

RESEARCH ARTICLE

Ameliorating Effects of Foliar Applied Salicylic Acid on Growth, Chlorophyll Contents and Antioxidant Enzymes of Wheat Genotypes under Salinity Stress

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ARTICLE INFO

Received: Sep 04, 2018

Accepted: May 31, 2019

Keywords

Catalase

Chlorophyll content

Growth

Salinity

Superoxide dismutase

Wheat

ABSTRACT

Salinity is an important abiotic stress affecting agricultural productivity in Pakistan. In order to meet the country's requirement, the sustainability of crop production is pre-requisite. A hydroponic experiment was conducted to check the response of wheat cultivars against salinity with salicylic acid application. There were four wheat varieties (Lasani-2008, MH-97, SARC-1 and Iqbal-2008), two levels of NaCl (75 mM and 150 mM) and two levels of salicylic acid (0.25 mM and 0.50 mM) along with their respective controls. This experiment was conducted in a completely randomized design with factorial arrangement and five replications of each. After one month of transplanting in hydroponic culture, plant growth parameters and chlorophyll contents were determined. The results showed that plant growth was highly affected at both levels of NaCl treatments, while a decrease in chlorophyll contents and K⁺ concentration was observed. However, the application of salicylic acid (0.25 mM and 0.50 mM) overcame the adverse effects of salinity and improved the plant growth significantly ($P \leq 0.05$) by increasing superoxide dismutase and catalase activities. The application of 0.25 mM salicylic acid in the presence of 75 mM NaCl enhanced shoot dry weight by 21% and 30.68% in Lasani-2008 and Iqbal-2008, respectively as compared to their respective control. The activity of superoxide dismutase was enhanced from 2.67 to 3.15 ($\mu\text{mol/g Fw}$) in SARC-1 when salicylic acid was applied in the presence of 150 mM NaCl. Under tested condition, SARC-1 performed better than others tested wheat genotypes. In conclusion, salicylic acid improved the performance of wheat varieties in saline conditions.

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INTRODUCTION

Soil salinity is an environmental problem, which affects the growth and development of many crops in arid and semi-arid regions (Yusuf et al., 2008). The salinity problem is being increased due to usage of poor-quality water along with poor drainage (Chinnusamy et al., 2005). Salinity affects physiological and biochemical processes of plants thereby decreasing the plant growth and

productivity (Khan et al., 2009). Different plant functions like protein synthesis and photosynthesis etc. are also affected by high levels of salts (Parida and Das, 2005).

Under salinity stress, there is enhancement in the formation of reactive oxygen species (Borsani et al., 2001). These cytotoxic oxygen species are strongly toxic to plants, may cause lipid peroxidation, damage to cellular membranes and protein denaturation (Liu et al., 2009). However, plants have different antioxidant

enzymes such as catalase (CAT), superoxide dismutase (SOD), etc. for protection against the harmful effects of reactive oxygen species. Superoxide dismutase scavenges O_2^- resulting in the formation of H_2O_2 and O_2 . Catalases and peroxidases scavenge hydrogen peroxide and convert it into water (Saffar et al., 2009). Salt stress affects the amount of enzymes that scavenge the oxygen radicals (Hernandez et al., 1993). These reactive oxygen species disturb the normal functioning by damaging mitochondria and chloroplasts (Mittler, 2002).

Salicylic acid (SA) is a well-known phenolic compound naturally found in plants (Aberg, 1981). It is considered a signaling molecule that plays role in stress tolerance and defense mechanisms of plants (Kang et al., 2012; Senaratna et al., 2000). Under salinity stress, the accumulation of reactive oxygen species in plant cells activates the scavenging pathways, which lead to the enhancement of antioxidant enzymes and depletion of reactive oxygen species (Beauchamp and Fridovich, 1971). Salicylic acid application modulates the antioxidant enzyme activity (Sakhabutdinova et al., 2003) under saline conditions.

