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Full Length Article

Identification of Salicylic Acid Conferred Resistance Genes against Gray Leaf Spot Disease in Tomato

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Abstract

Tomato gray leaf spot is a main disease in tomato production. Induced resistance to pathogen infection is a widely accepted environmentally friendly strategy. In this study, we aimed to discover the key disease resistance genes induced by exogenous applications of the hormone salicylic acid (SA). Our results showed that, under treatment with 0.2 m*M* SA, strong resistance to gray leaf spot was induced in the leaves of tomato seedlings, and a significant reduction in the number of lesions and in disease index accompanied the application of SA. The content of active oxygen and enzyme activities increased with SA treatment, including those of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD). In addition, RT-PCR results showed that the expression levels of a series of defense genes, such as members of the WRKY65, pathogenesis-related (PR), MAPK and abscisic acid (ABA) receptor PYR/PYL families, significantly increased in response to SA treatment. The results indicated that applications of the exogenous hormone SA can enhance the resistance to *S. lycopersici* in the susceptible cultivar "Moneymaker" by regulating the expression of genes related to disease resistance.

Keywords: Tomato; *S. lycopersici*; Induced resistance; Enzyme activities; PR genes

Introduction

Plant diseases are a serious threat to modern agriculture, and it has been confirmed that *S. lycopersici* causes tomato gray leaf spot disease (Graham and Zeiders 1960). Currently, the disease has been detected in pepper (Cho *et al.* 2001), cotton (Francovig *et al*. 1999) and spinach (Koike *et al*. 2001) crops and tomato gray leaf spot has been reported in many countries, including the United States (Hendrix and Frazier 1949) and Korea (Min *et al.* 1995; Kim *et al.* 1999).

At present, the most common solution to gray leaf spot is the use of fungicides (Becker *et al*. 2000). However, this measure poses a serious threat to the natural environment and human health. Elicitors are available for activating defense mechanisms that respond to the invasion of pathogens, and the use of these elicitors is considered a more environmentally friendly method for disease resistance than are chemical methods (Yu *et al*. 2014). Hormones such as ethylene (ET), abscisic acid (ABA), indole-3-acetic acid (IAA), jasmonic acid (JA) and salicylic acid (SA) are important signaling molecules in plants. A study showed that IAA was the main molecule affecting parthenocarpy and that application of IAA to unpollinated plants induced parthenocarpy (Takisawa *et al*. 2019). Jasmonates play an important role in regulating the signal transduction process. Moreover, a previous study indicated that SA is involved in plant-pathogen interactions (Loon 2000). Pretreatment with SA could increase the activity of alternative oxidase in plants (Rhoads and McIntosh 1992). SA is a key regulatory factor in the activation of certain plant defense responses, including the expression of pathogenesis-related (PR) genes (Gaffney *et al*. 1993; Klessig and Malamy 1994). It was recently reported that an increase in SA was associated with the induction of several defense responses in tomato (Hammond-Kosack *et al*. 1993; Shang *et al.* 2019).

When applied exogenously, the hormone SA affects the regulation of various physiological and biochemical reactions in plants. SA is an important endogenous signaling molecule that plays an important role in signal transduction systems, inducing enzymes to catalyze various biochemical reactions and playing a crucial role in systemic acquired resistance (SAR) (Loon and Antoniw 1982). Studies have shown that the activities of phenylalanine ammonia-lyase and polyphenol oxidase increased with SA treatment. These are important ingredients involved in phenolic compounds, protecting plant tissues by increasing cell wall thickness (Mandal *et al.* 2009). The exogenous hormone SA can induce the activities of phenylalanine ammonia-lyase and polyphenol

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oxidase to increase the resistance in crops (Chen *et al*. 2006).

This study was mainly to explore the effect of salicylic acid in resistance mechanisms of tomato. Additionally, this study identified the key disease resistance genes induced by salicylic acid, providing a theoretical basis for the study of the molecular mechanism of pathogens that cause gray leaf spot. Experiments were carried out to investigate the expression levels of resistance-related genes and the changes in the activities of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in tomato leaves.

Materials and Methods

Plant material

Plant material used in this study was the susceptible cultivar "Moneymaker". The tomato seedlings were grown in sterile soil under 60% relative humidity and 22 to 24°C temperature regimen.

