

Research Article



Physiological and Bio-Chemical Responses of Two Different Wheat Genotypes to Applied Salicylic Acid under Salt Stress

Muhammad Suhaib^{1*}, Ijaz Ahmad², Masooma Munir³, Badar-Uz-Zaman¹, Bushra Atta⁴ and Muhammad Khubaib Abuzar⁵

¹Land Resources Research Institute, NARC, Park Road, PO Box # 45500, Islamabad, Pakistan; ²Ecotoxicology Research Institute, NARC, Islamabad, Pakistan; ³Food Science and Product Development Institute, NARC, Islamabad, Pakistan; ⁴Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan; ⁵Earth and Environmental Sciences Department, Babria University, Islamabad, Pakistan.

Abstract | A hydroponic study was conducted to figure out the response of two different wheat genotypes supplemented with salicylic acid (0.25 and 0.5 mM) under salt stress (75 and 150 mM). It's an obvious factor that under salt stress certain reactive oxygen species are produced that damage the vital plant organelles causing disturbances in the normal functioning of the plant and even may lead to death in severe cases. The results of this study conferred that there were genotypic variation found in two genotypes regarding salinity tolerance. SARC-4 was found to tolerate more salt stress as compared to Parwaz-94 and performed better at both salinity levels than Parwaz-94. Salicylic acid induced improvement is significant regardless of stress environment or normal growth conditions. While regarding salicylic acid induced amelioration it was notable that both levels of salicylic acid had significant effects on the physiology and biochemical parameters of crop plants however lower level of salicylic acid 0.25 mM was found more efficient than the higher level of salicylic acid. Salicylic acid induced improvement is significant regardless of stress environment or normal growth conditions.

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***Correspondence** | Muhammad Suhaib, Land Resources Research Institute, NARC, Park Road, PO Box # 45500, Islamabad, Pakistan; **Email:** sahirsuhaib@gmail.com

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Introduction

Salinity a major abiotic stress accounts for the elevated production of reactive oxygen species (Borsani et al., 2001). These ROS are extremely reactive and if unchecked, they significantly disturb and deteriorate the normal plant functioning via affecting and damaging the lipids and protein bodies (Davies, 1987). These ROS further affect the mitochondria and chloroplasts at the cellular level (Mittler, 2002). Usually, plants have a natural defense mechanism in the form of antioxidant enzymes that protects the plants from

the harmful effects of reactive oxygen species. Among this chain of antioxidant enzymes defense machinery, Super-oxide-dismutase (SOD) is the major component enzyme that reacts with O_2^- and converts it into H_2O_2 and O_2 . The H_2O_2 produced in the above reaction is then alleviated by a number of enzymes like peroxidases and catalases (Dionisio-Sese and Tobita, 1998). Under salt stress the scavenging efficiency of these enzymes is also disturbed (Hernandez et al., 1993).

Under such conditions plants have naturally developed certain mechanisms at cellular and biochemical

levels to withstand highly saline environment. The processes involved in biochemical salinity tolerance mechanism induce the salinity tolerance in plants (Iyenger and Reddy, 1996) by bringing certain modifications in plant respiratory and photosynthetic processes along with the structural changes in crucial organelles like cell wall (Botella et al., 1994).

The mitigation of harmful effects of salinity could be achieved by the application of salicylic acid to the plants under saline environment (Borsani, 2001) as it improves the reactive oxygen species defense mechanism by improving the activity of essential antioxidant enzymes (Sakhabutdinova et al., 2004).

The biotic and abiotic stress tolerance induced by salicylic acid involves the reactive oxygen species (ROS) in primary signaling events that activate a number of signal transduction pathways. When salicylic acid is applied, NO is required for the induction of defense mechanisms (Zhang et al., 2007).

Abiotic stresses result in both altered levels of phyto-hormones and decreased plant growth (Morgan, 1990). An alternative strategy to ameliorate salt stress could be to use exogenous application of plant growth regulators (Tuna et al., 2008).

