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Research Article

Assessment of salicylic acid-induced resistance against *Septoria tritici* blotch disease on wheat using real-time PCR

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Abstract: *Septoria tritici* blotch (STB) caused by *Zymoseptoria tritici* is one of the most important wheat diseases in the world and causes significant annual damage to wheat crops around the globe. The use of resistant cultivars is the most effective method for the management of this disease. Recently, the use of acquired systemic induced resistance has been proposed to manage wheat leaf blotch. In this study, the effect of salicylic acid (SA) on the relative changes of *PAL* and *PR2* gene expression was investigated using qPCR technique. The expressions of catalase, peroxidase, and ascorbate peroxidase enzymes were also assessed in a sensitive wheat cultivar. Controlled and contaminated plants were sampled and compared at 0, 12, 24, 48, 96, and 240 hours after inoculation. The results showed that treatment with salicylic acid significantly reduced the level of disease compared to control plants. Comparison of gene expression patterns also showed that the expression of both *PAL* and *PR2* genes in control and SA pre-treated plants increased after fungal inoculation; however, their expression was significantly higher (2.6 and 1.3 folds respectively for *PAL* and *PR2*) in plants treated with salicylic acid than the control. Moreover, treatment with salicylic acid significantly affected the activity of all three enzymes. It can be concluded that the high response of *PAL* and *PR2* genes to salicylic acid pre-treatment, as well as increased activity of peroxidase along with the reduced activity of catalase and ascorbate peroxidase enzymes indicate the effective role of SA in inducing wheat resistance against STB.

Keywords: Salicylic acid, Septoria leaf blotch, Real time PCR, Induced resistance

Introduction

Septoria tritici blotch (STB), caused by the ascomycete fungus *Zymoseptoria tritici* (Desm) (Teleomorph: *Mycosphaerella graminicola*) is one of the most important foliar diseases of wheat (Quaedvlieg *et al.,* 2011). A significant annual loss of wheat crop productivity worldwide is related to this disease (Goodwin *et al.,* 2003; Bearchell *et al.,* 2005; Jørgensen *et al.,* 2014). Although studies have shown that most Iranian *Zymoseptoria tritici* isolates are susceptible to new fungicides (Saeidi *et al.,* 2012) and use of chemical fungicides is still one of the most common ways of controlling this disease (Goudemand *et al.,* 2013), various adverse effects of chemical pesticides on humans and animals, and the environmental damages, as well as their economic disadvantages, cannot

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be overlooked (Loughman and Thomas, 1992). Furthermore, the use of resistant cultivars is an effective the method in the STB management (Brown *et al.,* 2015) and research indicates specific resistance among different wheat genotypes to pathogenic isolates of *Zymoseptoria tritici* (Makhdoumi *et al.,* 2014). However, this pathogen can overcome plant resistance and become resistant to chemical fungicides as well (Torriani *et al.,* 2009). Biological control (Lynch *et al.,* 2016), as well as induction of systemic acquired resistance (SAR) (Ghanei *et al.,* 2017), have been proposed to counter the development of STB pathogen resistance to fungicides.

The plant immune system is a combination of the immunity triggered by pathogen-associated molecular patterns (PAMP) and effector-mediated immunity (ETI) (Jones and Dangl, 2006). The major difference between these pathways is the nature of the pathogen-derived molecules and the severity and potency of the immune responses. Effector-mediated immunity is a basic resistance against major diseases used to modify resistance to many biotrophic and necrotrophic pathogens (Thomma *et al.,* 2011; Tsuda and Katagiri, 2010).

Following local infection, plants show a wide spectrum of resistance to various agents, including fungal, bacterial, and viral pathogens. Since such induced resistance is present throughout the plant, it is called SAR. This phenomenon begins with the extensive accumulation of salicylic acid (SA) and a wide range of mRNA and their encoded proteins (Chen *et al.,* 2009). The SAR is a form of induced resistance that is activated throughout a plant after being exposed to elicitors that stimulate the activity of pathogen-responsive genes and induce resistance to pathogens (Vlot *et al.,* 2009). An important type of such chemical elicitors is salicylic acid. The latter is a known plant hormone and as the most important factor in the SAR plays a crucial role in systemic acquired resistance to pathogens (Nie, 2006). It is also a natural regulator of plant growth and its intrinsic concentration increases during infections (Hayat *et al.,* 2010). Salicylic acid is a phenolic compound that plays a role in regulating plant growth and response to biotic and abiotic stresses (Miura and Tada, 2014). It plays an important role in plant molecular, biochemical, and physiological processes such as photosynthesis, nitrogen and proline metabolism, signal transduction pathway related to the synthesis of defense compounds such as polyphenols, alkaloids, and pathogenic proteins, antioxidant enzyme activity, and expression of defense-related genes (Miura and Tada, 2014). Moreover, during pathogenic attacks, plants show several defensive reactions. By inducing the synthesis of antioxidant defensive enzymes to provide greater protection against damages, they can respond to a wide range of stresses such as temperature, drought, salinity, ozone, UV radiation, and pathogen attacks. Catalase, peroxidase, and ascorbate peroxidase are among the major enzymes involved against H_2O_2, one of the major reactive oxygen species (ROS) (Liu *et al.,* 2008).