SA Treatment and pathogen inoculation

An aqueous solution of SA (200 μM) in distilled water was uniformly sprayed on the surface of the leaves of tomato seedlings. The control seedlings were treated with the same volume of distilled water.

Isolated and purified *S. lycopersici* inocula were cultured in a Petri dish that contained PDA and incubated at 28°C for 7 to 10 days under a typical photoperiod. The conidial suspension (1 x 10^4 cells/mL) was for inoculation. All treated seedlings were grown in a light incubator (temperature: 28°C, relative humidity: greater than 85%) (Sun *et al*. 2016). Leaf samples were collected at 0, 12, 24, 48 and 72 h post inoculation.

Sample treatment for enzyme activity and gene expression analyses

We carried out the experiment in a greenhouse from November to December 2018. This test contained forty uniformly sized "Moneymaker" tomato seedlings. Twenty strains were treated with 0.2 m*M* SA, ten strains were inoculated with a conidial suspension of 1×10^4 conidia/mL and the rest were treated with distilled water. Three days after SA treatment, half of the twenty seedlings were inoculated with *S. lycopersici*. The treatments were as follows: controlled, sprayed with distilled water; GLS, tomato seedlings inoculated with *S. lycopersici*; SA, seedlings treated with 0.2 m*M* SA; and SA + GLS, seedlings treated with SA followed by *S. lycopersici* inoculation. Leaves were harvested from seedlings at different intervals (0, 12, 24, 48 and 72 h), immediately frozen in liquid nitrogen and stored at -80°C until used for enzyme assays and gene expression analysis. There were three biological replicates per sample.

Assays of enzyme activity in "moneymaker" tomato leaves

The leaf tissue and liquid nitrogen were first mixed together in a mortar to cool and grind the tissue and then a corresponding reagent (from a kit) for extracting the crude enzyme solution or physiological saline was added to the leaf tissue at a ratio of 1:9. The tissue homogenate was centrifuged at 12000 x g for 20 min at 4°C, after which the supernatant was removed as the crude enzyme extract for enzyme activity measurements.

In this test, Assay Kits (Nanjing Institute of Bioengineering, China) were used to determine the main enzyme activities following the manufacturer's instructions. According to the measurement principle of CAT, the reaction by which CAT breaks down hydrogen peroxide (H_2O_2) was quickly terminated by the addition of ammonium molybdate, and the remaining H_2O_2 reacted with ammonium molybdate to form a pale yellow complex. The changes were measured at 405 nm to calculate the CAT activity. The unit of enzyme activity was defined as units per milligram of protein (1×10^{-3} mol H_2O_2/minute/g fresh weight). The method by which H_2O_2 is catabolized with POD was used and the changes in absorbance at 420 nm were for the activities of POD. The amount of enzyme used to catalyze one microgram of substrate per minute per milligram of tissue protein was defined as one enzyme activity unit at 37°C (U/mg protein). SOD scavenges superoxide anion radicals ($O_2{\cdot}^-$) to protect cells from damage, and its activity was defined as the amount of SOD corresponding to one unit of SOD per gram of tissue in a 1 mL reaction solution whose SOD inhibition rate was 50%.

PAL catalyzes the cleavage of L-phenylalanine to trans-cinnamic acid, which has a maximum absorption at 290 nm and ammonia. The PAL activity was estimated by measuring the amount of change in absorbance and was defined as the absorbance value at 290 nm that varied by 0.1 to one enzyme activity unit per gram of tissue per minute in each milliliter of the reaction system. Polyphenol oxidase (PPO) is capable of catalyzing the production of ruthenium from phenol, which has a characteristic light absorption at 420 nm.

Microscopy

To further investigate the changes in the hypersensitive response (HR) and resistance of seedlings inoculated with *S. lycopersici* after treatment with SA and treatment with *S. lycopersici*, the leaves were sampled from seedlings with different treatments at 3 days post inoculation (dpi) and fixed in Farmer solution (Zhao *et al*. 2018) for 12 h. The samples were then stained with trypan blue (Colon *et al*. 1992; Zhao *et al*. 2016) for microscopic observations.

