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Evaluation of Salinity Induced Modification in Growth, Biochemical and Yield Characteristics of Spring Wheat (*Triticum aestivum* L.) Cultivars

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ABSTRACT

Salt stress impact was appraised on different antioxidative enzymes, MDA and H2O2 in ten spring wheat cultivars i.e., S-24, Lasani, Fsd-2008, Saher-2006, Inqlab-91, AARI-10, P.B-18, S.H-20, M.P-65, and G.A-20 when salinity applied at the seedling stage. The wheat cultivars were grown under saline (150 mM) and non-saline regimes (0 mM) in pots filled with sand. Diverse response in all wheat cultivars was observed in different studied attributes. Saline stress markedly decline SOD, CAT and POD conc. in different wheat cultivars. While some cultivars (S-24, Lasani, AARI-10 and GA-20) showed increase in these attributes under saline condition as compared to control. MDA and H2O2 content were increased in different wheat cultivars due to imposition of salt stress at the seedling stage. Whereas decrease in some cultivars was recorded in these attributes under saline regime than in non-saline conditions. Of all wheat cultivars, S-24, Lasani, AARI-10 and GA-20 showed high antioxidative activity, less lipid peroxidation and H2O2 content in plant shoot when salt stress applied at the seedling stage. On the basis of higher antioxidative activity and less MDA and H2O2 content, these four cultivars (S-24, Lasani, AARI-10 and GA-20) could be categorized as salt tolerant as compared to others.

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INTRODUCTION

The global agriculture is facing severe challenges for catering food demand, together with large consumption, land allocation for alternative uses in addition to environmental hazards (Curtis, *et al*., 2014). Currently, food security principally depends on the production of 3 cereals: *Triticum aestivum*, *Oryza sativa* and *Zea mays*. Across the world, wheat (*Triticum aestivum* L.) is one of the key cereals and is one of the most important sources of proteins, calories and other supermolecules. Roughly 82% -85% of the world's population relies on wheat to fulfill basic food need. Additionally, this cereal is used in the production of multiple products like sourdough bread, steamed and flat breads, pasta, noodles, cakes, couscous, biscuits, and brewages (Chaves, *et al*., 2013). Besides its use for human diet, it is also used for the non-food products like fuel. Attributable to its high-level adaptation, it is cultivated in climatic zone and tropical regions under both irrigated and rain-fed cultivation. Though, adverse environmental stresses severely affect the yield production of wheat crop (Curtis, *et al*., 2014).

The main stresses which affected crop productivity worldwide included drought, salt, heat, cold, chemicals, oil, pathogens, insects, nutrient deficiency, and ozone (O3) (Kanwal, *et al*., 2013). In these stresses, the soil salinity is an important factor distressing development and growth of plants. High saline conditions may lead to yield reduction or even death of the plant. Globally, about 20% of agricultural land is intensely affected by salinity. According to the current estimation, more than 50% of

cultivated land will be alkaline and saline by 2050. High salinity causes multiple damages to plants including yield reduction, decrease in growth of shoot and root eventually leads to the death (Kanwal, *et al*., 2013). Detrimental properties of high salt stress cause many changes in physiological mechanisms of plant including osmotic stress, oxidative stress, ion toxicity, and nutrient deficiency. Within plant cells, the synthesis of ROS is induced due to high salinity, and accumulation of ROS in excess amount results in oxidative damage of lipids, nucleic acids and proteins present in membranes (Gill and Tuteja, 2010).

An effective system of enzymatic and non-enzymatic antioxidants is responsible to scavenge high levels of ROS in plants (Karuppanapandian, *et al*., 2011). Under salt stress the improved activities of POX and CAT stress have been studied in salt-tolerant cultivars of wheat (Triticum aestivum) and millet (Setaria italica) seedlings. The high salt stress influences negatively to different plant developmental stages. Certain developmental periods are more responsive to salt stress including seedling emergence, germination, and flowering (Kanwal, *et al*., 2013). Osmotic and salt stresses control the delay or inhibition of seed germination and reduction in seedling growth and development (Fridovich, 1989).

The salt tolerance ability of wheat cultivars differs at different growth stages and it is reported that seedling growth stage as more sensitive to saline stress than the other growth stages (Kanwal, *et al*., 2013). Therefore, in present experiment 10 wheat genotypes were examined to observe the salt stress effect on antioxidant, MDA and H2O2 at the seedling growth stage.