Among cereal crops, wheat (*Triticum aestivum* L.) has prime importance and a principal diet of almost one third of the world's population. Wheat is ranked as a moderately salinity tolerant crop; however, yield parameters like tillering and grain weight were greatly affected by elevated external salinity (Naz et al., 2015; Iqbal et al., 2017).

Keeping in view the above facts, the present research was conducted to determine the effect of different levels of salicylic acid for increasing the tolerance of wheat to NaCl toxicity and to explore the differences in response of different wheat genotypes to salicylic acid application under NaCl stress.

MATERIALS AND METHODS

Seeds of four wheat genotypes (Lasani-2008, MH-97, SARC-1 and Iqbal-2008) were germinated in trays containing sand and the uniform size seedlings were transplanted in foam plugged holes of polystyrene sheet floating over $\frac{1}{2}$ Hoagland's nutrient solution (Hoagland and Arnon, 1950). Aeration was provided with the help of aeration pump and solution pH was maintained at 6 ± 0.5 , throughout the experiment. The salinity was developed by adding the NaCl salt according to respective treatment except control. Salicylic acid was applied as foliar spray by dissolving it in 95% Ethanol. There were five replications of each treatment.

There were nine treatments including control having no NaCl and salicylic acid. The treatments included: T_1 = control, T_2 = 0.25 mM SA, T_3 = 0.50 mM SA, T_4 = 75 mM NaCl, T_5 = 75 mM NaCl + 0.25 mM SA, T_6 = 75 mM NaCl + 0.50 mM SA, T_7 = 150 mM NaCl, T_8 = 150 mM NaCl + 0.25 mM SA, T_9 = 150 mM NaCl + 0.50 mM SA.

The chlorophyll contents of intact plants were determined before harvesting with SPAD-502 meter (Minolta, Osaka, Japan). After one month of transplanting, the crop was harvested. Shoots and roots of plants were cut into pieces, put into paper bags separately and sun dried. Then these bags were put into an oven at 65 °C till the constant weight obtained and weights of the sample were recorded.

For the extraction of antioxidant enzymes CAT and SOD, after homogenizing fresh leaves (0.5 g) in 5 ml of 50 mM cooled phosphate buffer of pH 7 and filtration, mixture was centrifuged at 15000 rpm for 20 minutes at 4°C, and then the supernatant was extracted in another test tube. This extract was used to determine SOD and CAT activity.

For SOD determination method of Giannopolotis and Ries (1977) was used. Catalase activity was determined by Chance and Maehly (1955) method.

For the determination of Na^+ and K^+ , the leaf sap was extracted by the method of Gorham et al. (1984). After dilution, sodium and potassium concentration in sap was measured by using Sherwood 410 Flame Photometer with the help of self prepared standard solution using reagent grade salts NaCl and KCl.

Statistical analysis

The data obtained from experiment were statistically analyzed using M-STATC Version 1.10 computer software package. Treatments having significant difference ($P \leq 0.05$) were subjected to Least Significant Difference (LSD) calculation for comparisons of their means (Steel et al., 1997).

RESULTS

Plant growth and chlorophyll contents

A significant ($P \leq 0.05$) effect of treatment and variety interaction for roots and shoots growth was observed. The roots and shoots dry weight of all genotypes was significantly decreased by NaCl salinity (Fig. 1a,b). The higher level of NaCl (150 mM) reduced the roots and shoots dry weight more as compared to the lower level of NaCl (75 mM). There was more reduction in shoots growth by salinity than roots growth. In saline conditions, the salicylic acid treatments (0.25 and 0.50 mM) improved the roots and shoots dry weight significantly. Among genotypes, reduction in roots and shoots dry weight was less in SARC-1 than other genotypes. The application of salicylic acid at lower level (0.25 mM) proved better for the alleviation of NaCl toxicity in wheat than its higher level.