The positive effects of exogenous salicylic acid for inducing resistance to viral, bacterial, and fungal pathogens have been extensively tested in plants (Hayat *et al,* 2010). Pre-treatment of plants with SA increases their resistance to biotic and abiotic stresses (Miura and Tada, 2014). External treatment of plants with salicylic acid and other substances including polyacrylic acid, acetylsalicylic acid, 2 and 6-dichloro iso-nicotinic acid, methyl salicylate, benzothiadiazole derivatives, DL-B amino-butyric acid and oxalic acid can induce accumulation of proteins associated with Pathogenicity and reduce damages caused by several pathogens in different crops (Gozzo, 2003). The study of salicylic acid effects on the inhibition of wheat powdery mildew has shown that it can reduce the disease symptoms by inducing plant defense responses, such as expression of defense genes, as well as altering the levels of

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antioxidant defensive enzyme activities (Ahangar *et al.*, 2017). Studies have shown that the application of salicylic acid as seed treatment and seedling inoculation can reduce the severity of *Z. tritici* disease symptoms in different wheat cultivars (Gholamnejad *et al.*, 2015). Also, the effect of salicylic acid on reducing the severity of STB in three tetraploid and three hexaploid wheat cultivars at the seedling stage showed that application of 1 mM can induce systemic resistance and reduce the severity of STB in both groups (Ghanei *et al.,* 2017). Also, Zamani *et al.*, (2016) demonstrated that the concentration of 4 mM salicylic acid not only completely inhibits the germination of *Z. tritici* conidia in the culture medium, but also significantly reduces the severity of disease symptoms in wheat leaves as well. Comparison of the activities of antioxidant enzymes in two SA-treated wheat cultivars (resistance and susceptible) in the presence or absence of *Z. tritici* demonstrated that the application of SA was effective in antioxidant activities and in the earliest time of infection, these activities were weaker in susceptible cultivar than in the tolerant one (Gholamnejad *et al.*, 2016b). In this study, we intended to investigate the effects of salicylic acid on the relative changes in the expression of *PAL* and *PR2* genes as well as the expression of antioxidant defense enzymes in wheat infected with foliar septoriosis.

Materials and Methods

Isolation of the pathogen and the condition of plant growth

This study was conducted on a susceptible wheat cultivar, Kouhdasht, at the Botanical Laboratory of Gonbad Kavous University. The experiment was carried out with 3 replications and two concentrations of salicylic acid (0 and 2 mM). Plastic pots containing a mixture of peat moss and soil in the ratio of 1: 1 were used to sow seeds disinfected with 1% sodium hypochlorite solution. The pots each containing about 5-7 seeds were placed in trays with a certain amount of water.

Z. tritici samples isolated from wheat fields of Golestan province were obtained from Gorgan Agricultural and Natural Resources Research Center. The fungal inoculum was prepared on YMD broth medium (yeast extract 4 g, malt extract 4 g and, dextrose 5 g per liter). The fungal suspension containing about 10^6 spores /ml was prepared and three drops of tween 20 per 100 ml inoculum suspension were added. Pathogenicity of the isolate was tested by spraying its spore suspension containing 10^6 spores/ml on two-leaf seedlings of Kouhdasht susceptible cultivar. Following inoculation, seedlings were transferred into plastic bags to keep high humidity and maintained at 20 ± 2 °C under dark conditions for 48 hours. Subsequently, they were removed from the plastic bags and kept in the same greenhouse with high humidity (> 75% RH) and after 21 days, the percentage of pycnidium coverage of the seedlings' first leaves was determined (Kema *et al.,* 1996).

Plant treatment with salicylic acid and pathogen infection

24 hours before inoculation with the *Z. tritici* suspension, the ten-day-old wheat seedlings were manually sprayed with salicylic acid at concentrations of 2 mM (dissolved in Ethanol). The inoculum was sprayed using a hand-held sprayer. Sterile distilled water was sprayed on control plants. Following inoculation, plants were transferred to a greenhouse under controlled conditions as mentioned above. Disease severity was evaluated on the first leaves; 21 days post-inoculation by visual assessing the percentage of leaf area with necrotic lesions bearing pycnidia (Kema *et al.,* 1996; Brown et al., 1999; Chartrain *et al.,* 2004).

RNA extraction, cDNA synthesis, and measurement of gene expression

A sampling of control and inoculated plants were carried out at 0, 12, 24, 48, 96, and 240

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hours after inoculation. To evaluate the expression of genes associated with pathogenicity (Table 1), 500 mg of leaf tissue was removed and milled in liquid nitrogen. RNA extraction was performed using Sinaclone's RNX-Plus kit (Cat. No.: EX6101). The quality of the extracted RNA was measured on 1.2% agarose gel and quantified by a Spectrophotometer (Model 6300Jenway-Uk). To remove possible DNA contamination, the DNase I, RNase-free kit manufactured by Sinaclone (cat. No.MO5401) was used according to the manufacturer's instructions. The cDNA synthesis kit (Cat. No: RT5201) made by Sinaclone was used to cDNA synthesize according to the manufacturer's instructions with a final volume of 20 µl.

Table 1 List of primers and their sequences.