METHODS AND MATERIAL

An experiment was conducted to examine the salt tolerance in 10 wheat cultivars, five newly developed (S-24, Lasani, Inqlab-91, Fsd-2008 and Saher-2006) and five candidate (AARI-10, S.H-20, P.B-18, G.A-20 and M.P-65) using antioxidants (SOD, POD and CAT), MDA and H_2O_2 as selection criteria. Of these 10 cultivars, the seeds of nine cultivars (Inqlab-91, Fsd-2008, Lasani, Saher-2006, P.B-18, AARI-10, M.P-65, G.A-20 and S.H-20) were collected from the Ayub Agricultural Research Institute, Faisalabad, Pakistan and that of cv. S-24 from the Department of Botany, University of Agriculture, Faisalabad. The experiment was performed in the research area of the Botanical Garden, of University of Agriculture, Faisalabad, Pakistan. All wheat cultivars were grown in plastic pots, each pot having diameter of 30 cm filled with 11 kg well washed river sand. Two salt (NaCl) levels (0 m*M* and 150 m*M*) prepared in full strength Hoagland's nutrient solution were applied. All pots were arranged in a completely randomized design with four replicates. after germination six plants were kept in each pot and salt stress application started after 15 days of germination and maintained throughout the experiment. Data for different attributes was recorded after 15 days of last salt stress applied at the boot stage.

Malondialdehyde

Malondialdehyde (MDA) in leaf tissues was analyzed by following the protocol of Carmak and Horst (1991). 0.1 g of fresh leaf sample was ground in 1.0 % trichloroacetic acid (5 ml) in ice to maintain temperature and then centrifuged for 10 min at 15000 g. 0.5 ml of supernatant was reacted with 3 ml of mixture of TCA and TBA comprising 0.5% thiobarbituric acid in 20 % TCA. This mixture was heated in a water bath at 95 C for 50 min. then test tubes were cooled on ice. After centrifugation, the optical density was measured at 532nm and these readings were corrected by measuring the non-specific absorbance at 600 nm and subtracting from optical densities at 532 nm.

Hydrogen peroxide

Hydrogen peroxide of the plant samples was estimated by the protocol of Velikova *et al.* (2000). Fresh leaf tissues (0.5 g) were homogenized in 5 ml of 0.1 % (w/v) trichloroacetic acid (TCA) then centrifuged for 15 min. To the 0.5 ml of supernatant, 0.5 ml of phosphate buffer of pH 7.0- and 1-ml potassium iodide was added. Then vortexed it and its absorbance were measured at 390 nm by using a UV visible spectrophotometer (IRMECO U-2020).

Activities of antioxidant enzymes

The extraction of antioxidant enzymes (SOD, POD and CAT) was done by grinding 0.5 g fresh leaf in 5 ml potassium phosphate buffer (50 mM) having pH 7.8 in ice bath. This homogenized material was centrifuged for 15 min at 10 000 g at 4 °C. The supernatant was used for enzymes assays activities. Superoxide dismutase (SOD) activity was estimated by appraising enzyme's extract ability to inhibit the photochemical reduction of NBT (nitroblue tetrazolium) at 560 nm following the protocol of Beauchamp and Fridovich (1971). Peroxidase (POD)

and catalase (CAT) activities were estimated following the method Chance and Maehly (1955). Catalase activity was measured by adding enzyme (100 µl) extract to reaction solution (3 ml) having 20 mM H_2O_2 in potassium phosphate buffer having pH 7.0 (50 mM) and decrease in optical density was read at 240 nm. Guiacol oxidation method was used to measure the peroxidase activity. 100 µl enzyme extract was added to reaction mixture (3 ml) having H_2O_2 (40 mM) and guaiacol (20 mM) in 50 mM potassium phosphate buffer (pH 7.8). Optical densities of the reaction mixtures were read at 470 nm. Different enzyme activities were determined as an enzyme unit per mg protein.

Total soluble proteins

Fresh leaf sample (0.5 g) was ground in 10 ml 50mM phosphate buffer of pH 7.8. Then centrifuged at 6000×g for 20 min. The supernatant was used to determine the total soluble proteins by following Bradford (1976).

Statistical analysis

Bartlett's test was performed on data of each variable by using the MSTAT computer package (MSTAT, 1989) to work out analysis of variance. All means within a variable were compared using the LSD test at 5% probability.

RESULTS

In present study, the assessment of ten candidate and newly developed wheat (*Triticum aestivum* L.) cultivars was done on the basis of antioxidant (SOD, POD, CAT) activity in response to salinity stress. This assessment also includes the influence of saline stress on MDA and H_2O_2 activity at the seedling growth stage of all ten cultivars of wheat. And the activity of MDA and H_2O_2 showed a different pattern in all wheat cultivars under saline stress applied at the seedling stage. Same cultivars showed an increase in these attributes while, others showed decrease in values of these attributes under saline stress condition as compared to non-saline regime when stress applied at the seedling stage.