Staining of H_2O_2 and $O_2{\cdot}^-$

As plants encounter stress, their defense system enables cells

Table 1: Primers used in the quantitative real-time PCR analysis of gene expression followed by a number of different cycles

Gene Name	Forward Primer Sequences (5′-3′)	Reverse Primer Sequences (5′-3′)	Expected size (bp)
Solyc02g072190.2.1	CAAGTTGAAAGGAGCCGTGC	GATCGGCGAAAAGTGACACG	484
Solyc04g079420.2.1	ATCTGAAAAGGCTTCCCCCG	ACACAAAAGAAGCCCAACGC	348
Solyc07g053170.2.1	TCCTACATTTGACGGACGGC	AAGATTCGGCGCGTTTATGC	243
Solyc10g085310.1.1	AGCTCCTGTCTCCACCGTAT	GACCGGAAATCACACGGACT	140
Solyc03g114210.2.1	GGCCAGAGGGTTGAGTTACC	TTTGTCAGGGTTAGCGTCCC	403

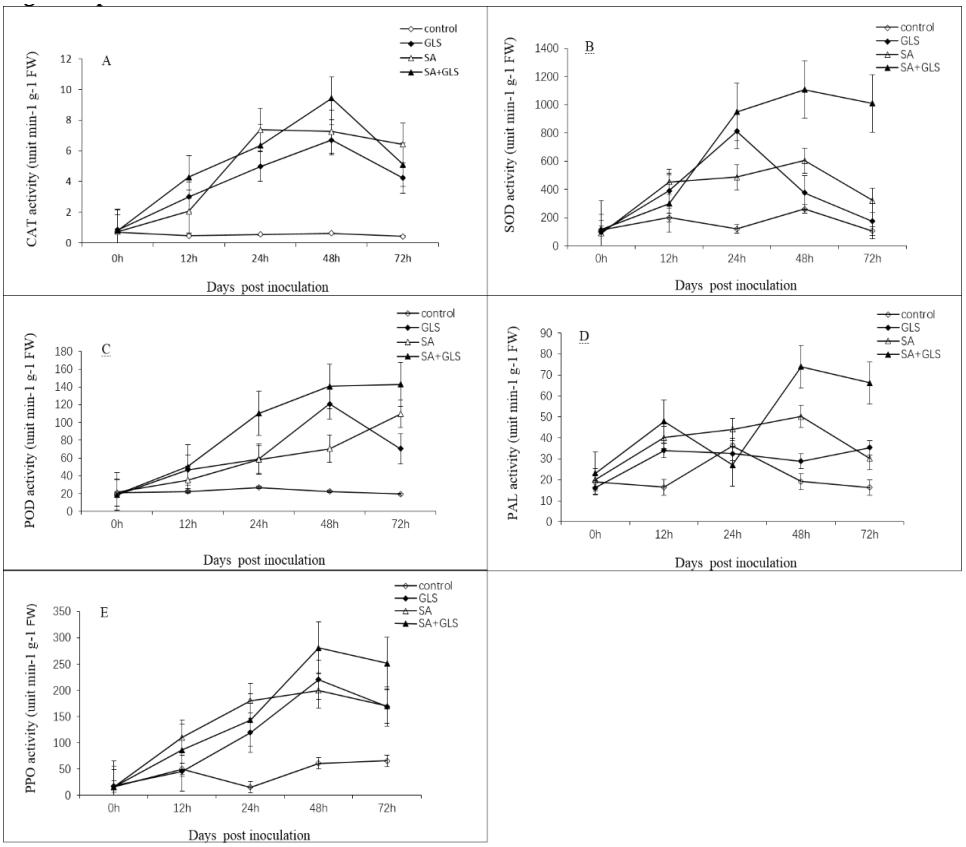

Fig. 1: Effect of salicylic acid (SA) on antioxidant enzyme activities in "Moneymaker" tomato leaves after inoculation with *S. lycopersici*. SA (0.2 m*M*) or distilled water was sprayed on the tomato leaves. After 2 dpi of the treatment, with the exception of the controls, the leaves were inoculated with an *S. lycopersici* conidial suspension at 1 x 10^4 cells/mL. After the various intervals, leaf tissues were collected to determine the activities of catalase (CAT; **A**), superoxide dismutase (SOD; **B**), peroxidase (POD; **C**), phenylalanine ammonia-lyase (PAL; **D**) and polyphenol oxidase (PPO; **E**). The data represent the means of two separate experiments with six replicates and the error bars represent the standard errors