Genes	Sequence Primer	Product
TaActin	F: AGCCCAATCATAGGAAAAGTGC R: AGTGTCTGGATCGGTGGTTC	150
Ta PR2	F: AGCAGAACTGGGGACTCTTCT R: CACATACGTACCGCATACACG	150
TaPAL	F: CCAATGTTCTGTCCGTCCTT R: GAGCTTCCCTCCAAGATGTG	155

For the RT-PCR reaction, 2 µL of diluted cDNA and actin-associated primers were used as the reference gene. These primers were designed using BioEdit 7.0.9.0 and Oligoexplorer V1.4 software. Real-time polymerase chain reaction was performed using Applied C1000TM Thermal Cycler with Cyberblue Kit (SinaSYBR Blue HS-qPCR Mix, 2X. Cat. No.: MM2171). RT-PCR reaction was performed in a final volume of 25 µL including 2 µL cDNA, 12.5 µL cyber blue mixtures, 0.3 µM of each primer, and RNase-free water. Amplification reactions consisted of initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds, and 60 °C for 1 min. For analysis of the specific function of PCR reactions, the melting curve was immediately drawn with a temperature program of 0.5 °C per cycle between 95 and 60 °C. The amplification level was measured at the end of each cycle. Then, the relative changes in the expression of the studied genes were normalized to the *Actin* gene as a housekeeping gene. The expression level before infection was considered as calibrator. Gene expression changes were also measured according to $2^{\Delta\Delta ct}$ method (Livak and Schmittgen 2001).

Measurement of enzymatic activity

Peroxidase activity was assayed according to In *et al.,* (2007), catalase activity was measured as stated by Aebi (1984), and measurement of ascorbate peroxidase activity was performed according to the method of Nakano and Asada (1987). The activity of these enzymes was determined in micromoles per gram of fresh tissue per minute by spectrophotometer at 470, 240, and 290 nm respectively.

Data analysis

Gene expression and enzyme assays were performed in 3 replicates and the results were analyzed by the student's T-test in each time, using SAS (9.1) software.

Results

Evaluation of the ability of salicylic acid to induce resistance to STB

Symptoms on the Kouhdasht susceptible cultivar first appeared as yellow spots on leaves 14 days post-inoculation and reached their maximum level with the appearance of fungal pycnidia 21 days after inoculation. The results showed that in terms of Septoria leaf blotch symptoms, there was a significant difference ($P < 0.01$) between 0- and 2-mM salicylic acid-treated plants. According to the results, the application of salicylic acid resulted in a 31% reduction in the area covered with fungal pycnidium compared to the control (Fig. 1).

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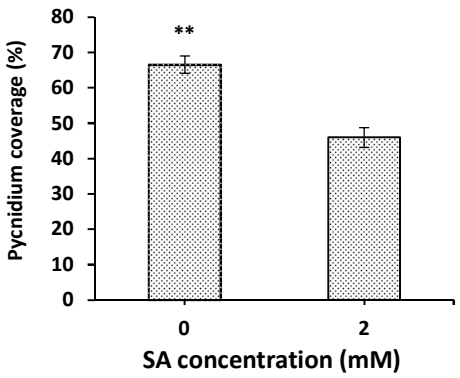

Figure 1 Assessment of pycnidium coverage percentage at different concentrations of salicylic acid (SA). Values are significantly different at $p \leq 0.01$.

Gene expression pattern

Melting curve analysis

The melting point of PCR products for *Actin*, *PR2* and *PAL* genes were about 84.77, 84.02, and 85.02 respectively (Fig. 2). The absence of any additional peaks in the results of the melting curves indicate the specificity of the primers, which confirms the specific amplification of the genes.

Phenylalanine ammonia-lyase (*PAL*) gene expression pattern

The results of phenylalanine ammonia-lyase (*PAL*) gene expression analysis in susceptible cultivar infected with *Z. tritici* showed that there was a significant difference between the control and SA pre-treated plants (Fig. 3-A). Increasing the *PAL* gene expression in SA pre-treated plants at the start point of the experiment, which was significantly different from control ($P < 0.05$), demonstrated that, this gene has a key role in the SA pathway. After inoculating with the pathogen, expression of this gene increased in both control and treated plants. Maximum expression of *PAL* gene was observed in the control and SA treated plants, 24 hours after inoculation (HAI). Gene expression rate at 24 HAI in salicylic acid pretreated plants were 4.3 folds and in the control plants were 2.5 folds higher than at zero time. Although both groups showed maximum expression at the one-time interval, a comparison of *PAL* gene expression at its peak showed that the transcript rate of this gene was 2.6 folds higher in SA pre-treated plants than untreated ones and this increase in expression were significant. The same pattern of gene expression was observed 48 HAI which was 1.8-fold higher in SA pre-treated plants again ($P < 0.01$), but the expression rate decreased in both groups of plants after that time and continued to decrease until 240 hours after inoculation.