Wheat is considered as the most demanding and important cereal crop as it holds the position of staple food for the one third population of the world and is ranked number one among the cereals in Pakistan (FAO, 1998). According to a survey wheat covers an area of 8.75 million hectares yielding 22.57 million ton per year (FAO Statistics, 2012) and adding up 2.6 percent to the gross domestic product of the country (ESP, 2012).

The major production area of wheat is Punjab province that contributes an area of 6.91 hectares with production of 19041 tons from 2010-2011 (ASP, 2006).

Under these conditions this study was planned to assess the genotypic variation in two wheat genotypes regarding salt stress and extent of salicylic acid induced salt tolerance in two genotypes.

Materials and Methods

A one month saline hydroponic study was conducted in University of agriculture Faisalabad on two

genotypes of wheat crop (SARC-4 and Parwaz-94) supplied with two levels of salicylic acid (0.25 mM and 0.50 mM) to find the ameliorative efficiency of salicylic acid in wheat under imposed salinity levels (75 mM and 150 mM). Seeds of the wheat genotypes were sown in sand containing trays with maintained optimum moisture for germination. At two leaf stage the healthy seedlings were transplanted in 100 L tubs with 5 replicates containing $\frac{1}{2}$ strength Hoagland solution (Hoagland and Arnon, 1950) with thermo pore sheets floating over surface of solution having a pH maintained at 6 ± 0.5 on daily basis. Saline environment was imposed in the respective treatment tubs in three increments using NaCl within 10 days of transplantation. Salicylic acid treatments were applied using ethanol dissolved salicylic acid in respective treatment tubs.

Chlorophyll contents were measured 5 days before harvest with the help of chlorophyll meter and after one month the plants were harvested and growth parameters (shoot length, root length, shoot dry biomass and root dry biomass) were recorded manually. The bio-chemical parameters (total protein contents, superoxide dismutase and catalase activity) was measured analytically by following the standard procedures. Total soluble proteins in the plant extract were determined by following the standard procedure described by Bradford in 1976 using Bradford reagents and reading the absorbance at 595 nm on spectrophotometer. Plant extract was obtained by grinding the leaf sample in phosphate buffer with pH 7.8. Supernatant was obtained by subjecting the extract to centrifuge at 1200 rpm.

Further catalase activity was measured by the method described by Chance and Maehly (1955) using the same supernatant of plant extract, 50 mM phosphate buffer and 5.9 mM H_2O_2 and measuring the rate of H_2O_2 disappearance at 240 nm. Superoxide dismutase activity was recorded on spectrophotometer by measuring the inhibition of Nitro-blue-tetrazolium at 560 nm using the method of Giannopolotis and Ries (1977).

The data of the experiment was then statistically analyzed using CRD-Factorial arrangement (Steel and Torrie, 1987).

Results and Discussion

Effect of salicylic acid and salinity on root length (cm)

There was a significant effect of all treatments on root length of the maize plants. Under saline treat-

ments alone, the root length was significantly reduced as compared to control. A reduction of 21.60 and 28.92% in root length of SARC-4 and Parwaz-94 respectively was observed under 75 mM NaCl concentration while that under 150 mM NaCl was 21.60 and 50% respectively for both varieties. A significant improvement in growth under saline and non-saline conditions with the application of salicylic acid was quite clear. Lower level of salicylic acid 0.25 mM was found more effective with a recorded increase of 55.77 and 65.66% in root length of SARC-4 and Parwaz-94 respectively as compared to 0.50 mM salicylic acid level (51.26 and 44.58%) with respect to control. Same improvement factor was observed under salinity treatments as 83.09 and 95.12% (SARC-4 and Parwaz-94 respectively) increased root growth with 0.25 mM applied salicylic acid under 150 mM NaCl stress. The results for 0.50 mM S.A was also significant but lesser than that of 0.25 mM S.A treatment.

