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Full Length Research Paper

Effects of salicylic acid on wheat salt sensitivity

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Salicylic acid (SA), a plant phenolic compound, is now considered as a hormone-like endogenous regulator, and there is a great interest to clarify its role in the defence mechanisms against biotic and abiotic stressors. In this study, investigations on the effects of foliar-applied SA on salt sensitivity, hydrogen peroxide (H_2O_2) generation and activities of antioxidant enzymes like peroxidase (POX) and catalase (CAT) in plant tissues under salt stress was performed. SA treatment significantly increased the fresh and dry weights in both root and shoots of wheat plants under salt stress. Similarly, POX and CAT activities were also augmented by SA treatment. While the highest POX activity was recorded at SA+120 mM NaCl, CAT activity also exhibited an increase compared to salt treatment without SA. In parallel to increasing antioxidative activity, SA treatment decreased H_2O_2 content when compared to plants growing under salt stress without SA. The results revealed that salt-induced deleterious effect in wheat seedlings were significantly alleviated by the SA treatment. SA can be used as a signal molecule to investigate plant defense to abiotic stress. After the application of SA, increasing tolerance of wheat seedlings to salt stress may be related to increases in antioxidative enzyme activitiy.

Key words: Wheat, salicylic acid, antioxidative enzyme activities, peroxidase (POX), catalase (CAT), hydrogen peroxide (H_2O_2) content.

INTRODUCTION

Plants are continuously exposed to biotic and abiotic stresses, and salt (NaCl) stress is one of the most severe abiotic stresses limiting plant productivity. If excessive amounts of salt enter the plant, it eventually rises to toxic levels in the older transpiring leaves, causing premature senescence, and reduces the photosynthetic leaf area of the plant to a level that cannot sustain growth (Munns, 2002). Salt stress can affect several physiological processes, from seed germination to plant development.

Salt stress increases the formation of reactive oxygen species causing deterioration of membrane functions (Erdal et al., 2010). Plants have evolved enzymatic and non-enzymatic defense mechanisms in order to reduce oxidative damages by detoxifying free radicals. The enzymatic defense system includes peroxidase (POX) and catalase (CAT). These enzymes detoxify H_2O_2, an active oxygen species, to H_2O. Elucidation of the mechanisms by which plants perceive and transduce these stresses is critical if the plant response and introduced genetic or environmental improvement to stress tolerance are understood (Borsani et al., 2001).

The role of salicylic acid (SA) in defense mechanisms as a signal molecule that induces the plant defense response has been known for several years. In recent years its role has been widely investigated in both biotic and abiotic stresses. A large body of evidence has shown that SA is a key endogenous signal involved in plant defense responses to environmental stressors such as ozone, low temperature, and salinity as well as pathogen infection. In contrast, high levels of SA are known to potentiate the oxidative damage caused by ozone (O_3) exposure and

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Abbreviations: SA, Salicylic acid; **POX,** peroxidase; **CAT,** catalase; **SOD,** superoxide dismutase; **ROS,** reactive oxygen species.

Table 1. Effect of SA on fresh and dry weights of roots and shoots of wheat seedlings.

Treatment	Fresh weight shoot (g)	Dry weight shoot (g)
0	0.141±0.0075	0.0202±0.0006
80 mM NaCl	0.11±0.0025	0.0187±0.0007
120 mM NaCl	0.09±0.0036	0.0144±0.0003
SA	0.184±0.0061	0.0255±0.0012
80 mM NaCl+SA	0.141±0.0065	0.0228±0.0008
120 mM NaCl+SA	0.12±0.0032	0.0195±0.0006

All values are mean of at least three determinations with 2 replicates. Mean±SD.

chilling temperature. SA has been reported to show apparent contrasting effects, that is, the reduction or promotion of stress tolerance (Sawada et al., 2008).

The aims of the present work are to investigate the effects of foliar-applied SA on salt sensitivity, H_2O_2 generation and antioxidative enzyme activities in wheat seedlings.