A significant increase in the concentration of superoxide dismutase (SOD) was recorded in six cultivars including S-24, Lasani, M.P-65, S.H-20, AARI-10 and G.A-20 when saline stress applied at the seedling stage than controlled conditions. While a slight increase in SOD activity was observed in P.B-18 cultivar under saline conditions as compared to control. However, the maximal increase in SOD conc. was observed in AARI-10. Inqlab-91, Saher-2006 and Fsd-2008 wheat cultivars showed decline in SOD conc. under salinity stress when applied at the seedling stage (Figure 1; Table 1).

Catalase (CAT) conc. was increased in three cultivars including S-24, Lasani, P.B-18 and AARI-10 under salinity stress when applied at the seedling stage than under controlled conditions. The CAT conc. was decreased significantly in cvs. Inqlab-91, Fsd-2008, M.P-65 and S.H-20 cultivars under stressed conditions while a slight decrease was observed in cvs. Saher-2006 and G.A-20 cultivars under salinity stress application at the seedling stage. While the maximum decline in this attribute was observed in cv. Fsd-2008 as compared to other cultivars (Figure 1; Table 1). A significant increase was observed in conc. of POD in cvs. S-24, Inqlab-91, Saher-2006, Lasani and AARI-10 under saline stress applied at the seedling stage as compared controlled. Whereas POD conc. was decreased in all other cultivars (M.P-65, S.H-20, Fsd-2008, P.B-18 and G.A-20) under salinity as compared to controlled conditions. While the highest decrease in POD conc. was observed in cv. G.A.-20 (Figure 1; Table 1).

A significant increase was observed in leaf soluble proteins in all wheat cultivars except in cvs. Fsd-2008 and G.A.-20 in which reduction in this attribute was recorded under saline stress applied at the seedling stage as compared controlled. While a higher value for leaf soluble proteins was recorded in cvs. Saher-2006, M.P-65 and AARI-10 cultivars as compared to other cultivars (Figure 1; Table 1).

Leaf MDA content was decreased in cultivars S-24, Lasani, AARI-10 and G.A-20 under salinity stress when applied at the seedling stage than under controlled conditions. Whereas its conc. was increased in all other wheat cultivars i.e., Inqlab-91, Saher-2006, Fsd-2008, P.B-18 and S.H-20 under saline stress applied at the seedling stage. While a slight increase in MDA content was recorded in cv. M.P-65 under stressed condition. The reduction in conc. of H_2O_2 was recorded in six wheat cultivars (S-24, Fsd-2008, P.B-18, M.P-65, AARI-10 and G.A-20) under saline conditions as compared to controlled. While increase in H_2O_2 activity was recorded in all other wheat cultivars under salinity stress applied at the seedling stage. However, cv. Lasani showed a slight increase in this attribute due to salinity application with comparison to control (Figure 1; Table 1).

