranian Journal of Plant Protection*, 37(3): 466-474.
- Morkunas, I. and Ratajczak, L. 2014. The role of sugar signaling in plant defense responses against fungal pathogens. *Acta Physiologiae Plantarum*, 36(7): 1607-1619.
- Nafisa, Shoaib, A., Iqbal, J. and Khan, K. A. 2020. Evaluation of phenotypic, physiological and biochemical attributes connected with resistance in tomato against *Alternaria solani*. *Acta Physiologiae Plantarum*, 42(5): 88. Available from: doi: 10.1007/s11738-020-03076-2.
- Parveen, A. and Siddiqui, Z. A. 2022. Impact of silicon dioxide nanoparticles on growth, photosynthetic pigments, proline, activities of defense enzymes and some bacterial and fungal pathogens of tomato. *Vegetos*, 35(1): 83-93.
- Pineda, M., Pérez-Bueno, M. L. and Barón, M. 2018. Detection of bacterial infection in

- melon plants by classification methods based on imaging data. *Frontiers in Plant Science*, 9: 164. Available from: doi: 10.3389/fpls.2018.00164. eCollection 2018.
- Rabiei, Z., Hosseini, S., Dehestani, A., Pirdashti, H. and Beiki, F. 2022. Exogenous hexanoic acid induced primary defense responses in tomato (*Solanum lycopersicum* L.) plants infected with *Alternaria solani*. *Scientia Horticulturae*, 295: 110841. Available from: doi: 10.1016/j.scienta.2021.110841.
- Rajendran, D. K., Park, E., Nagendran, R., Hung, N. B., Cho, B.-K., Kim, K.-H. and Lee, Y. H. 2016. Visual analysis for detection and quantification of *Pseudomonas cichorii* disease severity in tomato plants. *The Plant Pathology Journal*, 32(4): 300-310.
- Ramezani, M., Karimi Abdolmaleki, M., Shabani, S. and Dehestani, A. 2017. The role of potassium phosphite in chlorophyll fluorescence and photosynthetic parameters of downy mildew-challenged cucumber *Cucumis sativus* plants. *Archives of Phytopathology and Plant Protection*, 50(17-18): 927-940.
- Ray, S., Mondal, S., Chowdhury, S. and Kundu, S. 2015. Differential responses of resistant and susceptible tomato varieties to inoculation with *Alternaria solani*. *Physiological and Molecular Plant Pathology*, 90: 78-88. Available from: doi: 10.1016/j.pmpp.2015.04.002.
- Rodríguez-Moreno, L., Pineda, M., Soukupová, J., Macho, A. P., Beuzón, C. R., Barón, M. and Ramos, C. 2008. Early detection of bean infection by *Pseudomonas syringae* in asymptomatic leaf areas using chlorophyll fluorescence imaging. *Photosynthesis Research*, 96(1): 27-35.
- Saleh, A., Withers, J., Mohan, R., Marqués, J., Gu, Y., Yan, S., Zavaliev, R., Nomoto, M., Tada, Y. and Dong, X. 2015. Posttranslational modifications of the master transcriptional regulator *NPR1* enable dynamic but tight control of plant immune responses. *Cell Host & Microbe*, 18(2): 169-182.
- Shao, D., Smith, D. L., Kabbage, M. and Roth, M. G. 2021. Effectors of plant necrotrophic fungi. *Frontiers in Plant Science*, 12: 995. Available from: doi: 10.3389/fpls.2021.687713.
- Shinde, B. A., Dholakia, B. B., Hussain, K., Aharoni, A., Giri, A. P. and Kamble, A. C. 2018. *WRKY1* acts as a key component improving resistance against *Alternaria solani* in wild tomato, *Solanum arcanum* Peralta. *Plant Biotechnology Journal*, 16(8): 1502-1513.
- Suárez, J. C., Vanegas, J. I., Contreras, A. T., Anzola, J. A., Urban, M. O., Beebe, S. E. and Rao, I. M. 2022. Chlorophyll fluorescence imaging as a tool for evaluating disease resistance of common bean lines in the western amazon region of Colombia. *Plants*, 11(10): 1371. Available from: doi: 10.3390/plants11101371.
- Tiwari, A. and Upadhyay, R. S. 2016. *Alternaria alternata* infection in tomato adversely affects the nutritional parameters of tomato. *Technology*, 12(6): 1083-1098.
- Trouvelot, S., Héloir, M.-C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., Combier, M., Trdá, L., Daire, X. and Adrian, M. 2014. Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. *Frontiers in Plant Science*, 5: 592. Available from: doi: 10.3389/fpls.2014.00592.
- Wang, F., Lin, R., Feng, J., Chen, W., Qiu, D. and Xu, S. 2015. *TaNAC1* acts as a negative regulator of stripe rust resistance in wheat, enhances susceptibility to *Pseudomonas syringae*, and promotes lateral root development in transgenic *Arabidopsis thaliana*. *Frontiers in Plant Science*, 6: 108. Available from: doi: 10.3389/fpls.2015.00108. eCollection 2015.
- Wang, X., Basnayake, B. M. V. S., Zhang, H., Li, G., Li, W., Virk, N., Mengiste, T. and Song, F. 2009. The *Arabidopsis ATAF1*, a NAC transcription factor, is a negative regulator of defense responses against necrotrophic fungal and bacterial pathogens. *Molecular Plant-Microbe Interactions*, 22(10): 1227-1238.
- Wu, Y., Deng, Z., Lai, J., Zhang, Y., Yang, C., Yin, B., Zhao, Q., Zhang, L., Li, Y., and