Pathogenicity related gene 2 (*PR2*) expression pattern

The status of the *PR2* gene expression pattern indicates a significant increase ($P < 0.05$) in its transcript levels in the early hours after infection in salicylic acid pre-treated plants relative to the controls so that its transcript level at 12 HAI with *Z. tritici* was 3.8 times higher than control plants. Maximum expression of the *PR2* gene in SA pre-treated plants and the control was at 24 hours after inoculation with *Z. tritici*. Its maximum transcription levels in pre-treated and control plants increased by 4.3 and 6 folds of those at zero time respectively. Comparison of control plants with SA pre-treated plants at the time of peak expression showed that pretreatment with salicylic acid increased the expression of the *PR2* gene by 1.3 folds (Fig. 3-B). The expression pattern of this gene at 48 HAI indicated that *PR2* transcript level decreased in both groups but there was a significant difference ($P < 0.01$) between treated and untreated plants at 48 HAI. Like the *PAL* gene, the decrease in gene expression rate continued up to 96 and 240 HAI with no significant difference.

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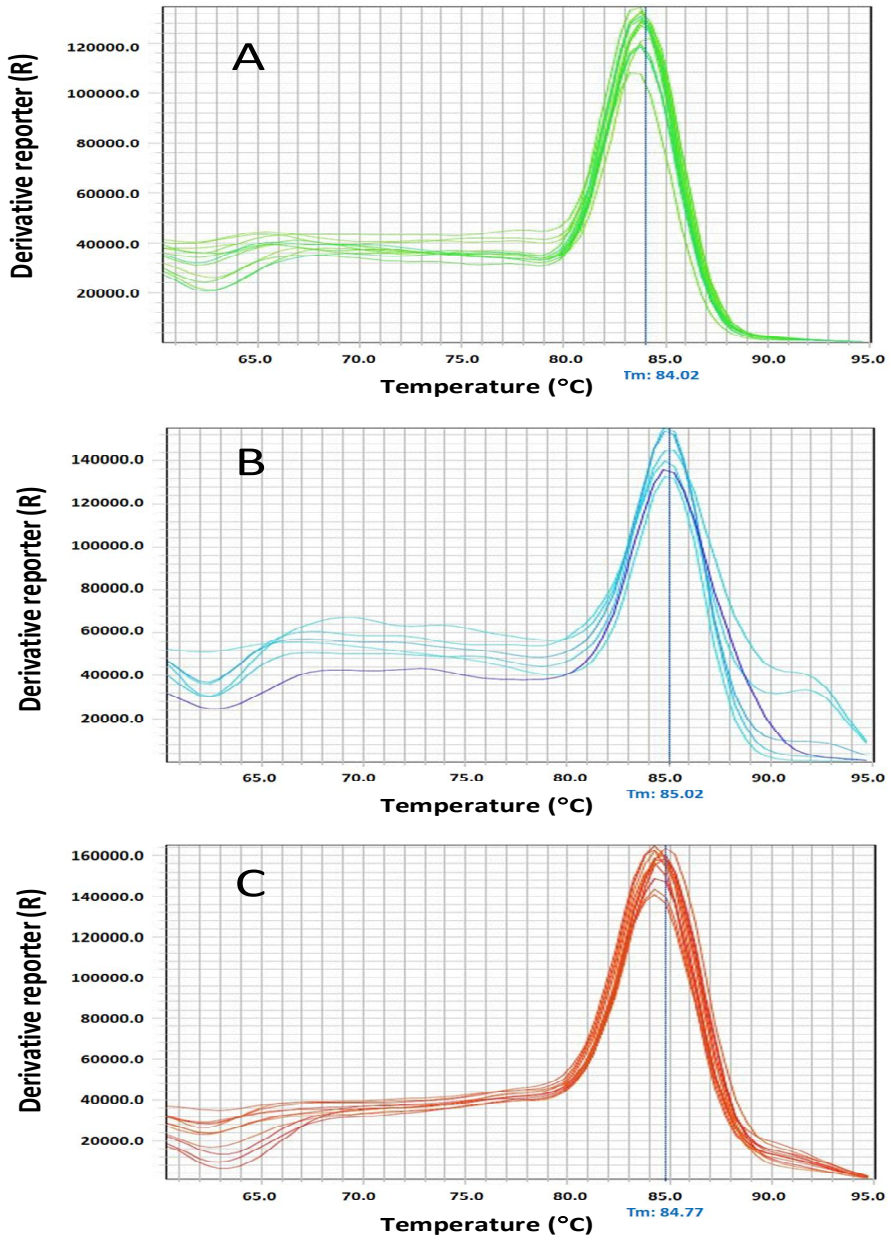

Figure 2 Melting curve of PCR products for (A) *PAL*, (B) *PR2* and (C) *Actin* genes, confirming their specific amplification.

Enzyme activity

Peroxidase (POX) Analysis of variance showed significant differences between SA pre-treated and control plants in terms of POX enzyme activity. Following infection with *Z. tritici*, the activity of this enzyme increased significantly in both groups of plants. POX activity pattern demonstrated that the activity of this enzyme starts at 12 HAI, continues to increase at 24 HAI, and reaches its maximum activity at 48 HAI (Fig. 4-A). Comparison of pre-treated and control plants showed that the POX enzyme activity in infected plants was significantly higher than those of control plants at all-time intervals.