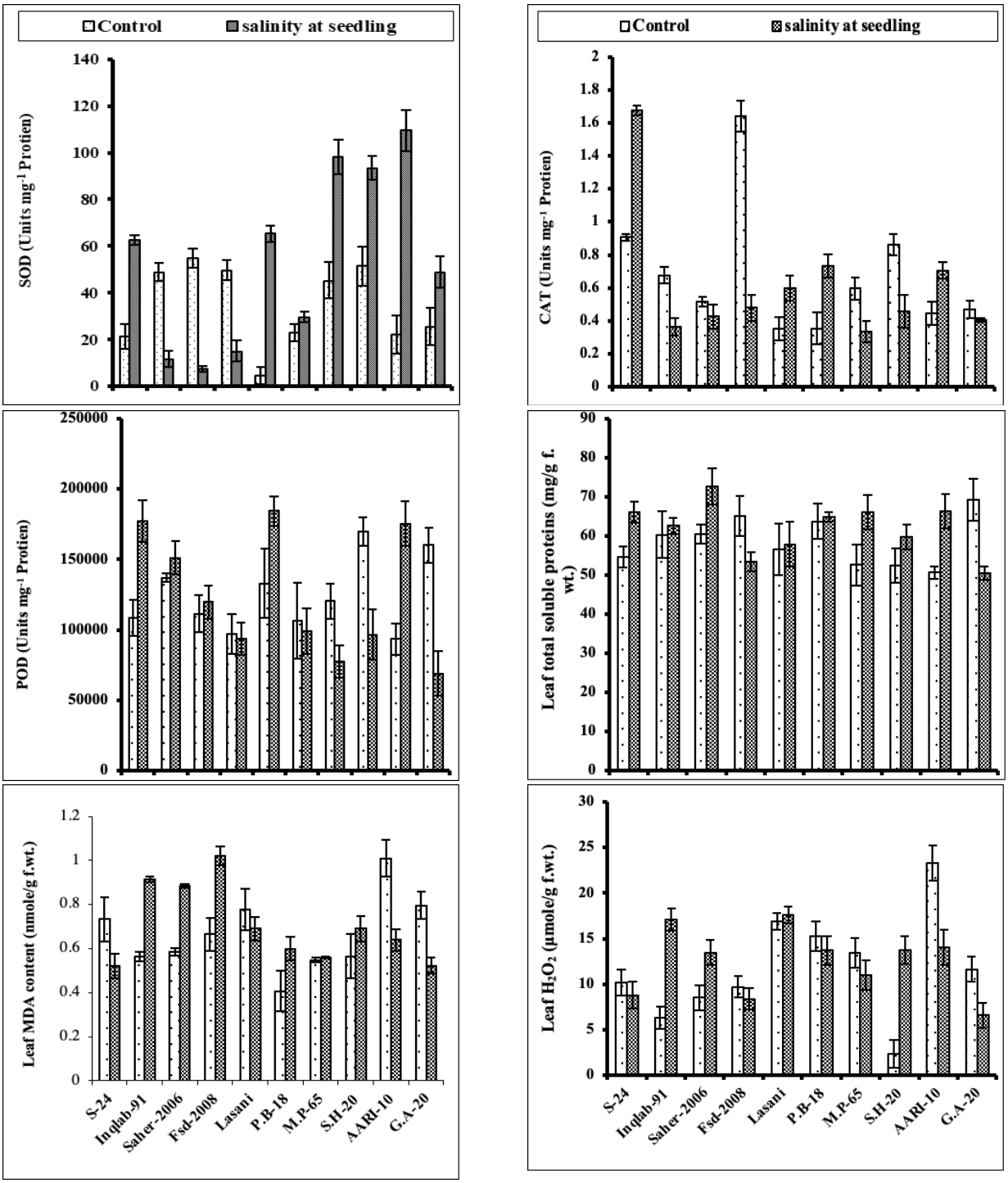

Figure 1. Leaf antioxidants (SOD, POD and CAT), total soluble proteins, MDA and H_2O_2 of different cultivars of wheat (*Triticum aestivum* L.) as influenced by salt stress (150m*M*) applied at the seedling stage (mean ± S.E.)
SOD= Superoxide dismutase, POD= Peroxidase, CAT= Catalase

Table 1. Mean square values from analyses of variance of data for different antioxidants, total soluble proteins, MDA and H_2O_2 of 10 wheat cultivars under salt stress.

Source	Df	SOD	POD	CAT
Salt stress (S)	1	261.79*	1.306*	0.0013ns
Cultivars (CV)	9	1754.24***	2.825*	0.046*
S × Cv	9	4049.53***	3.55*	0.126*
Error	40	186.7	3.262	0.044
Source	Df	Total soluble proteins	MDA	H_2O_2
Salt stress (S)	1	168.26*	0.034ns	209.71*
Cultivars (CV)	9	157.05*	0.478*	65.39*
S × Cv	9	72.42*	0.299*	50.695*
Error	40	87.104	0.308	19.208

*, **, *** = significant at 0.05, 0.01, and 0.001 level, respectively; ns = non-significant

DISCUSSION

Salt stress has been frequently stated as one of the main reasons of oxidative injury in plant tissues (Jalali-e-Emam, *et al*., 2011). Whereas, by developing a resilient defence system together with antioxidant enzymes such as SOD, POD and CAT plants can prevent themselves from the destructive possessions of reactive oxygen species (ROS) (Joseph, *et al*., 2011). In this research, the activity of antioxidant enzymes such as SOD, POD and CAT is affected by the salinity stress. Generally, in plant cells the production of ROS including H_2O_2 and $O^{2\bullet -}$ is enhanced when they are exposed to salt stress, which results in substantial damage in the composition of cell membranes because of lipid peroxidation (Blokhina *et al*., 2003).