Both levels of NaCl salinity reduced the chlorophyll contents (SPAD value) in all genotypes significantly ($P \leq 0.05$). There was found a remarkable increase in chlorophyll contents in the treatments where salicylic acid was applied (Fig. 1c). The application of 0.25 mM and 0.50 mM salicylic acid increased the chlorophyll

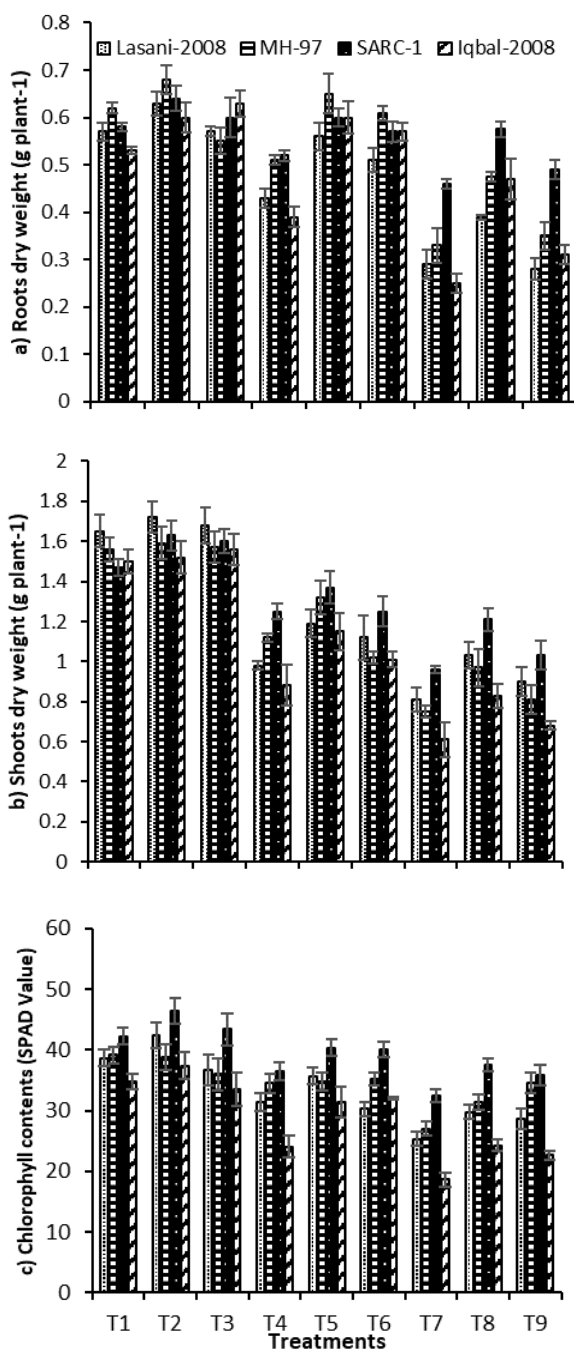


Fig. 1: Effect of salinity and SA application on wheat genotypes a) roots, b) shoots dry weights and c) chlorophyll contents. T₁ = control, T₂ = 0.25 mM SA, T₃ = 0.50 mM SA, T₄ = 75 mM NaCl, T₅ = 75 mM NaCl + 0.25 mM SA, T₆ = 75 mM NaCl + 0.50 mM SA, T₇ = 150 mM NaCl, T₈ = 150 mM NaCl + 0.25 mM SA, T₉ = 150 mM NaCl + 0.50 mM SA.

contents in saline conditions. The genotype SARC-1 maintained high chlorophyll contents and reduction in chlorophyll contents under saline condition was less in this genotype as compared to others tested genotypes.

