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EXOGENOUS APPLICATION OF SALICYLIC ACID ENHANCES ANTIOXIDATIVE CAPACITY IN SALT STRESSED SUNFLOWER (*HELIANTHUS ANNUUS* L.) PLANTS

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Abstract

Salicylic acid (SA) is a growth regulator that promotes growth of plants under stress and non-stress conditions. The present study was conducted to assess alteration in antioxidative capacity of salt stressed sunflower plants due to foliar applied SA. Two hybrid lines of sunflower (Hisun-33 and SF-187) were grown under non-saline (control) or saline (120 mM NaCl) conditions. Varying levels of salicylic acid (0, 100, 200, 300 mg L^{-1}) were applied foliarly. Activities of antioxidant enzymes (superoxide dismutase, catalase and peroxidase) of both cultivars increased due to salt stress. Foliar applied SA caused a significant increase in leaf SOD and POD activity. However, leaf CAT activity remained almost unchanged due to SA application under both control and saline conditions, particularly in SF-187. Furthermore, increase in growth and photosynthetic capacity of both cultivars due to exogenously applied SA may have been due to SA-induced increase in activity of peroxidase.

Introduction

Salicylic acid (SA) acts as a potential non-enzymatic antioxidant as well as a plant growth regulator, which plays an important role in regulating a number of plant physiological processes including photosynthesis (Fariduddin *et al.*, 2003; Singh & Usha, 2003; Waseem *et al.*, 2006; Arfan *et al.*, 2007). Some earlier reports show that exogenous SA could ameliorate the damaging effects of heavy metals in rice (Mishra & Choudhuri, 1999), drought stress in wheat (Waseem *et al.*, 2006), and salt stress in wheat (Arfan *et al.,* 2007). These observations suggest that SA being an oxidant could be linked to oxidative stress.

Both mitochondria and chloroplasts through their respective electron transport systems can generate reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and singlet oxygen ($^1O_2$) (Mittler, 2002). However, ROS are scavenged by plant antioxidant defense systems, comprising both enzymatic and non-enzymatic components (Ashraf, 2009). Environmental stresses, such as salt stress, may lead to an imbalance between antioxidant defenses and ROS levels, resulting in oxidative stress (Foyer & Noctor, 2003). Salt induced high production of ROS can cause damages to mitochondria and chloroplasts (Apel & Hirt, 2004; Smirnoff, 2005). In addition, from a number of studies it is evident that the efficiency of the antioxidative systems is correlated with tolerance to salt stress (Athar *et al.*, 2008; Munns & Tester, 2008). Other studies have shown that exogenous SA can regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stress (Li *et al.*, 1998; He *et al.,* 2002). In view of these reports, it is suggested that salt tolerance can be induced by enhancing antioxidant capacity of plants. However, SA cannot induce abiotic stress

tolerance in all types of plants or in other words the effectiveness of SA in inducing stress tolerance depends upon type of species or concentration of SA applied (Borsani *et al.*, 2001; Németh *et al*., 2002; Waseem *et al.*, 2006; Arfan *et al*., 2007). In our previous studies, it was found that exogenous application of SA improved the growth of sunflower plants (Noreen & Ashraf, 2008). Therefore, the present study reveals whether foliar applied SA could alter the activities of antioxidant enzymes which might have been involved in salt tolerance of sunflower plants.

Materials and Methods

The study was conducted under greenhouse conditions at the Department of Botany, University of Agriculture, Faisalabad Pakistan under the growth conditions described earlier (Noreen & Ashraf, 2008). Achenes of sunflower SF-187 and Hisun-33 hybrid lines were obtained from the Regional Office of the Pakistan Seed Council Faisalabad, Pakistan.

The design of the experiment was a completely randomized with four replicates. Two levels of NaCl i.e., 0 and 150 mM in full strength Hoagland's nutrient solution were applied to 18-day old sunflower plants. Varying concentrations of salicylic acid (SA) (M. wt. = 138.1) [(0, 0.1% Tween 20 solution; 100, 200 and 300 mg L-1 in 0.1% Tween-20 {polyoxyethylene sorbitan monolaurate} solution)] were applied as a foliar spray at the vegetative stage. Five ml of SA solution was sprayed to each plant with a manual pump. The plants were sprayed once on the leaves early in the morning. After three weeks of foliar SA application plants were harvested and data for fresh biomass recorded. These plants were then oven-dried at 65^{o}C for 72 h and dry biomass recorded. However, before harvest, following physiological parameters were also measured:

Extraction of antioxidant enzymes: Fresh leaves (0.5 g) from plants of both sunflower lines harvested at the vegetative stage were triturated in 8 ml of 50 m*M* cold phosphate buffer (pH 7.8) and centrifuged at 15000 x *g* for 20 min at 4°C. The supernatant was used for appraising the activities of antioxidant enzymes.