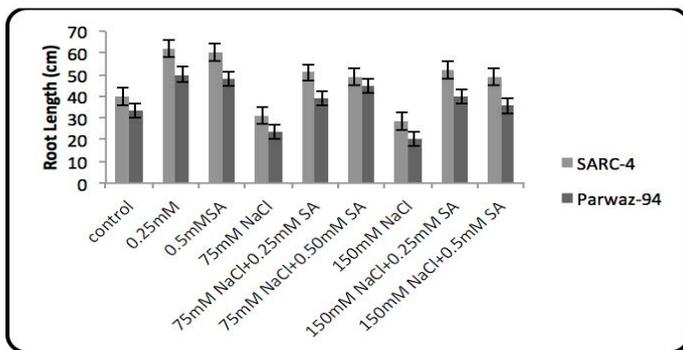


Figure 1: Root length.

Turkiyalmaz (2012) argues in favor of these results that salt stress causes a drastic drop in root length and shoot's height of wheat seedlings. But salicylic acid ameliorates the harmful effects of salinity and supports the plant to grow better under adverse salinity conditions. Waseem et al. (2006) also found the parallel results that application of salicylic acid to the soil improves the root growth and other physiological functions of wheat. These results are further supported by the findings of Szepesi et al. (2009) who reported that with the application of S.A under 100 mM NaCl in potato crop the root length was increased.

Effect of salicylic acid on shoot length (cm)

Effect of all treatments was significant on shoot length of both wheat varieties utmost showing the same trend as root length. A considerable reduction in shoot length was observed at both salinity levels 75 mM and 150 mM (see Figure 2). A reduction of 12.12 and 15.15% (SARC-4 and Parwaz-94 respec-

tively) in shoot height was found at 75 mM NaCl stress while that for 150 mM was 11.55 and 18.18% for SARC-4 and Parwaz-94 with respect to control.

Under control conditions the application of S.A (0.25 mM) shoot length was improved by 15.55 and 21.12% (SARC-4 and Parwaz-94 respectively). While same level of S.A(0.25 Mm) when applied to the saline treatment (150 mM NaCl)an increment of 33.16% and 48.14% was observed in SARC-4 and Parwaz-94 respectively with respect to the saline treatment. Both levels of S.A (0.25 mM and 0.50 mM) improved the plant shoot length in both saline and non-saline treatments.

From the above results it could be assessed that the application of salicylic acid under stress conditions improves the crop growth. These results are absolutely parallel to those of Jazi et al. (2011) who observed and concluded that under stress conditions (heavy metal) application of S.A in *Brassica napus* increases the shoot growth. Furthermore these results are strengthened by the argument of Turkiyalmaz (2012), that wheat plant growth was increased with the application of S.A as compared to those not supplemented with S.A. Alleviation of salinity hazards with S.A is well supported by Cornelia et al. (2011) who reported the stunted growth of shoot under salt stress in wheat plants while this effect was overcome when the wheat plants were supplemented with S.A.

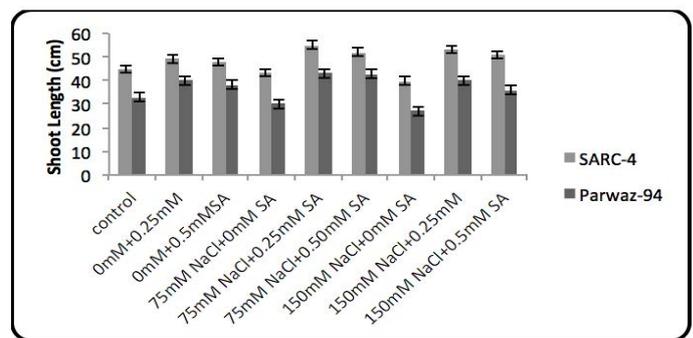


Figure 2: Salicylic acid and salinity effect on shoot length.