MATERIALS AND METHODS

Plant material, salicylic acid and salt treatment

Sterilized seeds of wheat (*Triticum aestivum* L., Mv Emma) were germinated in an incubator at 30°C for 48 h in the dark and then transferred to a growth chamber under a 12 h photoperiod (25/20 8C; day/night, 200 mmol m^2 s^{-1}) and 60% relative humidity for 2 weeks. For the SA treatment and salt stress, the seedlings were pretreated by spraying 0.5 mM SA solution (pH 6.8) 1 day before the salt stress with an atomizer onto the leaf blades until it ran off. Distilled water (pH 6.8) was applied to the leaves of control plants. In the standard nutrient solution (Hogland), NaCl was added to 14 day old seedlings to adjust to 0 (control), 80 and 120 mM concentrations, respectively. After, plant root and leaves were harvested, frozen in liquid nitrogen, and then stored at -80°C for determination of antioxidant enzyme activities (peroxidase and catalase) and hydrogen peroxidase contents.

Determination of peroxidase and catalase enzyme activities

Harvested root and leaves samples (500 mg) were homogenized in 5 ml 10 mM potassium phosphate buffer (pH 7.0) containing 4% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 12,000 x *g* for 30 min at 4°C, and the supernatant obtained was used as an enzyme extract (Erdal and Dumlupinar, 2010a, b; Erdal and Demirtas, 2010; Erdal et al., 2010).

The POX activity was measured by monitoring the increase in absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5) containing 1 mM guaiacol and 0.5 mM H_2O_2 (Janda et al., 2003). One unit of POX activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01/min.

The CAT activity was measured by monitoring the decrease in absorbance at 240 nm in 50 mM phosphate buffer (pH 7.5) containing 20 mM H_2O_2. One unit of CAT activity was defined as the amount of enzyme that used 1 µmol H_2O_2/min (Gong et al., 2001).

Determination of H_2O_2 content

Hydrogen peroxide levels were measured according to He et al. (2005). Absorbance values were calibrated to a standard curve generated with known concentrations of H_2O_2.

Statistical analysis

Each experiment was repeated at least three times with two replicates. Statistical analysis was performed using one way analysis of variance (ANOVA). $P \leq 0.05$ was considered as significant. Error bars are in figures, mean ± SD.

RESULTS

Fresh and dry weights

Fresh and dry weights decreased significantly with exposure to NaCl salinity and reduction was severe at 120 mM of NaCl treatment without SA in both root and shoots. Exogenously treated 0.5 mM SA increased fresh and dry weights in both saline and non-saline conditions (Table 1). Compare to control level, SA without NaCl was the highest fresh and dry weight group.

Catalase activity

CAT activity was determined in both root and leaves of wheat seedlings to see the further effect of SA on antioxidant enzyme activities under saline conditions. During NaCl treatments, CAT activity was inhibited by both 80 and 120 mM NaCl levels on wheat leaves (Figure 1). Statistically, most significant inhibition was recorded at 120 mM NaCl application. The highest CAT activity was recorded on solely SA treatment. Similarly, CAT activity was also strongly repressed with SA+80 mM NaCl and SA+120 mM NaCl treatments compared to control plants. Althought the variation of CAT activities on the wheat root samples was not statistically important unlike the leaves where the activities obtained in SA+80 mM NaCl and SA+120 mM NaCl treaments were high compared to solely NaCl treated plants without SA.