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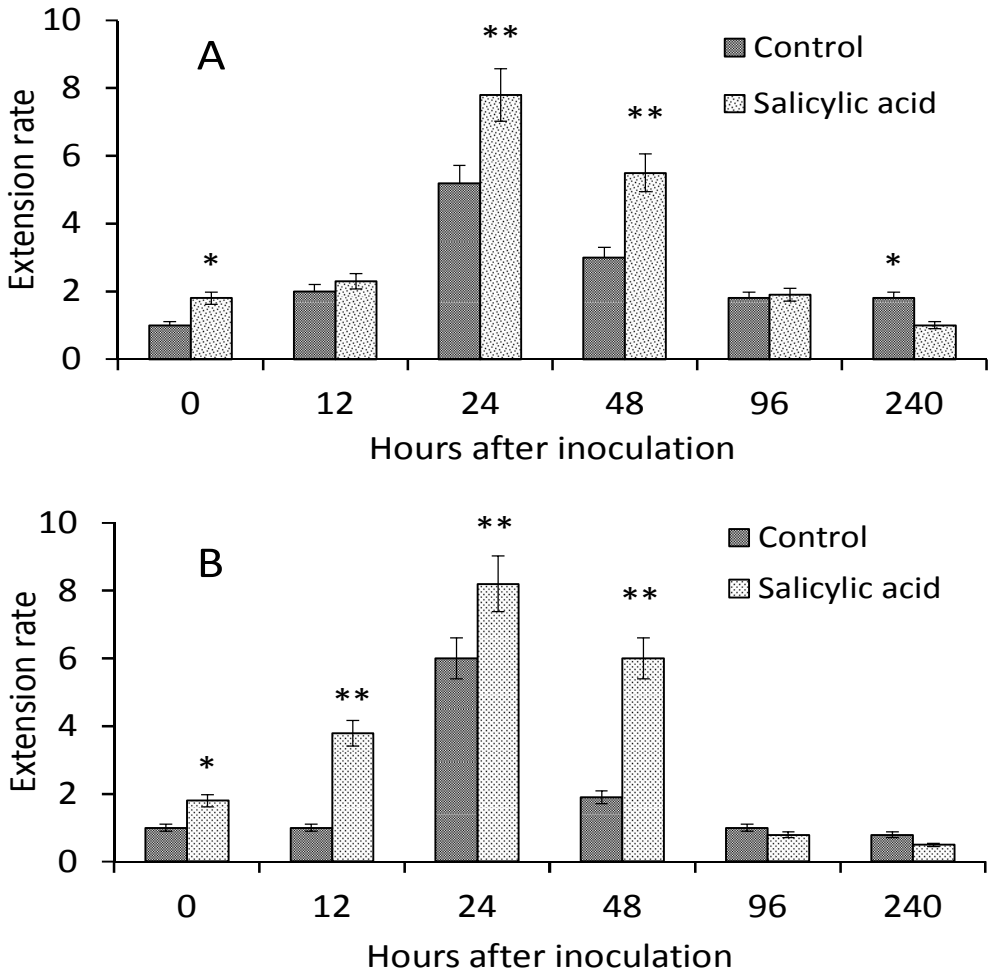

Figure 3 Expression levels of (A) *PAL* and (B) *PR2* genes in wheat plants pretreated with salicylic acid and control confronted by *Zymoseptoria* tritici. * and ** indicate significant differences at 0.05 and 0.01 levels, respectively.

Catalase (CAT) Evaluation of catalase activity pattern exhibited an upward trend up to 24 hours after infection in control plants, whereas its activity in SA pre-treated plants displayed a very slow upward trend. A significant difference ($P < 0.01$) was observed in the activity of this enzyme in pre-treated and control plants at all time intervals following *Z. tritici* inoculation. The highest CAT activity occurred at 24 HAI in SA pre-treated plants, which, however, was significantly lower than that in control plants. 48 HAI, both groups of plants exhibited a decreasing trend in catalase activity (Fig. 4-B).

Ascorbate peroxidase (APX) Analysis of APX enzyme activity after infection with *Z. tritici* showed a significant difference ($P < 0.01$) in SA pretreated plants compared to control plants (Fig. 4-C). The activity pattern of this enzyme in control plants displayed that its production is induced at 12 HAI, the upward trend in its production continues until 24 h, and starts to decrease after this time. Conversely, in SA-treated plants, there was a decreasing trend in APX enzyme activity, and the lowest activity was observed at 12 hours after fungal inoculation and then increased slowly so that the highest difference between pre-treated and control plants was observed 24 h after inoculation.