Effect of salicylic acid on root dry weight

Effect of S.A on dry-biomass was found significant under both stress and non-stress conditions. The saline treatments (75 mM and 150 mM) drastically decreased the root dry-biomass as compared to the control conditions. Negative impact of 150 mM salt stress on root dry bio-mass was more pronounced rather than that of 75 mM saline treatment. While the application of S.A (0.25 mM and 0.50 mM) healed the harmful effects of salinity and improved the

root dry biomass as compared to the control and saline treatments (Figure 3). At lower level (75 mM) of NaCl stress reduction of 12.93 and 6.97% in root dry weight of SARC-4 and Parwaz-94 respectively was recorded as compared to control. While the extent of hazard was multiplied with increasing level of salt stress (150 mM NaCl) causing a marked reduction of 20.12 and 22.37% in root dry biomass for SARC-4 and Parwaz-94 respectively. An increase of 18.96 and 16.51% in root dry biomass was recorded for SARC-4 and Parwaz-94 respectively with 0.25 mM S.A under non-saline conditions. While with higher level of salicylic acid application (0.50 mM) under normal soil conditions an increase of 12.07 and 9.30% root dry weight was observed in both genotypes (SARC-4 and Parwaz-94 respectively) as compared to control.

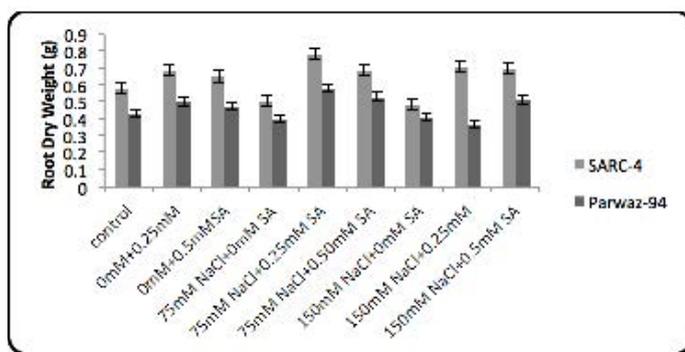


Figure 3: The salicylic acid effect on root dry weight.

The S.A application (0.25 mM) under 150 mM salt stress caused an increase (47.91 and 9.75%) in root dry weight in SARC-4 and Parwaz-94 respectively as compare to 150 mM saline treatment. While lower level of S.A (0.25 mM) in the presence of 75 mM NaCl improved the root dry biomass (54 and 45%) in SARC-4 and Parwaz-94 respectively as compare to 75mM salt stress. Both levels of S.A (0.5mM and 0.25mM) improved the root dry biomass but the increase was more pronounced in 0.25mM as compared to 0.5mM S.A application.

These results are supported by the findings of El-Tayeb and Ahmed (2010), who reported an increase in wheat plant dry biomass with the application of S.A under drought stress. Bhupinder and Usha (2003) findings also favors the improved dry biomass with application of S.A. The increase in dry biomass under salt stress indicates the alleviating effect of S.A and the findings of Baghizadeh et al. (2009) that the S.A application in okra under salt stress produced higher bio mass as compared to stress condition alone.

Effect of salicylic acid on shoot dry weight (g)

Salinity significant negative impact on the shoot dry weight of both cultivars but the adverse effect of 150 mM NaCl was more prominent as compare to 75 mM NaCl stress. At 75 mM, a reduction of 19.08% in shoot dry biomass was observed for Parwaz-94 as compared to control. But with the application of S. A adverse effects of salinity were mitigated and shoot dry biomass was improved under both saline and non saline conditions (see Figure 4). 9.30% and 7.94% increase in shoot dry weight was observed for SARC-4 and Parwaz-94 when supplemented with 0.25 mM S.A under control conditions. While under saline conditions treatment effect of 0.25 mM was also found significant with 32.66% and 51.03% increase in shoot dry weight for SARC-4 and Parwaz-94 respectively under 75 mM NaCl stress followed by 0.5 mM S.A application.