Peroxidase activities

Compared to control, salt stress caused a significant

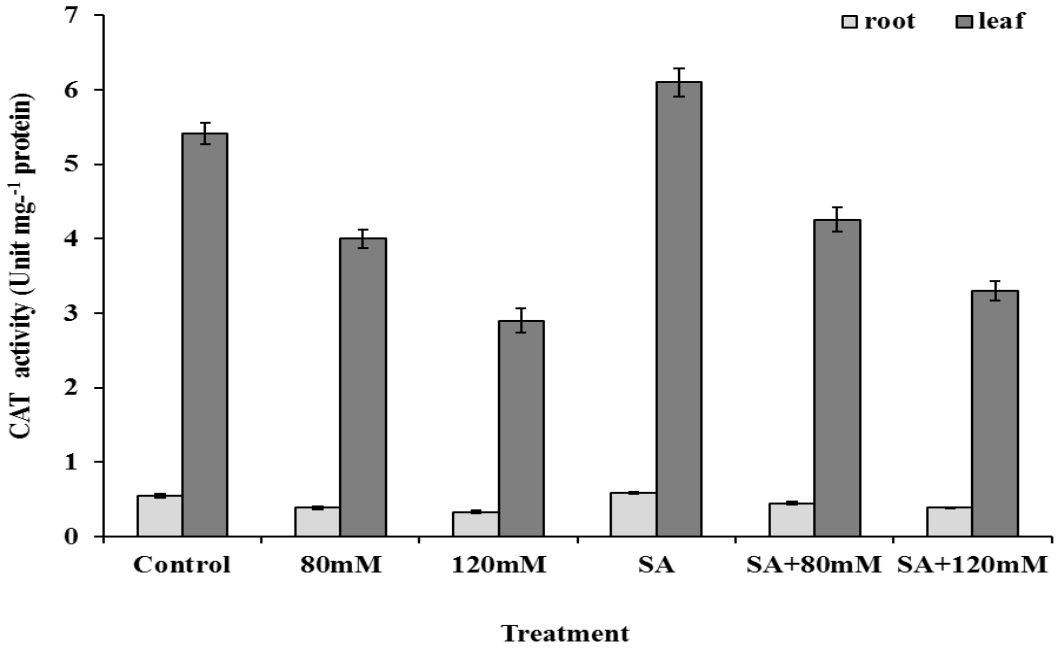

Figure 1. Effect of SA on changes in CAT activity in root and leaves of wheat seedlings under increasing concentration of NaCl.

increase ($P < 0.05$) in the POX activity in both organs. Exogenous SA combined with NaCl increased more than in only NaCl (Figure 2). In the wheat roots, POX activity began to increase with NaCl treatments and continued to increase throughout the SA addition however, it was an over activity on SA+120 mM NaCl. The highest activities of POX were calculated on 120 mM NaCl and SA+120 mM NaCl groups of wheat leave samples.

H_2O_2 content

The H_2O_2 content of wheat was statistically enhanced as a result of all treatments groups in leaves and roots samples. On the salinity conditions, H_2O_2 contents of leaves and roots increased but at first, SA level was sharply inhibited before this increase (Figure 3). The highest H_2O_2 contents were recorded at 120 mM NaCl in both organs.

DISCUSSION

Plants growing in saline soils face three main problems: High salt concentrations in the soil solution (that is, high osmotic pressure and correspondingly, low soil water potential "drought stress"), high concentrations of potentially toxic ions (such as Na^+ and Cl^-), and nutrient imbalance as a result of depressed uptake, impaired internal distribution and shoot transport of minerals (Gunes et al., 2007).

Since SA is necessary for the induction of antioxidant defenses and maintaining the redox state of the gluthatione pool, it has been shown to be essential for the plant protection against oxidative stress (Borsani et al., 2001). Therefore, in the present study, the effects of exogenously treated SA on wheat seedlings growing under salt stress was investigated.

These results demonstrated that salt stress decreased wheat seedlings' fresh and dry weights. However, SA treatments positively affected them when compared to their salt-treated controls (Table 1). Exogenous application of SA through the rooting medium had an ameliorative effect as well as growth promoting effect under non-saline and saline conditions (Arfan et al., 2007). These results were similiar to earlier studies which showed that exogenous application of SA promotes growth and counteracts the stress-induced growth inhibition in some crop species (Tari et al., 2002; Singh and Usha, 2003). While working with wheat, Singh and Usha(2003) reported that foliar spray with SA counteracted growth inhibition caused by water stress, one of the major factors caused by salinity stress in plants. Increase in growth of wheat under non-saline or saline conditions from which SA treatment resulted, can be attributed to an increase in photosynthesizing tissue, that is, the leaves (Dhaliwal et al., 1997), which is in agreement with the results obtained.