to continuously produce reactive oxygen species (ROS), which are a product of aerobic metabolism in plants. The accumulation of ROS severely damages plant cell tissues and even leads to cell death. Our experiment used tissue chemistry to detect two important ROS: H_2O_2 and $O_2\cdot^-$ anions. 3,3'-Diaminobenzidine (DAB) and nitrotetrazolium blue chloride (NBT) were used as chromogenic substrates in tomato. Under the catalysis of CAT, DAB is oxidized by H_2O_2 to produce a reddish brown precipitate in plant tissues. $O_2\cdot^-$ reacts with NBT to form a deep blue insoluble compound (Klessig and Malamy 1994). In the present study, DAB (1 mg/mL, pH 3.8) or NBT (0.2%) was used for leaf staining. On the basis of this coloration principle, the accumulation of H_2O_2 and $O_2\cdot^-$ in tomato leaves could be examined.

Analysis of Gene Expression by RT-qPCR

Reverse transcription of RNA into cDNA was performed according to the manual of a reverse transcription kit (Thermo). The obtained cDNA was used as a template for RT-qPCR. Fluorescence RT-qPCR first required an initial denaturation of 5 min at 94°C. Each cycle then consisted of denaturing at 94°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 30 sec and there was a final extension at 72°C for 7 min. Information concerning the primer sequences used in RT-qPCR is shown in Table 1. Three biological replicates were evaluated for the RT-qPCR experiments for each set of genes.

Results

In vitro antifungal activity assay of SA against gray leaf spot

The SA solution added to the PDA medium did not significantly affect the growth of the mycelia. Five SA concentrations (50 μM, 80 μM, 150 μM, 250 μM and 400 μM) were also tested and no inhibition in the growth of *S. lycopersici* was observed on the plates containing SA compared with the control plates.

Effects of exogenous SA on CAT, POD and SOD activities

Enzyme activities were monitored over a certain time course. Samples were gathered at 0, 12, 24, 48 and 72 h to evaluate the changes in CAT, SOD and POD activities. In the control leaves, the activity of none of the three enzymes evidently changed throughout the experimental period, whereas the enzyme activities enhanced in the other three treatments ($P < 0.05$).

The CAT activity in the leaves with SA treatment or *S. lycopersici* inoculation enhanced substantially and peaked at 24 and 48 h, respectively (Fig. 1A). However, the CAT activity in the leaves that were inoculated with *S. lycopersici* and treated with SA exhibited a more significant increasing trend and peaked at 48 h, which was 30.0% and 40.7% higher than the maximum CAT activity in the leaves with SA treatment or *S. lycopersici* inoculation, respectively.

Compared with that in the control samples, the activity of SOD in the three treatments significantly changed within 0~12 h, while an evident increase in SOD activity was detected after 12 h, especially in the leaves inoculated with *S. lycopersici* or in those inoculated with *S. lycopersici* after SA treatment (Fig. 1B). The SOD activity was higher in the leaves inoculated with *S. lycopersici* after SA treatment than in the leaves in the other treatments. However, the SOD activity in the leaves inoculated with *S. lycopersici* began to decrease after 24 h, while the SOD activity in the leaves treated with SA tended to increase. Although the SOD activity in the other three treatments decreased, the activity in the leaves inoculated with *S. lycopersici* and treated with SA increased substantially and peaked at 48 h.

Compared with the POD activity in the control treatment, the POD activity in the leaves of the other three treatments increased from 0 to 48 h (Fig. 1C). The POD activity in the leaves treated with SA was lower than that in the leaves inoculated with *S. lycopersici* at 0–24 h, but the POD activity in the leaves inoculated with *S. lycopersici* after SA treatment was significantly greater than that in the leaves receiving the other two treatments at the same time. In addition, the activity in the leaves inoculated with *S. lycopersici* began to decrease at 48 h, whereas it was elevated in the leaves inoculated with *S. lycopersici* and treated with SA during the same period. Therefore, it was speculated that SA may induce resistance in tomato leaves.

Effect of exogenous SA on PAL and PPO activities in "Moneymaker" tomato leaves

Compared with that in the control treatment, the PAL activity in the leaves in the three other treatments increased (Fig. 1D). The PAL activity in the leaves inoculated with *S. lycopersici* and treated with SA began to decrease after 12 h. However, PAL activity markedly increased and was greater than that in the other two treatments at 24 h and its activity reached a maximum that was 3.8 times that of the control groups at 48 h. Although the activity in the leaves inoculated with *S. lycopersici* after treatment with SA decreased after 48 h, its activity was still high.