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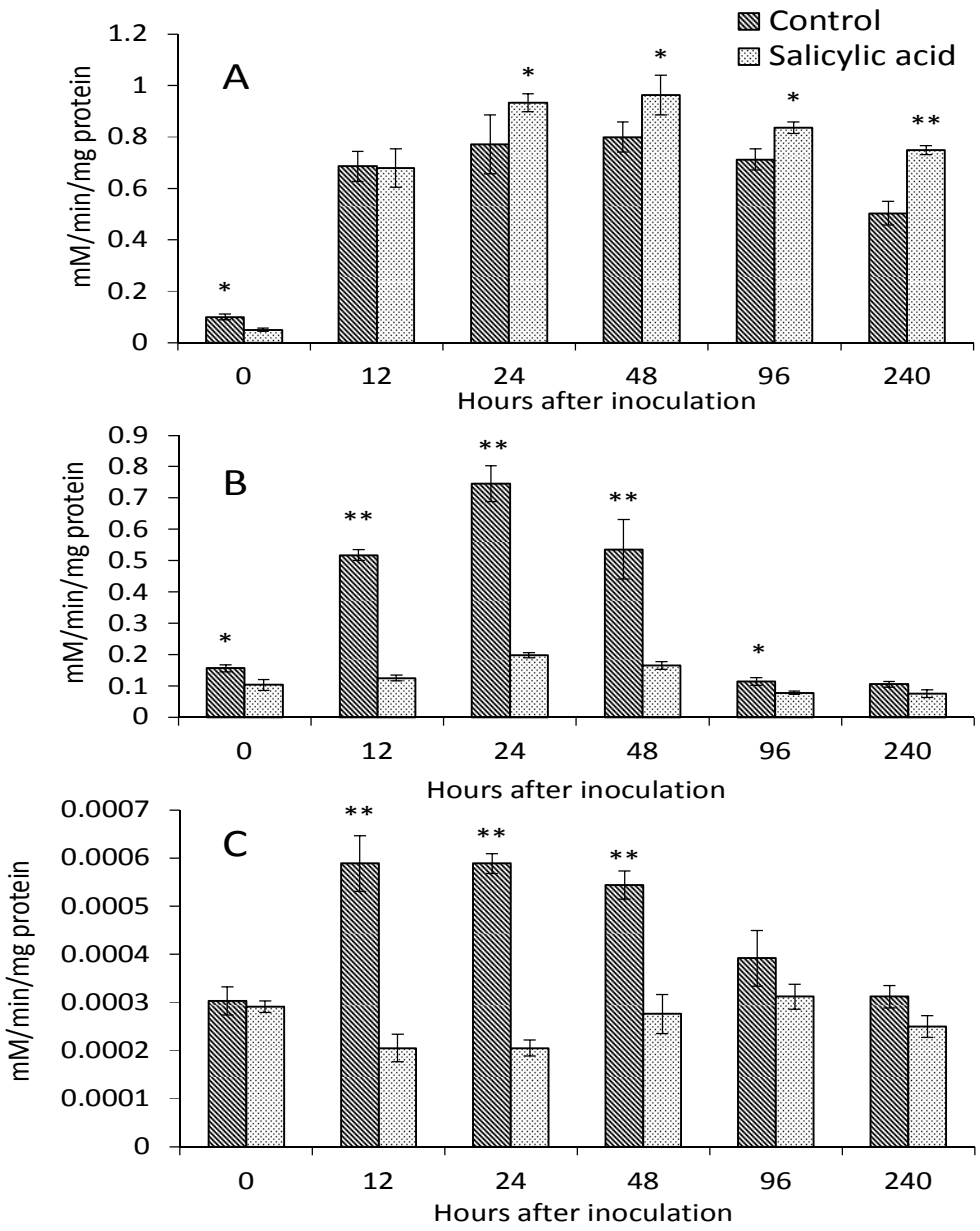

Figure 4 Changes in the activity of peroxidase (A), catalase (B) and ascorbate peroxidase (C) in control and salicylic acid pretreated plants at different hours after inoculation with *Zymoseptoria tritici* (in micro mole per gram fresh tissue). * and ** indicate significant differences at 0.05 and 0.01 levels, respectively.

Discussion

In the present study, the possibility of induction of wheat resistance to septoria leaf blotch by SA was investigated. Results showed that wheat treatment with salicylic acid had a significant effect on the severity of STB symptoms. Based on our results, the SA application reduces the pycnidium-covered area by 31% compared to the control (Fig. 1), which is indicative of salicylic acid's ability to induce resistance to the disease. This finding is

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in line with the results of Gholamnejad *et al.* (2016a) reported the reduction of foliar STB severity by spraying salicylic acid. Research has also shown that the application of 2- and 4-mM salicylic acid on susceptible wheat cultivar reduces the severity of STB symptoms, although it does not completely stop the development of the disease (Zamani *et al.,* 2016; Ghanei *et al.,* 2017). The effect of this substance in reducing the severity of disease symptoms has also been reported in the pathogenesis of Fusarium head blight (Sarahinobar *et al.,* 2015) and wheat powdery mildew (Ahangar *et al.,* 2017).

Our evaluation of *PAL* gene expression patterns in salicylic-acid treated plants indicates increased gene expression in early hours after inoculation (Fig. 3-A), and consequently rapid induction of resistance genes in plants to prevent fungal infiltration and growth in host cells. This expression reached its maximum transcript 24 hours after infection; apparently, inducing cell death to prevent further growth of fungal mycelium in plant cells (Eichmann and Hückelhoven 2008). Nevertheless, the expression of the *PAL* gene had a decreasing trend at 48 hours after inoculation, but this decrease was more apparent in control than treated plants (Fig. 3-A). Similarly, Gholamnejad *et al.* (2016a) showed that the expression level of the *PAL* gene in wheat tolerant variety increased after infection with STB and intensified with salicylic acid treatment. Increases in *PAL* gene expression after inoculation with the pathogen have also been reported in other pathosystems (Huang *et al.,* 2010; Sarahinobar *et al.,* 2015). The product of the *PAL* gene, phenylalanine ammonium enzyme, catalyzes the conversion of phenylalanine to trans-cinnamic acid (the precursor of various phenolic compounds), which is the first step in the phenylpropanoid pathway and is an important regulatory bottleneck step between primary and secondary metabolism (Huang *et al.,* 2010). *PAL* enzyme activity induces plant resistance to stress by regulating the metabolism of trans-cinnamic acid and various phenolic compounds (Wen *et al.,* 2005). It has been found that *PAL* is a gene involved in resistance through a direct effect on the production of salicylic acid, activation of the *NPR1* gene and subsequent *PR* gene expression, and induction of rapid cell death (Fitzgerald *et al.,* 2004). Also, *PAL* is the first enzyme in the biosynthetic pathway of monolignol and phenylpropanoid. The enzyme activity increases with fungal infection and leads to the deposition of lignin and phenylpropanoid compounds in the cell wall, which prevents the fungus from infiltration into the plant tissue (Jaeck *et al.,* 1992).