Figure 1 displayed the CAT activities of the root and leaves in the wheat stressed by NaCl treatment. The CAT

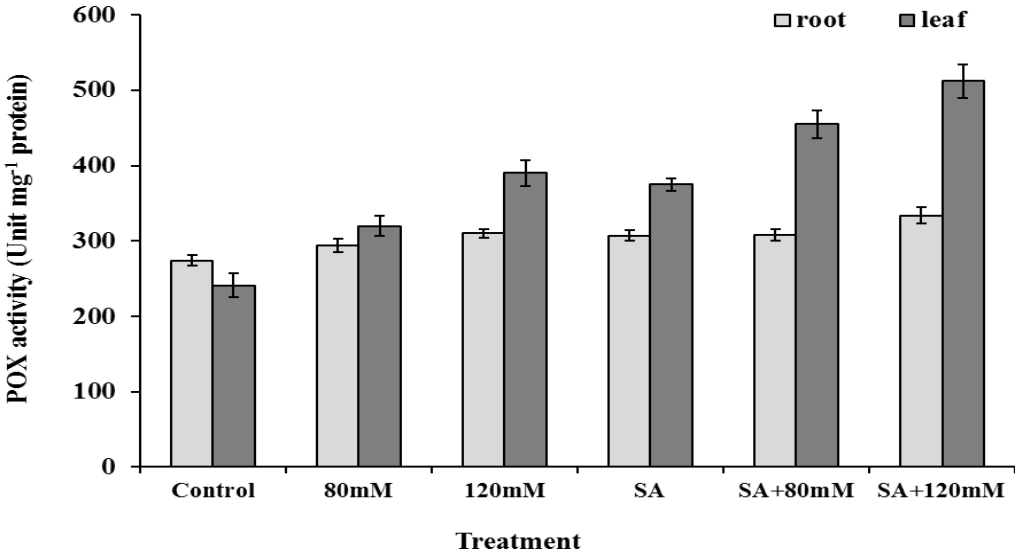

Figure 2. Effect of SA on POX activity in wheat seedlings under increasing concentration of NaCl.

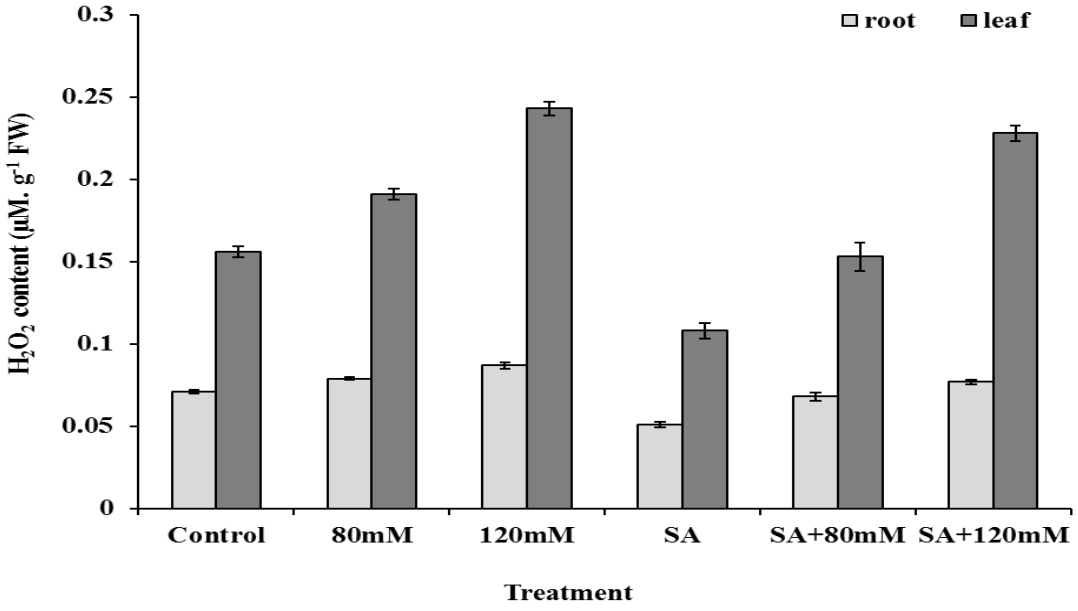

Figure 3. Effect of SA on H_2O_2 content of in root and leaves wheat seedlings under increasing concentration of NaCl.