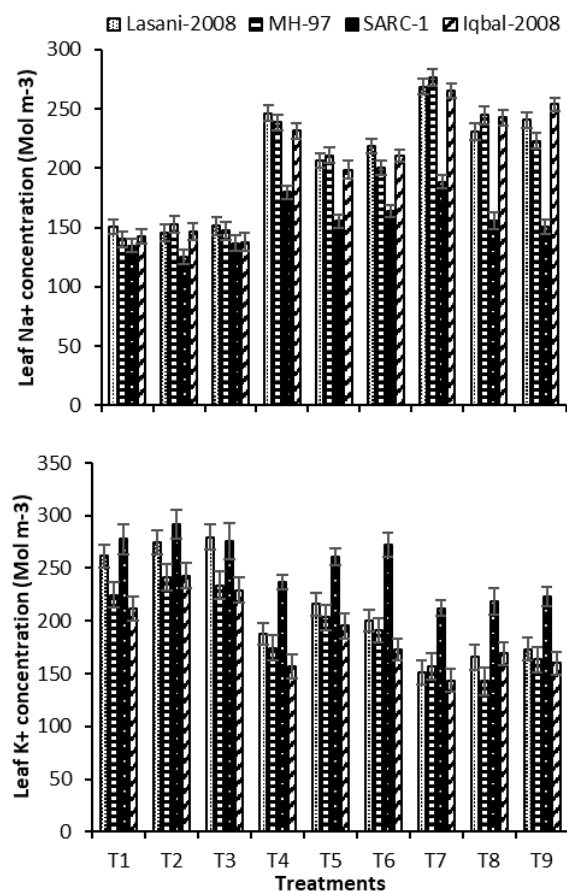


Fig. 2: Effect of salinity and SA application on a) leaf Na⁺ and b) leaf K⁺ concentration of wheat genotypes. T₁ = control, T₂ = 0.25 mM SA, T₃ = 0.50 mM SA, T₄ = 75 mM NaCl, T₅ = 75 mM NaCl + 0.25 mM SA, T₆ = 75 mM NaCl + 0.50 mM SA, T₇ = 150 mM NaCl, T₈ = 150 mM NaCl + 0.25 mM SA, T₉ = 150 mM NaCl + 0.50 mM SA.

Na⁺ and K⁺ concentration in leaf sap

A significant ($P \leq 0.05$) effect of treatment and variety interaction was found for leaf Na and K⁺ concentration. The salinity treatments significantly increased the Na⁺ but decreased K⁺ concentration in all wheat genotypes than their respective controls (Fig. 2a,b). However, applied salicylic acid remarkably reduced the Na⁺ and increased K⁺ concentration in wheat leaves. Under 75 and 150 mM NaCl levels, Na⁺ concentration increased in all genotypes as compared to control with simultaneous decrease in K⁺ concentration. However, in wheat genotype SARC-1, increase in Na⁺ was less than other genotypes and a relatively higher K⁺ concentration was found in it. The higher level of NaCl (150 mM) resulted in higher increase in Na⁺ and more reduction in K concentration in wheat leaves than lower level of NaCl (75 mM). In the absence of salinity both levels (0.25 mM and 0.50 mM) of salicylic had no effect on the leaf Na⁺ and K⁺ concentration. The