The PPO activity markedly increased after 12 h, and the leaves inoculated with *S. lycopersici* presented significantly greater PPO activity than did the leaves of the control (Fig. 1E). The PPO activity in the leaves inoculated with *S. lycopersici* after SA treatment and in the leaves treated with SA markedly increased after 24 h; however, the PPO activity was significantly greater in the leaves inoculated with *S. lycopersici* and treated with SA than in the leaves of the controls and the activity also peaked at 48 h, while the maximum was 4.6 times the activity of the control. After 48 h, the activity of PPO in the different treatments tended to decrease, but the PPO activity in the leaves inoculated *S. lycopersici* and treated with SA was more readily detected.

Direct observation and microscopic analysis of leaves

The leaves inoculated with *S. lycopersici* (Fig. 2b) had more necrotic lesions than did the leaves in the *S. lycopersici* and SA treatment (Fig. 2c). The control displayed no changes (Fig. 2a). Microscopic analysis revealed strong HR symptoms of necrotic lesions on the leaves inoculated with *S. lycopersici* after SA treatment (Fig. 2f). Considerable hyphal and lesion perforations were observed on the leaves inoculated with *S. lycopersici* (Fig. 2e). No obvious symptoms were observed on the leaves of the controls (Fig. 2d). These findings indicate that exogenous applications of the hormone SA can increase resistance to *S. lycopersici* in the susceptible tomato cultivar "Moneymaker".

Effects of exogenous SA on H_2O_2 and O_2^- in tomato leaves

According to the color changes of DAB and NBT, the accumulation of ROS induced by exogenous applications of the hormone SA could be detected in tomato leaves. There were no obvious symptoms in the stained control leaves (Fig. 3a and d). The leaves inoculated with *S. lycopersici* after three days showed significant coloration changes in the lesions of the leaves (Fig. 3b and e). However, the color of the lesions in the leaves inoculated with *S. lycopersici* after

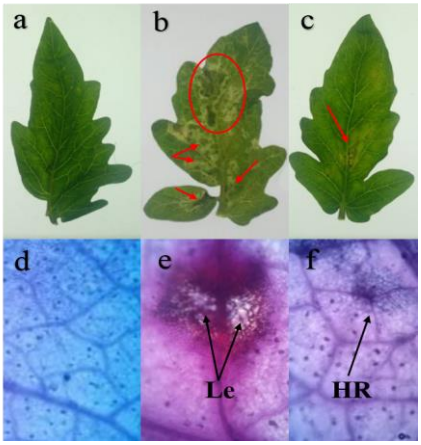

Fig. 2: Phenotypic observations and trypan blue staining results. Leaf of control seedlings (**a**). Leaf of seedlings inoculated with *S. lycopersici* showing typical gray spot disease symptoms (**b**). Leaf of seedlings inoculated with *S. lycopersici* after SA treatment, showing a small number of lesions (**c**). Microscopic image of a control leaf (**d**). Necrotic lesions and a mass of hyphae are shown along with a perforation of lesions on a leaf inoculated with *S. lycopersici* (**e**). Few hyphae and strong HR symptoms are shown on a leaf with *S. lycopersici* and SA treatment (**f**)
HR: hypersensitive response. Hy: hyphae

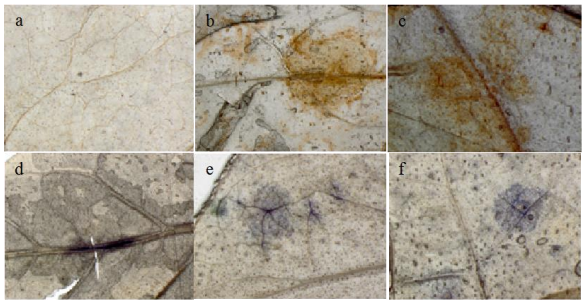

Fig. 3: Color changes in "Moneymaker" tomato leaves stained with DAB and NBT. No obvious symptoms were observed in the control leaves subjected to DAB staining (**a**), but light brown was observed in the leaves inoculated with *S. lycopersici* (**b**). Darker brown was presented in the leaves treated with SA and inoculated with *S. lycopersici* than in the leaves of the control (**c**). Moreover, there were no symptoms in control leaves subjected to NBT staining (**d**). A light blue color was observed in *S. lycopersici*-inoculated leaves (**e**). The leaves inoculated with *S. lycopersici* after treatment with SA displayed a colouration of darker blue than did the leaves of the control (**f**)

SA treatment was intense (Fig. 3c and f). Thus, it was speculated that SA could induce the accumulation of ROS in the leaves inoculated with *S. lycopersici*.