According to our findings, *PR2* gene expression in control plants reaches its maximum level relatively slowly to prevent the penetration of fungal mycelium in control plants. However, in the SA pre-treated plants, rapid high expression of this gene was observed in the early hours (12 h) after *Z. tritici* infection and reached its maximum level at 24 HAI (Fig. 3-B). An increase of 2.2-fold expression of *PR2* gene at 24 h relative to 12 h after infection can indicate the role of this elicitor in inducing systemic resistance in wheat through early rapid induction of *PR2* gene to protect the plant against *Z. tritici*. Increased expression of the *PR2* gene after the infection has also been confirmed by previous studies (Sabbagh *et al.,* 2017; Molitor *et al.,* 2011). The role of *PR2* proteins in resistance to various diseases has been proven. This gene family is a group of pathogen-related proteins capable of hydrolyzing glucoside polymers. When the plant encounters a pathogen or during biological and abiotic stresses, beta-glucanases are induced and act as a synergist with the chitinase enzyme (Simmons, 1994). These enzymes destroy the fungi as the main fungal cell wall polymers are chitin and 1, 3-glucan (Simmons, 1994). Also, Hernandez *et al.,* (2005) reported that beta-glucanases release oligosaccharides by destroying the fungal cell wall, which activates the plant's secondary defense responses against the pathogen by producing phytoalexins. They also play a role in the formation of papillae at the site of fungal infiltration.

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Studies have demonstrated that the generation of reactive oxygen species is a major contributor to cell death because they modify the factors involved in the preservation of membrane constituents, anti-freezing agents, antioxidants, and numerous other processes (Hückelhoven *et al.,* 1999; Liu *et al.,* 2008). To reduce the damage caused by reactive oxygen compounds, plants activate enzymatic or non-enzymatic antioxidant defense systems (Apel and Hirt, 2004). Enzymes including ascorbate peroxidase, guaiacol peroxidase, catalase, etc., and compounds such as phenol-like secondary metabolites accumulate in plants in response to stresses and regulate H_2O_2 and other free radicals. The role of these compounds in plant defense systems has been demonstrated (Liu *et al.,* 2008).

In this study, we found that the expression of the peroxidase enzyme in wheat infected with *Z. tritici* increased in the presence and absence of salicylic acid, but the difference between the two treatments was significant (Fig. 4). POX activity is the primary and early response to plant-pathogen interactions and its increased activity is directly related to the level of H_2O_2 in stressed plants (Sasaki *et al.,* 2004). Bhuiyan *et al*. (2009) found that a few hours after infection, the level of peroxidase in a plant rises to prevent pathogen infiltration through the production of ROS, sedimentation of phenolic compounds, and lignification of the cell wall. It seems that the peroxidase enzyme participates in the process of peroxidation of their substrates earlier than other compounds, and eventually leads to a high accumulation of toxic constituents, such as oxidized phenolic compounds like quinones (Peng and Kuc, 1992). Increased expression of this enzyme has also been reported in Salicylic acid-treated wheat infected with the STB (Gholamnejad *et al.,* 2016b) as well as powdery mildew (Ahangar *et al.,* 2017). High peroxidase activity may be due to its role in cell wall crosslinking and lignin synthesis. In this way, plants can prevent fungal infiltration by blocking the cell wall pores through increasing wall thickness, elevating the level of their isoperoxidase activity and if the disease progresses, produce H_2O_2 and induce cell death to prevent further pathogen infiltration and development (Hückelhoven *et al.,* 1999).

In contrast, the results of this study showed a significant decrease in the activity of catalase in SA pre-treated wheat compared to the control plants (Fig. 4-B). This finding suggests that SA is involved in the induction of wheat resistance to STB via the catalase enzyme pathway. Studies have shown that SA can chelate iron located in the catalase molecule due to the presence of oxygen in its free hydroxyl group on its benzoic ring, thereby suppressing the H_2O_2 sorption activity of this enzyme specifically (Qinghua and Zhujun 2008). Our findings indicate that pretreatment of plants with salicylic acid leads to a significant decrease in catalase activity. Although the level of H_2O_2 in the tissue has not been measured in this experiment, evidence suggests that decreasing the catalase activity following infection, increases the H_2O_2 level in SA pretreated plants as a signal of cell death. This could activate disease-related genes (PR proteins) to protect the plant against the pathogen. It has been known that decreased catalase activity can elevate plant cell death in transgenic tobacco plants under pathogen infection (Mitler *et al.,* 1999). Similarly, decreased catalase activity in salicylic acid-treated wheat infected with powdery mildew has also been reported (Ahangar *et al.,* 2017).