- Yang, C. 2009. Dual function of Arabidopsis *ATAF1* in abiotic and biotic stress responses. *Cell Research*, 19(11): 1279-1290.
- Xing, F., Li, Z., Sun, A. and Xing, D. 2013. Reactive oxygen species promote chloroplast dysfunction and salicylic acid accumulation in fumonisin B1-induced cell death. *FEBS Letters*, 587(14): 2164-2172.
- Yao, Z., Rashid, K. Y., Adam, L. R. and Daayf, F. 2011. *Verticillium dahliae* 's VdNEP acts both as a plant defence elicitor and a pathogenicity factor in the interaction with *Helianthus annuus*. *Canadian Journal of Plant Pathology*, 33(3): 375-388.
- Zhang, G., Liu, Y., Ni, Y., Meng, Z., Lu, T. and Li, T. 2014. Exogenous calcium alleviates low night temperature stress on the photosynthetic apparatus of tomato leaves. *PLoS One*, 9(5): e97322. Available from: doi: 10.3389/fpls.2020.607029.
- Zhou, J.-M. 1999. Signal transduction and pathogen-induced *PR* gene expression : Pathogenesis-Related Proteins in Plants. CRC Press, 195-205.
- Zou, J., Rodriguez-Zas, S., Aldea, M., Li, M., Zhu, J., Gonzalez, D. O., Vodkin, L. O., DeLucia, E. and Clough, S. J. 2005. Expression profiling soybean response to *Pseudomonas syringae* reveals new defense-related genes and rapid HR-specific downregulation of photosynthesis. *Molecular Plant-Microbe Interactions*, 18(11): 1161-1174.

پاسخ‌های متفاوت فیزیولوژیکی و مولکولی ژنوتیپ‌های حساس و مقاوم گوجه‌فرنگی به بیماری لکه‌موجی

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چکیده: بیماری لکه‌موجی ناشی از *Alternaria solani* یکی از بیماری‌های مهم گوجه‌فرنگی *Solanum lycopersicum* می‌باشد که بخش قابل‌توجهی از تولید گوجه‌فرنگی را در سراسر جهان از بین می‌برد. کشت ارقام مقاوم به‌طور قابل‌توجهی سبب کاهش آسیب ناشی از بیماری لکه‌موجی می‌شود. بنابراین، درک پاسخ ژنتیکی به نفوذ بیمارگر می‌تواند سبب افزایش شناخت ما از مکانیسم‌های دخیل در سیستم دفاعی گیاه شود. در مطالعه حاضر، پاسخ افتراقی ژنوتیپ‌های حساس و مقاوم گوجه‌فرنگی به *A. solani* از جنبه‌های مولکولی و فیزیولوژیکی مورد بررسی قرار گرفت. نتایج نشان داد که میزان قند محلول در ژنوتیپ مقاوم پس از حمله پاتوژن افزایش یافته است. اگرچه میزان رنگدانه‌های فتوسنتزی مانند کاروتنوئید، کلروفیل a و کلروفیل b در هر دو ژنوتیپ حساس و مقاوم کاهش یافت، اما تغییرات شاخص‌های مرتبط با فلورسانس کلروفیل a پس از حمله پاتوژن در ژنوتیپ‌های مقاوم و حساس متفاوت برآورد شد. همچنین، تجزیه و تحلیل مولکولی نشان داد که بیان ژن *SIWRKY1* در رقم مقاوم، ۴۸ ساعت پس از مایه‌زنی به مقدار ۲/۵۸ برابر شاهد افزایش یافت. همچنین بیان ژن *SINAC1* در ژنوتیپ حساس ۲۴ ساعت پس از مایه‌زنی به مقدار ۶۹/۱۲ برابر شاهد افزایش یافت. یافته‌های این تحقیق می‌تواند سبب شناخت بهتری از مکانیسم‌های دفاعی گیاه گوجه‌فرنگی در برابر بیماری لکه‌موجی شود.

واژگان کلیدی: بیماری لکه‌موجی، فلورسانس کلروفیل a، *SIWRKY1*، *SINAC1*، qRT-PCR