Superoxide dismutase (SOD): The activity of SOD was assayed following the method of Giannopolitis & Ries (1977) by measuring the enzyme ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction solution (3 ml) contained 50 µ*M* NBT, 1.3 µ*M* riboflavin, 13 m*M* methionine, 75 n*M* EDTA, 50 m*M* phosphate buffer (pH 7.8), and 20 to 50 µl enzyme extract. The reaction solutions contained in test tubes were irradiated under a light (15-W fluorescent lamps) at 78 µmol m^{-2} s^{-1} for 15 min. The absorbance of the irradiated solution at 560 nm was read with a spectrophotometer (Hitachi U-2100, Tokyo, Japan). One unit of SOD activity was considered as the amount of enzyme which caused 50% inhibition of photochemical reduction of NBT.

Catalase (CAT) and peroxidase (POD): Activities of CAT and peroxidase (POD) were appraised using the method of Chance & Maehly (1955) with some modifications. The CAT reaction solution (3 ml) contained 50 m*M* phosphate buffer (pH 7.0), 5.9 m*M* H_2O_2, and 0.1 ml enzyme extract. The reaction was started by adding the enzyme extract. Changes in absorbance of the reaction solution at 240 nm were read spectrophotometrically every 20 sec. One unit CAT activity was defined as an absorbance change of 0.01 units per min. The POD reaction solution (3 ml) contained 50 m*M* phosphate buffer (pH 5.0), 20 m*M* guaiacol,

40 m*M* H_2O_2, and 0.1 ml enzyme extract. Changes in absorbance of the reaction solution at 470 nm were recorded every 20 sec. One unit POD activity was considered as an absorbance change of 0.01 units per min.

The activity of each enzyme was expressed on protein basis. Protein concentration of the crude extract was measured following Bradford (1976).

Statistical analysis: Analysis of variance of all parameters was computed using the Costat computer package.The least significance difference between the mean values was calculated following Snedecor & Cochran (1980).

Results and Discussion

Salt stress caused a significant reduction in shoot fresh weights of both sunflower lines. However, growth of both lines was appreciably promoted due to exogenous application of salicylic acid under both salt-stressed and non-stressed conditions.

Salinity stress caused a marked increase ($p<0.001$) in the activities of three antioxidant enzymes in the leaves. Foliar applied SA caused a significant ($p<0.001$) increase in the activity of leaf SOD in the salt-treated and control plants of sunflower lines, particularly at 300 mg L^{-1} SA level in salt stressed plants (Fig. 1). Leaf guaiacol peroxidase (POD) activity in both lines of sunflower also increased ($p<0.001$) due to foliar applied SA in both salt stressed and control plants (Fig. 1). Moreover, leaf POD activity was higher in SF-187 than that in Hisun-33 due to foliar applied 300 mg L^{-1} SA under saline conditions. The increase in POD activity in the salt stressed plants was associated with net CO_2 assimilation rate (*A*) and shoot fresh weight of sunflower lines (Fig. 1), suggesting that POD activity has a role in growth. In contrast, CAT activity remained almost unchanged due to SA application in the salt stressed plants of SF-187 (Fig. 1). However, leaf CAT activity in the salt stressed plants of Hisun-33 significantly increased due to foliar applied 300 mg L^{-1} of SA.