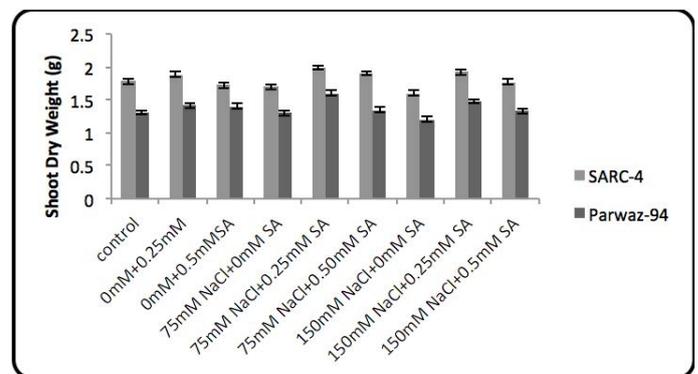


Figure 4: The salicylic acid effect on shoot dry weight.

The results are in alliance with Deef (2007), who found that pre-treatment with SA improved the growth and dry masses of plant root and shoot but the improvement was more pronounced in shoot rather than roots. While carrying a study on tomato plants Salehi et al. (2011) also reported a marked increase in the fresh and dry biomass of shoot with the application of SA. These results are also parallel to those of Fahad and Bano (2012) who found that under salt stress application of SA increased the shoot dry and fresh biomass in maize crop.

Chlorophyll contents (SPAD value)

Hazardous effect of salinity on plants is clear from the fact that both levels of salinity reduced the chlorophyll contents significantly in both cultivars. The salt stress had a drastic effect on physiological functioning and hence reduced the chlorophyll contents. A significant improvement in chlorophyll contents was recorded when SA was applied under salt stress (see

Figure 5). Both levels of SA (0.25 mM and 0.50 mM) improved the chlorophyll contents in both genotypes under control and stress environment. The NaCl toxicity of 75 mM reduced the chlorophyll contents as 11.11 and 14.86 in SARC-4 and Parwaz-94 respectively as compared to control. While 150 mM NaCl toxicity decreased the chlorophyll contents by 18.88 and 27.29% in SARC-4 and Parwaz-94 respectively with respect to control.

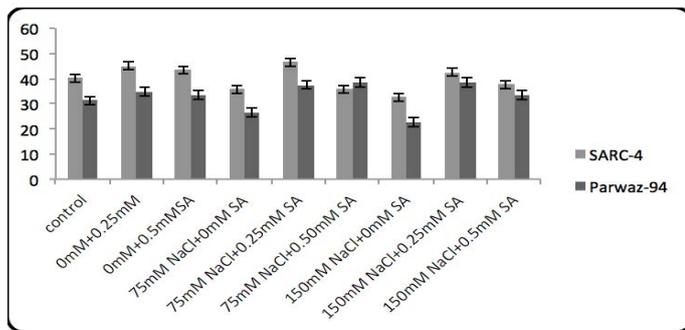


Figure 5: Effect of salicylic acid and salinity on chlorophyll contents.

The lower level of salicylic acid (0.25 mM) increased the chlorophyll contents as 11.95 and 11.82% in SARC-4 and Parwaz-94 respectively with respect to control. While 0.50 mM salicylic acid caused an increase by 8.34 and 7.50% in both varieties i.e SARC-4 and Parwaz-94 respectively as compare to control. The application of 0.25 mM under 75 mM NaCl toxicity increased the chlorophyll contents in SARC-4 (30.18%) and in Parwaz-94 (41.01%) as compare to 75 mM NaCl toxicity alone. The application of 0.5 mM salicylic acid in the presence of 150 mM NaCl enhanced the chlorophyll contents by 15 and 47% in SARC-4 and Parwaz-94 respectively as compare to 150mM NaCl alone.

El-Tayeb (2005) found remarkable decrease in chlorophyll contents in barley under salinity stress. But under salicylic acid treatments, he found an increase in chlorophyll contents (chlorophyll *a* and *b*). Yildirim et al. (2008) who indicated that chlorophyll content considerably decrease due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation under saline conditions. 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.