Effects of SA on the expression of members of the WRKY65, PR, MAPK and PYR/PYL families and of the Serine/Threonine-protein kinase gene CTR1

To determine the transcript levels of members of the WRKY65, PR, MAPK and ABA receptor PYR/PYL families and of serine/threonine-protein kinase gene CTR1, which are involved in SA-induced resistance, we used RT-qPCR to analyze the expression of these genes in "Moneymaker" tomato leaves during *S. lycopersici* infection.

The expression of the WRKY65 gene was significantly upregulated in the SA-treated seedlings compared with the control seedlings (Fig. 4A). The number of genes expressed in the seedlings treated with *S. lycopersici* peaked after 48 h. It can be inferred that the pathogenesis of gray leaf spot disease lasted approximately 48 h. The gene expression in the leaves inoculated with *S. lycopersici* after SA treatment was significantly greater than that in the leaves of seedlings in the other three treatments at 12 h, 24 h and 72 h. The expression level in the leaves inoculated with *S. lycopersici* after SA treatment was 153 times that of the control at 72 h. Therefore, exogenous applications of the hormone SA were capable of inducing the expression of the WRKY65 gene.

Similar regulatory expression effects were detected with the PR gene (Fig. 4B). Expression of the PR gene peaked at 48 h only in the seedlings inoculated with *S. lycopersici*. The expression level of the PR gene in the SA-treated leaves was upregulated and increased by 29-fold compared with the control at 48 h. Moreover, the expression of the PR gene in the leaves inoculated with *S. lycopersici* after SA treatment increased and was 11.6 times that of the control at 24 h after treatment.

MAPK exhibited a more pronounced regulatory effect (Fig. 4C). The expression level of the MAPK gene after SA treatment was like that in the leaves with *S. lycopersici* inoculation. However, the expression of MAPK significantly greater in the leaves inoculated with *S. lycopersici* after SA treatment than in the leaves of the controls, and its expression increased by 48.3 times at 72 h after treatment.

The expression of the PYR/PYL family gene was also affected by SA, and compared with that in the control leaves, its expression level in the leaves after SA treatment increased (Fig. 4D). Moreover, the expression level was highest at 48 h and was approximately 7 times that of the control. The expression of the PYR/PYL family gene in the leaves inoculated with *S. lycopersici* after SA treatment peaked at 24 h after treatment, which was 3.92 times that of the control plants. Although the regulatory effect of this gene did not significantly differ from that of the other genes in this study, SA regulated the expression of this gene.

The expression of the serine/threonine-protein kinase gene CTR1 was regulated by SA treatment (Fig. 4E). Compared with that in the control leaves, the CTR1 gene expression in the leaves treated with SA significantly increased by 66.8 times, 27.9 times, 238.4 times and 135 times at the four different time points measured. CTR1 expression in the leaves inoculated with *S. lycopersici* after SA treatment was greater than that in the leaves in the other three treatments and was 175.4 times that of the control leaves.