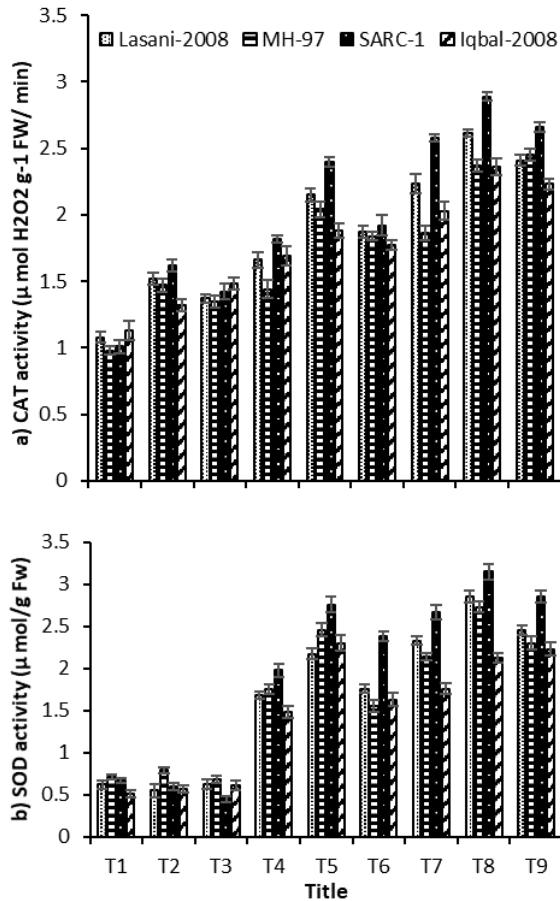


Fig. 3: Effect of salinity and SA application on activity of antioxidant enzymes of wheat genotypes: a) CAT and b) SOD. T₁ = control, T₂ = 0.25 mM SA, T₃ = 0.50 mM SA, T₄ = 75 mM NaCl, T₅ = 75 mM NaCl + 0.25 mM SA, T₆ = 75 mM NaCl + 0.50 mM SA, T₇ = 150 mM NaCl, T₈ = 150 mM NaCl + 0.25 mM SA, T₉ = 150 mM NaCl + 0.50 mM SA.

application of salicylic acid in the presence of salinity reduced Na⁺ and enhanced K⁺ concentration thereby alleviating the deleterious effects of salinity.

Catalase (CAT) and superoxide dismutase (SOD) activity

A significant treatment and variety interaction was recorded for CAT and SOD activity in wheat leaves. The activity of both CAT and SOD was significantly increased under saline conditions than control. Both levels of NaCl treatment (75 and 150 mM) enhanced CAT and SOD activity in all genotypes as compared to their respective controls (Fig. 3a,b). While the application of salicylic acid (0.25 mM and 0.50 mM) further increased the activity of both enzymes as compared to NaCl treatments. The activity of antioxidant enzymes CAT and SOD was higher in SARC-1 under saline conditions as compared to other genotypes. The lower level of salicylic acid was found

better than its higher level for providing defense against reactive oxygen species in wheat genotypes.

DISCUSSION

Soil salinity is an environmental problem, which affects the growth and development of many crops in arid and semi-arid regions (Yusuf et al., 2008). In present study, salinity negatively affected the growth of wheat and resultantly roots and shoots dry weight decreased in all wheat genotypes. It has been reported that salinity causes deleterious effects on plant growth by affecting physiological and biochemical processes of plants (Khan et al., 2009). Salt stress adversely affects plant growth such as fresh and dry weight of shoots and roots (Zhao et al., 2007) and plant height (Rui et al., 2009). Salinity stress causes the decline in relative leaf water content resulting in turgor loss, which in turn causes stomatal closure and reduces the carbon dioxide assimilation ultimately reduces the photosynthesis. Young seedlings are sensitive to salinity (Lutts et al., 1995). The leaf area is also negatively affected by salt stress (Yilmaz and Kina, 2008).

However, the application of salicylic acid alleviated the negative effects of salinity and enhanced roots and shoots dry weight under saline conditions in the present study. Salicylic acid induced tolerance against abiotic stresses has been reported previously e.g. in barley against heavy metals (Metwally et al., 2003) and salinity (El-Tayeb, 2005) and in wheat against drought (Singh and Usha, 2003). It is a natural plant hormone that affects many physiological and biochemical processes of plants (Arfan et al., 2007). El-Tayeb and Ahmed (2010) found that salicylic acid alleviated drought stress damages in wheat. Arfan et al. (2007) also reported that salicylic acid improved the roots biomass and overall plant growth. It also has influence on photosynthesis of plants (Khan et al., 2003). Salehi et al. (2011) also found significant increase in tomato plant growth due to the application of salicylic acid.

The chlorophyll contents of all wheat genotypes reduced in response to salt stress in the present study. The application of salicylic acid as foliar spray improved chlorophyll contents by mitigating the negative effects of salinity. El-Tayeb (2005) found that salinity stress decreased chlorophyll contents. But salicylic acid application increased chlorophyll contents. Yildirim et al. (2008) described that chlorophyll content considerably decrease under saline conditions due to proteolytic enzymes such as chlorophyllase, responsible for the chlorophyll degradation (Sabater and Rodriguez, 1978). Tari et al. (2002) reported that salicylic acid application at 100 mM NaCl decreased loss in chlorophyll a, b and carotenoids contents due to salinity. The foliar application of salicylic acid enhanced the photosynthetic

rate and chlorophyll content in maize under salt stress (Khodary, 2004). Yildirim et al. (2008) quantified the effect of foliarly applied salicylic acid on chlorophyll contents of cucumber plants grown under salinity stress and found that foliarly applied salicylic acid enhanced the chlorophyll contents by eliminating the deleterious effects of salinity.