The production of antioxidant enzymes such as SOD, POD, CAT and GR (glutathione reductase) can alleviate the harmful effects induced by salinity, these antioxidant enzymes can scavenge ROS effectively (Ashraf, *et al*., 2008). From these antioxidant enzymes, SOD functions at the membrane boundaries as the first line of defence mechanism against oxidation. Scavenging of ROS, particularly superoxide radical was completed properly when SOD activity was high at the membranes therefore, oxidative stress and membrane damage was decreased, resulting an increase in oxidative stress tolerant behaviour. The level of superoxide was increased in cells due to salinity stress. It is necessary to scavenge superoxide, if SOD does not scavenge these radicals then they disturb the normal functioning and production of vital biomolecules (Mittler, 2002). Furthermore, the superoxide inactivates or disturbs the normal functioning of some antioxidant enzymes such as peroxidases (Fridovich, 1989) and catalases (Joseph, *et al*., 2011) which are essential for scavenging of H_2O_2. As reported earlier in many researches, increased concentration of SOD in cvs. Lasani, S-24, AARI-10, S.H-20, G.A-20 and M.P-65 exhibited their ability to scavenge ROS in a better way under saline conditions (Joseph, *et al*., 2011).

Another significant antioxidant enzyme is CAT that is present in the peroxisomes and transforms H_2O_2 to water (McCord and Fridovich, 1969). In peroxisomes H_2O_2 is produced from photorespiration and β-oxidation of fatty acids (Morita, *et al*., 1994). In Plant cells the higher activity of APX and CAT increase the membrane stability and decrease the level of H_2O_2. In present study, Catalase (CAT) conc. was increased in three cultivars including Lasani, S-24, AARI-10 and P.B-18. Although, CAT conc. was decreased considerably at the seedling stage in cultivars Fsd-2008, S.H-20 Inqlab-91 and M.P-65 under saline conditions. However, increased CAT conc. and decrease in H_2O_2 level in cvs. S-24, Lasani, P.B-18 and AARI-10 indicates the salt tolerance capability of these cultivars by controlling the H_2O_2 level produced under stress (Bradford, 1976). These finding are also in agreement with different studies on different crops e.g., wheat, *Glycine max* L., Maize (Azooz, *et al*., 2009).

It is believed that POD together with CAT are two efficient scavenging systems for H_2O_2, in cells of plants these systems facilitate the rapid removal of H_2O_2 (Blokhina, *et al*., 2003; Mittler, 2002). In our experiment, POD activity in cvs. S-24, Lasani and AARI-10 was significantly enhanced when plants were grown under salinity stress (150 mM NaCl). The enhancement of POD activity by salinity has also been observed in rice (Lee, *et al*., 2001) pea and mulberry (Harinasut, *et al*., 2003). In tolerant plants, POD activity was found to be higher to protect plants against the oxidative stresses. These findings suggest cvs. S-24, Lasani and AARI-10 to be salt tolerant.

The substantial membrane damage is due to the decreased activity of SOD under salinity. Due to decreased SOD activity an increase in oxidative stress and MDA content occurred. Under salinity stress conditions membrane lipid peroxidation occurs which is an indication of membrane leakage and damage (Kanwal, *et al*., 2013). MDA is the secondary end-product of polyunsaturated fatty acid oxidation. Estimation of malondialdehyde (MDA) is done to measure the degree of lipid peroxidation which is the indicator of oxidative stress (Lin, *et al*., 2000). In plant cells the lipid peroxidation seems to be originated by a number of ROS. To evaluate the sensitivity as well as tolerance of plants to salt and oxidative stress, the rate of lipid peroxidation with respect to MDA can be used as an indication (Jain, *et al*., 2001). Our results showed that lipid peroxidation was influenced by salinity stress in all wheat cultivars which responded differently to leaf MDA contents under salt stress. However, there was a significant decline in MDA contents under salt stress in S-24, Lasani, AARI-10 and G.A-20 wheat cultivars. Reduced contents of MDA are an important indicator of stress tolerance as shown in some earlier studies e.g., in salt tolerant cultivars of barley (Liang, *et al*., 2003), sorghum (Brankova *et al*., 2005), wheat and Maize (Azooz, *et al*., 2009) and *Glycine max L*. From all these findings and results, it can be inferred that salt tolerance ability of different wheat cultivars was associated with higher antioxidant activity and lower levels of MDA and H_2O_2. However, all wheat cultivars responded differently to different parameter examined in this study. From all cultivars, S-24, Lasani, AARI-10 and G.A-20 were found to be tolerant on the basis of higher antioxidant activities and lower MDA and H_2O_2 content in plant tissues particularly salinity stress application at the seedling stage.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.