Total soluble proteins (µg/g FW)

Both levels of NaCl enhanced the total soluble proteins in both varieties (SARC-4 and Parwaz-94). The NaCl treatment of 75 mM enhanced the amount of total soluble proteins by 5.31 and 52.80% in SARC-4 and Parwaz-94 respectively as compare to control. While 150 mM NaCl increased the soluble protein contents in SARC-4 (51.10%) and Parwaz-94 (60.06%) with respect to control. While application of salicylic acid has given significant results regarding total soluble proteins (see Figure 6). Both levels of salicylic acid (0.25 mM and 0.50 mM) significantly enhanced the total soluble proteins. The lower level of salicylic acid under non-saline conditions enhanced the protein contents as 12.95, 25.86, 20.78 and 16.97% in SARC-4 and Parwaz-94 respectively as compared to control. Under saline conditions (75 mM and 150mM), salicylic acid also enhanced the total soluble protein. The salicylic acid (0.25) application under 150 mM NaCl toxicity enhanced the protein contents by 39.66 and 59.76% in both genotypes i.e SARC-4 and Parwaz-94 respectively as compare to 150 mM NaCl toxicity alone. While the application of 0.50 mM salicylic acid in 75 mM NaCl toxicity increased the concentration of total soluble proteins in SARC-4 (41.44%) and Parwaz-94 (38.06%) as compared to the treatment where 75mM NaCl was applied alone.

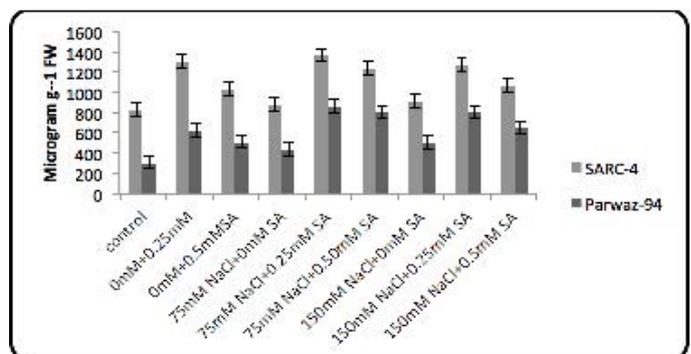


Figure 6: Total soluble proteins.

Fahad and Bano (2012) reported that under saline conditions the soluble protein contents increased in maize plant. Bartels and Sunkar (2005), reported that drought stress negatively affect many physiological and biochemical parameters of different plants such as wheat including Total Soluble Proteins but the application of salicylic acid eliminate these negative effects and increased the total soluble proteins. Cag et al. (2009) found an increase in total soluble proteins in excised cotyledon of sunflower because of application of salicylic acid to the cotyledons.

Catalase activity ($\mu\text{ mol H}_2\text{O}_2\text{ g}^{-1}\text{ FW/ min.}$)

NaCl treatments enhanced catalase activity as compare to control. The increment caused by 150 mM NaCl treatment was 60 and 51% in SARC-4 and Parwaz-94 respectively over control. While the application of salicylic acid (0.25 mM and 0.50 mM) further improved the catalase activity in both normal and saline conditions. Salicylic acid (0.25 mM) application in non-saline conditions enhanced the catalase by 14.28% and 17.14% in SARC-4 and Parwaz-94 respectively as compare to control. Whereas under saline conditions (75 mM), salicylic acid (0.25 mM) caused an increase in catalase activity in SARC-4 (90.32%) and Parwaz-94 (55.25%) with respect to 75 mM NaCl toxicity alone. Both levels (0.25 mM and 0.50 mM) of salicylic acid promoted the catalase activity under both levels (75 mM and 150 mM) of NaCl.

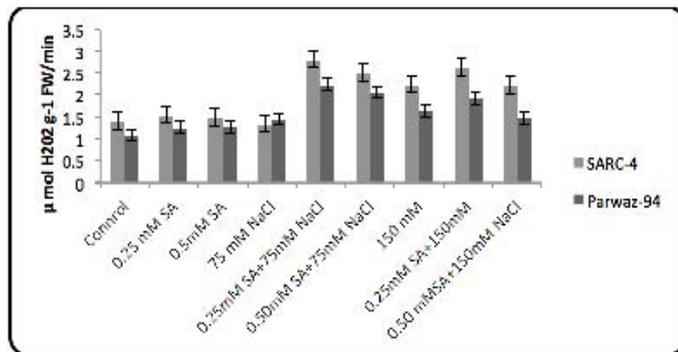


Figure 7: Catalase (CAT) activity.