Another antioxidant enzyme is ascorbate peroxidase, which is a key H_2O_2-destroying enzyme that plays a crucial role as a signaling mediator for activating downstream events including gene expression to induce resistance to Stress (Bowler and Fluhr, 2000). Ascorbate can be oxidized with hydroxyl radical by direct reaction with reactive oxygen species such as superoxide or be used as a reducing agent in alpha-tocopherol (a membrane-bound antioxidant) remodeling and protects the membrane against oxidative stress (Parida and Das, 2005). Studies have shown that SA inhibits H_2O_2 sorption by cytosolic ascorbate peroxidase and increases H_2O_2 levels in plants

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immediately after SA treatment (Dempsey *et al.,* 1999). Likewise, the results of our study indicate that the activity of APX enzyme is reduced in SA pretreated plants following infection with a pathogenic fungus, whereas its activity in the control wheat displays an increasing trend (Fig. 4-C). Its high level in control plants, especially at 24 hours after infection, is probably due to the successful infiltration of the fungus into the plant cells. El-Zahaby *et al.,* (1995) reported that in barley powdery mildew pathosystem, H_2O_2 level is reduced in susceptible plants by increasing the activity of APX enzyme to prevent plant cell death and enhance fungal growth. Also, a significant decline of this enzyme in the pre-treated cultivars can be justified by the rise in H_2O_2 level to induce plant cell death by fungal invasion. Indeed, transgenic cAPX-S2 and cAPX-S3 tobacco plants, in which APX activity is 50% and 75% lower than wild types respectively, showed high levels of H_2O_2 compared to the control plant, and this increased ROS induces high resistance to stress in these plants (Ishikawa *et al.,* 2005).

Conclusion

According to the results of this study, the application of salicylic acid reduces the symptoms of disease caused by *Z. tritici* in susceptible wheat cultivar at biochemical and molecular levels. It increases the expression levels of phenylalanine amino lyase and another pathogenicity related proteins, as well as altering the activities of defense enzymes such as catalase, peroxidase, and ascorbate peroxidase, indicating the efficacy of external SA application in inducing wheat defense mechanism against STB. These changes induce systemic resistance, thereby putting the plant on alert by preparing and expanding the immune system to counteract pathogens faster and more powerfully. Therefore, it is recommended to investigate the possible application of salicylic acid at the field level to reduce disease damages and adverse effects of chemical abuse.

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بررسی مقاومت القایی حاصل از اسید سالیسیلیک در برابر بیماری سپتوریوز برگی گندم با استفاده از روش Real-time PCR

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چکیده: بیماری سوختگی سپتوریایی برگ گندم که به‌وسیله قارچ *Zymoseptoria tritici* ایجاد می‌شود یکی از مهم‌ترین بیماری‌های گندم در جهان است که سالیانه خسارات قابل‌توجهی به محصول گندم در سراسر دنیا وارد می‌کند. استفاده از ارقام مقاوم مؤثرترین در مدیریت این بیماری است. اخیراً استفاده از مقاومت القایی سیستمیک اکتسابی در مدیریت این بیماری پیشنهاد شده است. در این مطالعه تأثیر سالیسیلیک اسید بر تغییرات نسبی بیان ژن‌های *PAL* و *PR2* با استفاده از تکنیک qPCR مورد بررسی قرار گرفت. هم‌چنین بیان آنزیم‌های دفاعی کاتالاز، پراکسیداز و آسکوربات پراکسیداز در رقم حساس گندم به بیماری سپتوریوز برگی مورد بررسی قرار گرفت. گیاهان شاهد و آلوده در زمان‌های ۰، ۱۲، ۲۴، ۴۸، ۹۶ و ۲۴۰ ساعت پس از آلودگی نمونه‌برداری شده و مورد مقایسه قرار گرفتند. نتایج نشان داد که تیمار با سالیسیلیک اسید به‌طور معنی‌داری سبب کاهش مقدار بیماری نسبت به گیاهان شاهد گردید. هم‌چنین مقایسه الگوی بیان ژن‌ها نشان داد که پس از تلقیح بیمارگر، بیان هر دو ژن در گیاهان شاهد و پیش تیمار شده با سالیسیلیک اسید افزایش یافت. اما میزان بیان هر دو ژن در گیاهان تیمار شده با سالیسیلیک اسید به‌طور معنی‌داری بیشتر از گیاهان شاهد بود (۲/۶ و ۱/۳ برابر به‌ترتیب برای ژن‌های *PAL* و *PR2*). هم‌چنین تیمار با سالیسیلیک اسید به‌طور معنی‌داری میزان فعالیت هر سه آنزیم را تحت تأثیر قرار داد. بنابراین پاسخ بالای این دو ژن در برابر پیش تیمار با سالیسیلیک اسید و نیز افزایش میزان فعالیت آنزیم پراکسیداز و به موازات آن کاهش فعالیت آنزیم‌های کاتالاز و آسکوربات پراکسیداز، حکایت از نقش مؤثر این ماده در القای مقاومت در گندم در برابر بیماری سپتوریوز برگی دارد.

واژگان کلیدی: سالیسیلیک اسید، سوختگی سپتوریایی برگ، مقاومت القایی، Real time PCR

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