In our previous study (Noreen & Ashraf, 2008), it was found that SA-induced increase in growth could be related to SA-induced considerable enhancement in net photosynthetic rate under salt stress, particularly at 200 mg L^{-1} SA level (Noreen & Ashraf, 2008). It has already been reported in a number of plant species that a decrease in CO_2 concentration inside the chloroplasts causes a decrease in $NADP^+$ concentration coupled with the generation of reactive oxygen species (ROS) (Foyer & Noctor, 2003; Souza *et al*., 2004). Among ROS, superoxide radicals are most damaging to cellular structures. Superoxide dismutase (SOD), a key enzyme in cellular defence, catalyses the dismutation of superoxide radicals to H_2O_2 and O_2 (Foyer & Noctor, 2000). In the present study, enhanced activities of SOD due to SA application might have been one of the factors contributing to improved growth in sunflower plants under saline conditions. Similar to our results, some reports have shown that salt stress induces an increase in SOD activity, and this has frequently been correlated with plant salt tolerance (Sreenivasulu *et al*., 2000; Sudhakar *et al*., 2001). For example, in a salt-tolerant rice cultivar, SOD activity was higher as compared to that in a salt sensitive cultivar (Dionisio-Sese & Tobita, 1998). Although leaf SOD activity and shoot fresh weight (mean data presented in Noreen & Ashraf, 2008) bear some degree of positive correlation, net CO_2 assimilation (a major growth controlling factor in our previous studies, Noreen & Ashraf, 2008) and activity of leaf SOD are not positively correlated with each other (Fig. 2).

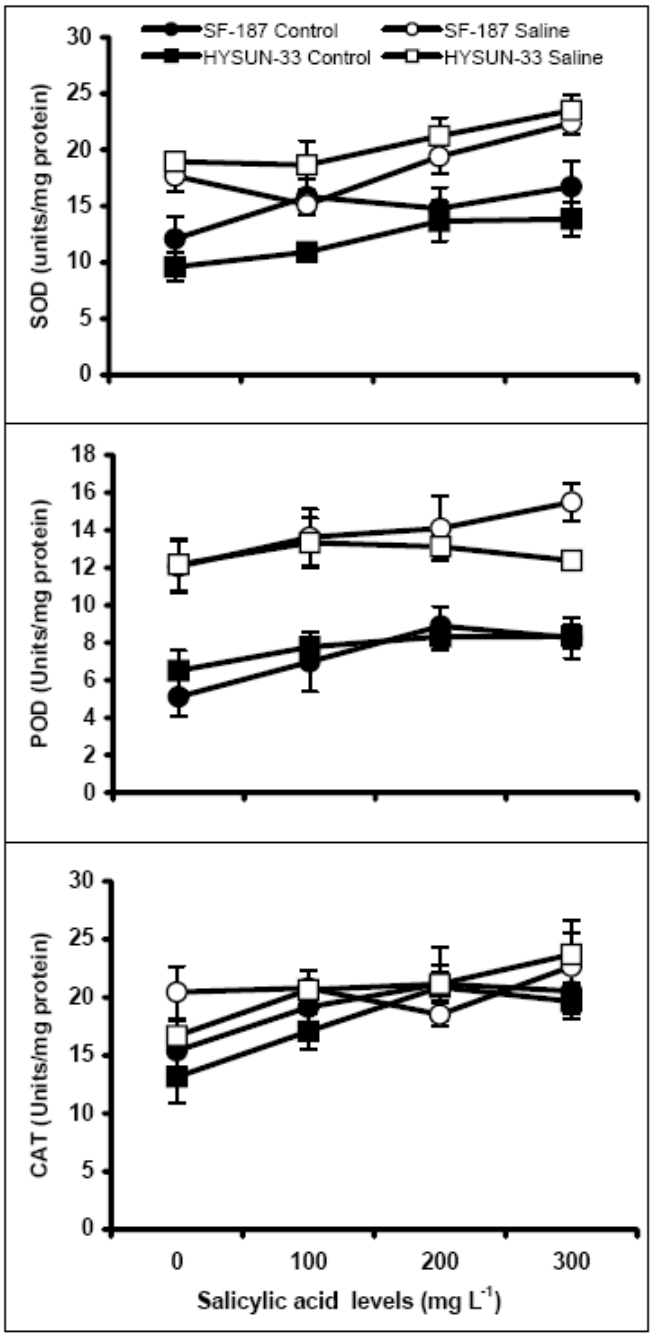

Fig. 1. Activities of antioxidant enzymes of salt stressed and non-stressed plants of two hybrids of sunflower when different concentrations of SA was applied as a foliar spray at the vegetative stage.