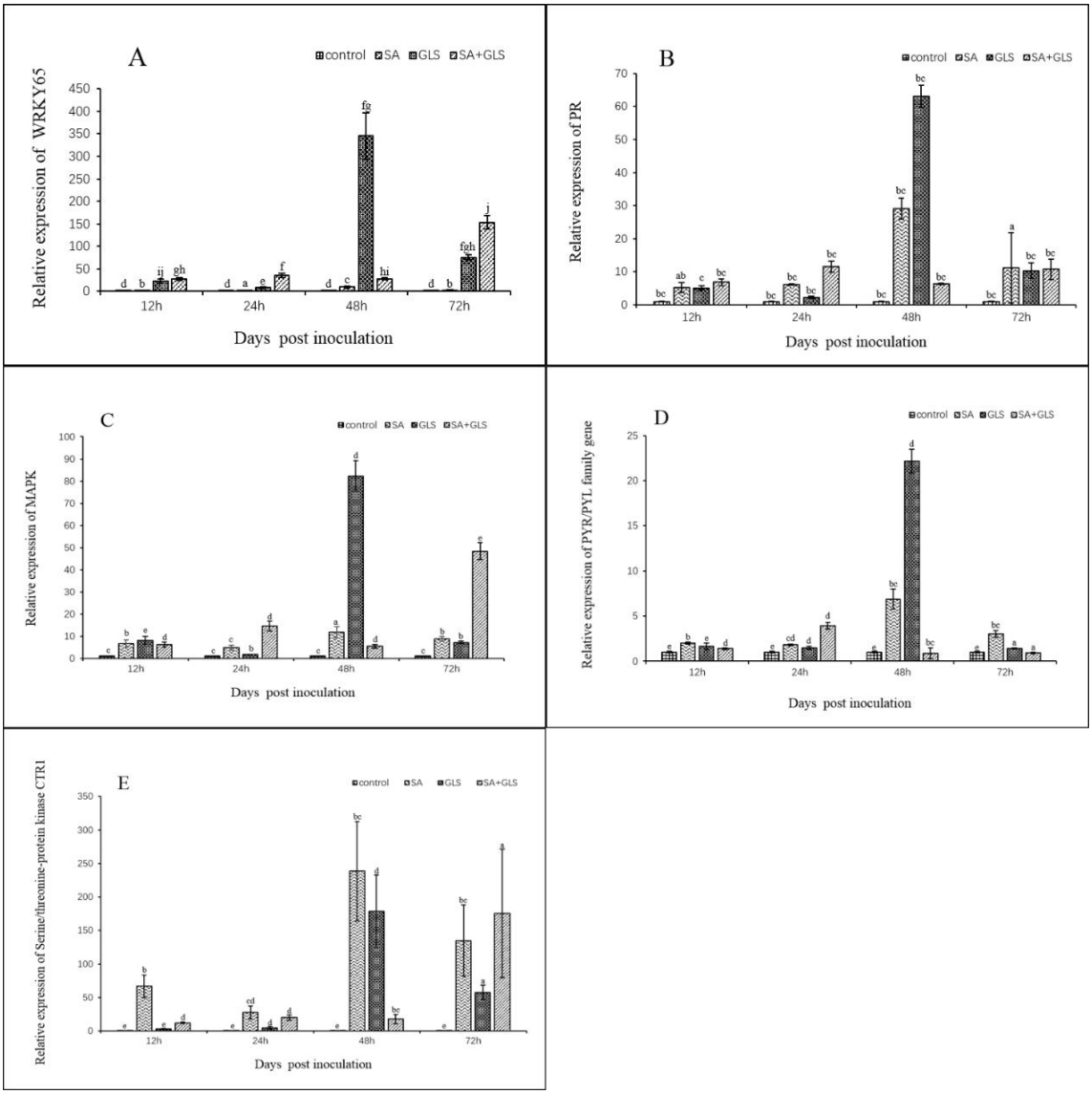

Fig. 4: Effects of salicylic acid (SA) on the transcript abundance of members of the WRKY65 (**A**), PR (**B**), MAPK (**C**), PYR/PYL families (**D**) and of the serine/threonine-protein kinase CTR1 gene (**E**) in "Moneymaker" tomato leaves after inoculation with *S. lycopersici*. Fully expanded leaves pretreated with 0.2 m*M* SA were inoculated with *S. lycopersici.* Total RNA was extracted from leaf tissues collected at different time points, converted to cDNA, and subjected to quantitative real-time PCR. Transcript levels were calculated and normalized to the expression of Malus EF 1a mRNA. The expression levels of WRKY65, PR, MAPK, PYR/PYL families and the serine/threonine-protein kinase CTR1 gene in the control leaves were set to 1. The data represent the means of two separate experiments with six biological replicates. The different letters indicate significant differences at each time point ($P < 0.05$) and the error bars represent standard errors

Discussion

Induced resistance has been identified as an environmentally friendly strategy for disease resistance (Wang *et al.* 2015). In this study, exogenous applications of the hormone SA could significantly improve the resistance to *S. lycopersici* in the leaves of the susceptible tomato cultivar "Moneymaker". The number of necrotic leaves and the degree of infection were reduced. Thus, we could conclude that SA was effective against the invasion of *S. lycopersici* by inducing host disease resistance in the seedlings, and the results were consistent with those of previous reports concerning the ability of SA to induce resistance (Mandal *et al*. 2009).