In present study the concentration of Na^+ in leaf sap increased under saline conditions while that of K^+ decreased. The application of salicylic acid enhanced K^+ concentration and decreased Na^+ concentration in leaf sap probably due to its effects on membrane stability. Similar results have been reported previously. Abdi et al. (2011) reported that salt stress increased the Na content in both organs i.e. roots and shoot of tomato plants. Gunes et al. (2007) reported that concentrations of the Na^+ and Cl^- were higher in salt stressed maize plants and increasing levels of applied salicylic acid significantly decreased the Na^+ and Cl^- concentrations as compared to control plants in saline conditions. The salt treatments increased Na^+ concentration and decreased potassium concentration in maize plants but the application of salicylic acid increased the K^+ concentration (Fahad and Bano, 2012). Waseem et al. (2006) found increased the roots K^+ in wheat by applying salicylic acid under drought conditions. Parizi et al. (2011) quantified an increase in sodium concentration in sweet basil under saline treatments but under salicylic acid treatments, they found an increase in K^+ concentration in leaves. It has been reported that salicylic acid application under abiotic stresses influences membrane permeability (Cutt and Klessing, 1992). As a result of improved membrane stability, the uptake of Na^+ and K^+ leakage is reduced. El-Tayeb (2005) also described that exogenously applied salicylic acid had significant role in the stability of membrane in barley.

The activity of antioxidant enzymes in present study increased in all wheat genotypes in response to salinity stress. The salicylic acid application as foliar spray enhanced SOD and CAT activity (Fig. 3 a, b) thereby providing tolerance to plants in salinity stress. Szepesi et al. (2009) reported that salt tolerance is associated with enhanced antioxidant enzyme activity i.e. SOD, CAT etc. in *Solanum lycopersicum*. Janda et al. (2007) reported that salicylic acid improves the antioxidant capacity and induces the synthesis of antioxidant compounds resulting in acclimation of salinity stress. Kumara et al. (2010) marked a significant increase in SOD activity due to pretreatment of salicylic acid in Gerbera. Singh and Usha (2003) also found a remarkable increase in SOD in wheat seedlings due to salicylic acid application under drought stress. Mutlu et al. (2009) concluded that the improved activity of SOD in salt tolerant wheat cultivar due to salicylic acid application is associated with the induction of

antioxidant enzymes that help to protect the plants from salinity induced damage. Yusuf et al. (2008) reported the increment in the activity of CAT in mustard plant. Mutlu et al. (2009) found enhanced activity of CAT in wheat cultivars because of salicylic acid application. Application of salicylic acid modulates the activities of antioxidant enzymes SOD and CAT (Sakhabutdinova et al., 2003) under saline conditions.

Conclusion

Salinity is an important abiotic stress that severely affected the growth of tested four wheat genotypes. However, the application of salicylic acid provided tolerance against salinity and ameliorated harmful effects of salinity by increasing roots and shoot growth, lowering leaf Na^+ concentration, increasing chlorophyll contents, leaf K^+ concentration and activity of antioxidant enzymes. Among wheat genotypes SARC-1 showed higher tolerance to salinity and maintained growth and high $\text{K}^+:\text{Na}^+$ ratio in leaves, higher chlorophyll contents. The higher activity of antioxidant enzymes in this genotype provided tolerance against salinity. Moreover, it is concluded that application of 0.25 mM salicylic acid as foliar spray is a cheap and easier way to combat toxic effects of salinity on plant growth. Furthermore, the in-hand outcomes from this study need to be confirmed in field experiments and economic viability must be worked out.

Acknowledgements

We are highly thankful to Saline Agriculture Research Centre (SARC), University of Agriculture Faisalabad for the provision of research facilities to complete this experiment.

Authors' contributions

All authors contributed equally in this study. All authors read and approved the final draft before publication.

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