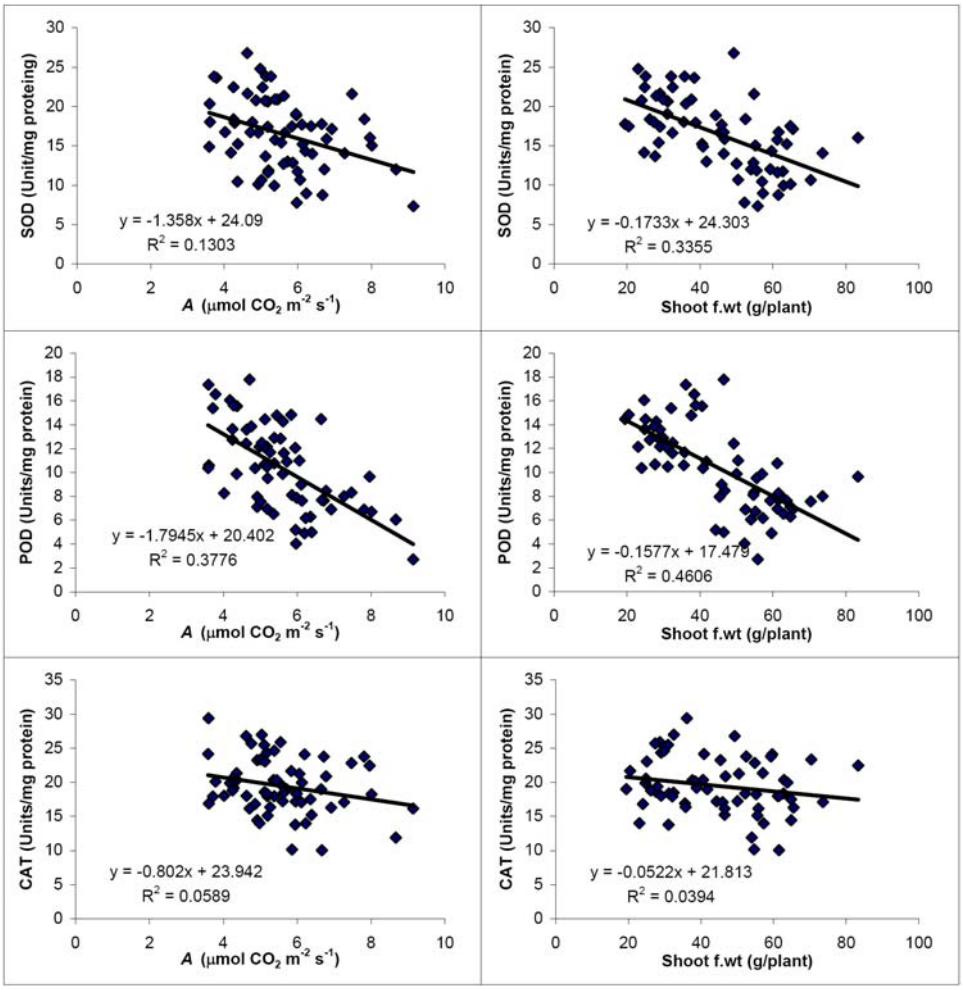

Fig. 2. Relationships between net CO_2 assimilation rate (*A*) or shoot fresh weight and activities of antioxidant enzymes of salt stressed and non-stressed plants of two hybrids of sunflower when different concentrations of SA applied as a foliar spray at the vegetative stage.

In contrast to the activity of SOD, leaf POD activity of sunflower lines is positively correlated with both shoot fresh weight and net CO_2 assimilation (Fig. 2). Increased POD activity in sunflower lines caused by salinity and SA application is well established (Dionisio-Sese & Tobita, 1998; Sudhakar *et al*., 2001; El-Tayeb, 2005), and appears to be caused by over-expression of genes coding for peroxidases (Mittal & Dubey, 1991).

Leaf CAT activity of both sunflower lines slightly increased under salt stress, but it remained almost unchanged due to SA application except in the salt stressed plants of Hisun-33. These results can be explained in view of the findings of Hertwig *et al*., (1992) that salt stress could provoke CAT protein degradation by endogenous proteases. In addition, if salt induced degradation of CAT protein exceeds biosynthesis, CAT activity will decrease (Feierabend & Engel, 1986). Furthermore, non-significant change in CAT

activity due to SA application might have been due to increase in endogenous level of SA, which might have inhibited the CAT activity -a phenomenon that occurs in many plant species exposed to oxidative stress (Shim *et al*., 2003).

In view of all these findings, it is suggested that SA-induced enhancement in growth of sunflower lines might have been due to SA-induced increase in antioxidant capacity, but this was mainly due to enhanced activity of leaf POD.

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