Exogenous hormone-induced resistance occurs primarily via the activation of biochemical pathways and plant tolerance mechanisms (Najafian *et al*. 2009). Enzyme activity associated with plant resistance has also been studied (Mandal *et al.* 2009). The results of our study showed that pretreatment with exogenous applications of

the hormone SA significantly improved the activities of enzymes such as CAT, SOD, POD, PPO and PAL in the leaves after inoculation with *S. lycopersici*, indicating that the increased resistance to *S. lycopersici* in the susceptible tomato cultivar "Moneymaker" is related to the induced resistance caused by exogenous SA.

Signaling molecules are involved in the signal transduction system and induce specific enzymes to catalyze biochemical reactions that form compounds such as phenols, alkaloids and PR proteins for protection against foreign invaders in plants (Hahlbrock and Scheel 1989; Ding *et al*. 2002). Specific chemical factors can stimulate the expression of PR genes to resist pathogens (Yu *et al*. 2014). In our study, applications of the exogenous hormone SA significantly increased the expression of the PR gene, and the expression of the PR gene was previously compared with the expression of other genes. In particular, compared with those in the other three treatments, the leaves inoculated with *S. lycopersici* after treatment with SA presented greater PR expression. These results suggested that, by inducing the expression of specific genes, SA (applied exogenously) could increase resistance against *S. lycopersici*.

SA plays an important role in inducing disease resistance (Maleck *et al*. 2000; Audenaert *et al*. 2002). The accumulation of ROS is an indicator of the defense system of plants in response to stress (Wang *et al*. 2014). In the present study, the accumulation of ROS in plant leaves was determined by NBT and DAB staining. Compared with the control leaves, the leaves treated with *S. lycopersici* and SA were darker blue and darker brown in color. These results indicated that SA pretreatment increased the accumulation of H_2O_2 and $O_2 \cdot^-$ in the leaves. SA plays an important role in the antioxidant system associated with the metabolism of ROS (Eraslan *et al*. 2007). The function of these antioxidant enzymes is controlled by SA (He and Liu 2002). The antioxidant system includes a variety of antioxidant enzymes, such as CAT, SOD and POD. These antioxidant enzymes can scavenge ROS and free radicals to protect plant tissues (Foyer *et al*. 1994). In this study, the activities of CAT, SOD and POD in the leaves treated with 0.2 m*M* SA significantly increased. However, the activities were greater in the leaves with *S. lycopersici* inoculation and SA treatment than in the leaves of the controls. These results were consistent with those of previous reports (Rao *et al*. 1997; Srivastava and Dwivedi 1998; Qin *et al*. 2003). However, the regulatory mechanism of ROS by SA requires further study (Cao *et al*. 2013). As an endogenous signaling molecule, SA is a major component of signal transduction systems and it can induce specific enzymes that catalyze biochemical reactions that are part of antioxidant systems and are critical in the development of SAR (Loon and Antoniw 1982). However, the resistance of plants to pathogens is the result of complex physiological and biochemical reactions, and changes in enzyme activities are related to various factors, such as crop type, disease severity and the time interval of treatment.

The results showed that the activity of resistance-related enzymes and gene expression in the leaves inoculated with *S. lycopersici* after SA treatment were greater than those treated with SA or inoculated with the pathogen. These results were consistent with those of a previous study (Spletzer and Enyedi 1999). In addition, the results of this experiment indicated that exogenous applications of the hormone SA could effectively induce resistance to *S. lycopersici* in the susceptible cultivar "Moneymaker". SA activated the defense system and produced corresponding responses in seedlings, such as increased activity of related enzymes and gene expression. These findings indicate the important role of exogenous applications of SA in resistance to tomato gray leaf disease.

Conclusion

Application of salicylic acid was effective to induce powerful resistance against *S. lycopersici* in tomato. The effect of salicylic acid owed to activate defence responses, such as enhanced activities of antioxidant enzymes and up-regulated expression of genes related to disease resistance. The discovery emphasizes the character of salicylic acid in enhancing resistance to gray leaf spot in tomato leaves, it is an alternative method to control gray leaf spot.

Acknowledgments

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