activity was reduced by salt stress and this indicated that CAT activity was affected by oxidative stress as well as salt stress. The decrease in CAT activity by salt stress is a phenomenon that occurs in many kinds of plant species, not only in the gramineous species like rice and wheat. This phenomenon has been reported in pea and rye treated with NaCl (Shim et al., 2003). In some cases, however, an increase in CAT activity after NaCl treatment was observed in rice (Lin and Kao, 2000) and cucumber (Lechno et al., 1997), indicating that the responses may be different according to the intensity of the stress, plant part, time assayed after stress treatment, and induction of new isozyme(s) (Shim et al., 2003). Since SA improved the photosynthetic performance of plants under stress conditions (Ananieva et al., 2002), and chlorophyll a fluorescence could give insight into the ability of a plant to

tolerate environmental stresses, Szepesi et al. (2005) determined the fluorescence induction parameters in SA-pretreated samples under salt and osmotic stress, and also declerated that SA pre-treatment decreased the CAT activity both in the roots and leaves. The amount of catalase activity and its sensitivity to SA were determined for two fractions by Paloma and Klessig (1994), and it was shown that cross-linking the catalase subunits may destroy much of the catalase activity. However, cross-linking appears to have much less effect on SA binding. Both tobacco and tomato extracts contained high SA binding activity, and most of the remaining CAT activity are inhibitable by SA. Cross-linking is probably due to the activity of phenol oxidases, and the extent of cross-linking may reflect differences in the levels of phenol oxidases or their substrates (phenolic compounds) in the leaves of the various plants.

POX activity showed an opposite trend to CAT, that is, it increased both in root and leaves tissues as the concentrations of salt increased in the growth medium (Figure 3). In general, at all the concentrations, leaves had a much higher POX activity than the roots. Salt-tolerant plants are better protected from oxidative stress by antioxidant enzymes, therefore, it is thought that increasing the antioxidant enzyme levels under salinity is an effective strategy to confer salt tolerance in salt-sensitive plants (Yamane et al., 2004). POXs should play a more significant role than CAT in detoxifying the produced H_2O_2 since the activity of POX increased, in contrast to that of CAT (Dey et al., 2007). Besides, it is well known that CAT is less efficient than POX in scavenging H_2O_2 because of its low substrate affinity (Mutlu and Atici, 2009; Erdal and Dumlupinar, 2010a, b). Therefore, as long as the stress level does not exceed the plant's defensive capacity, the main response to pollutants is provided by an increase in superoxide dismutase (SOD) and POX activities (Mutlu and Atici, 2009). The augmentation in POX activity which was used as a biomarker of stress might be due to increased release of peroxidases localized in cell walls as reported in rice under stress situations since it participates in lignin biosynthesis, causing a physical barrier against toxic pollutants (Verma and Dubey, 2003; Zhang et al., 2007; Mutlu and Atici, 2009; Kose et al., 2011).

There was an increase in H_2O_2 content in the seedlings under high salinity (Figure 3). Similar to this results, Velikova et al. (2000) reported that after stress, an increased in H_2O_2 content was observed, and they emphasized that H_2O_2 can diffuse relatively long distance causing changes in the redox status of surrounding cells and tissues, whereas at relatively low concentrations, it initiates an antioxidative response. Rather than just the scavenging capacity, a fine tuning of H_2O_2 levels was expected to be determined for efficient stress control. The rationale of this assumption is that H_2O_2, while it is deleterious to some cellular components, is also essential to plants in various biosynthetic reactions, and as suggested by some studies, are possibly involved in signal transduction pathways that could contribute to plant defense. It has been suggested that the accumulation of H_2O_2 levels caused by various environmental stresses would require the combined activity of catalase and peroxidase in order to protect plant cells. H_2O_2 generated at the intercellular space of the plant subjected to salt stress appears to diffuse first into the cytosol where cytosolic POXs are localized, and then into peroxisome where catalase is typically found (Lee et al., 2001).

Consequently, the present paper showed that reactive oxygen species (ROS) induced decreased in fresh and dry weights of root and leaves for wheat seedlings under salt stress and can be ameliorated exogenously when treated with SA. The findings support that SA treatment can enhance resistance of wheat seelings by increasing antioxidant enzyme activity and photosynthetic tissue. However, the effect mechanism of SA needs further investigations.

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