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. Mandhania et al. (2006) reported the stimulation of catalase activity by salt stress. Kumara et al. (2010) found an increase in catalase by exogenous application of salicylic acid in Gerbera under salinity stress conditions. Gautam and Singh (2011) reported that salicylic acid is involved in positive magnification of reactive oxygen species signaling pathways.

Superoxide dismutase (SOD) activity ($\mu\text{ mol/g Fw}$)

NaCl treatments (75 mM and 150 mM) enhanced the superoxide dismutase activity with respect to control. The lower level of NaCl (75 mM) caused an increase in SOD activity in Parwaz-94 (26.17%) as compared to control. The NaCl treatment of 150 mM increased the SOD activity by 71.14 and 49.30% in SARC-4 and Parwaz-94 respectively as compare to control. The salicylic acid treatments (0.25 mM and

0.50 mM) improved the superoxide dismutase activity as compare to control and NaCl treatments. Under non-saline conditions, the salicylic acid (0.25 mM) improved the SOD activity by 74% and 69.45% in SARC-4 and in Parwaz-94 respectively as compare to control. Under saline conditions (75 mM NaCl), salicylic acid (0.25 mM) application increased the SOD activity by 53.19 and 81.11% in SARC-4 and in Parwaz-94 respectively as compared to 75mM NaCl toxicity alone. The salicylic acid application alone and under NaCl levels enhanced the SOD activity.

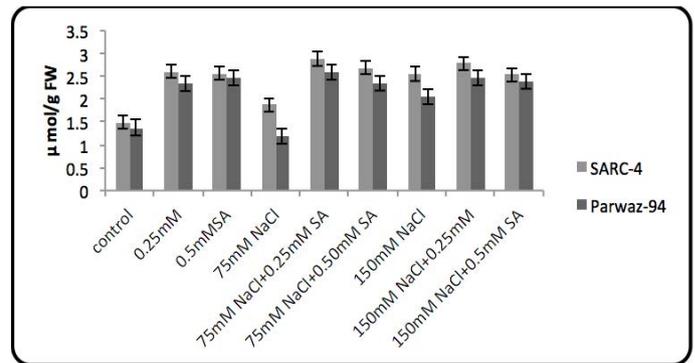


Figure 8: Superoxide Dismutase (SOD) activity.

Many investigators reported that increase in superoxide dismutase by salt stress is related to induction of salinity tolerance in plants (Sreenivasulu et al. 2000). Mutlu et al. (2009) concluded that the improved activity of superoxide dismutase in salt tolerant wheat cultivar due to salicylic acid is associated with the induction of antioxidant enzymes that help to protect the plants from salinity induced damage. Gautam and Singh (2011) also reported an increase in superoxide dismutase activity in maize plants under salinity stress conditions. Deef (2007) also marked an increase in SOD activity.

Conclusion

The results of this study conferred that there were genotypic variation found in two genotypes regarding salinity tolerance. SARC-4 was found to tolerate more salt stress as compared to Parwaz-94 and performed better at both salinity levels then Parwaz-94. The harmful effects of salinity were more prominent on Parwaz-94 as compared to SARC-4. While regarding salicylic acid induced amelioration it was notable that both levels of salicylic acid had significant effects on the physiology and biochemical parameters of crop plants however lower level of salicylic acid 0.25 mM was found more efficient then the higher

level of salicylic acid. From the results of the present study and former findings of the researchers it could be recommended that wheat crop should be supplemented with salicylic acid either grown under normal soil conditions or under saline environment. Salicylic acid induced improvement is significant regardless of stress environment or normal growth conditions.

Author's Contribution

Muhammad Suhaib and Bushra Atta conceived the idea and conducted research. Masooma Munir and Ijaz Ahmad did the analytical work. Badar Uz Zaman and Khubaib Abuzar collected the data.

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