IMPACT OF SALT STRESS ON GROWTH PARAMETERS, CHLOROPHYLL CONTENT, AND OXIDATIVE DAMAGE IN DIVERSE WHEAT CULTIVARS AT SEEDLING STAGE

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ABSTRACT

Plant growth and productivity are being adversely affected by several biotic and abiotic stresses that collectively lead to drastic yield losses globally. Increased soil salinization and climate change are posing serious threats to sustainable production of all the crops including wheat. This study explored the agronomic, physiological, and biochemical responses of fourteen diverse Pakistani wheat varieties to salt stress. In this study, plants were exposed to 150 mM saline conditions for ten days, after 30 days of growth under normal conditions. Key growth indicators, photosynthetic pigments and antioxidant parameters were evaluated to assess the genotypic variation in stress tolerance. The experiment was conducted in completely randomized design (CRD) with factorial arrangement, comprising fourteen wheat varieties and two salinity treatments (control and 150 mM NaCl), with three replications. Among growth indicators, highest variation was observed in their root length, with *Chakwal-86*, *Galaxy-2013*, and *Akbar-2019* exhibiting the higher tolerance and less affected root length, while highest reduction was observed in *Sehar 2006*, *Chakwal 97*, *Suleman 96*, and *Inqilab 91*. Photosynthetic pigments were affected more in *Pakistan 81*, *Auqab 2000*, *Chakwal 97*, *Suleman 96*, and *Ujala 2016* with up to 38% reduction in total chlorophyll contents while *Chakwal 86*, *Akbar 2019*, and *Pakistan 2013* showed less reduction under stress conditions with minimum reduction of up to 10%. *Chakwal 86*, *Akbar 2019* and *Galaxy 2013* showed higher increase in the activity of antioxidant enzymes under salinity with minimal lipid peroxidation while *Pakistan-81*, *Inqilab-91*, *Sehar-2000*, and *Ujala 2016* showed higher oxidative damage under stress. The increase in activity of antioxidant enzymes varied between 6% to 48% in case of Catalase, 7%-10% in case of superoxide dismutase (SOD), 2%-29% in ascorbate peroxidase (APX) and between 11% to 45% in case of peroxidase (POD). Higher malondialdehyde (MDA) contents of up to 62% were observed in *Pakistan 81*, *Margalla 99* under salinity, while less increase in MDA of up to 28% was observed in *Chakwal 86*, *Galaxy 2013*, *Pakistan 2013*. Overall, it was found that *Pakistan 81*, *Margalla 99* and *Suleman 96* were not able to perform well under salinity stress, while *Chakwal 86*, *Galaxy 2013* and *Pakistan 2013* were better able to deal with stress conditions. It is suggested that these tolerant genotypes can serve as valuable genetic resources for breeding programs with an aim to improve wheat performance under saline conditions.

Keywords: salt stress, chlorophyll, malondialdehyde, antioxidant activities, wheat

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crop, serving as the staple food for nearly 40% of global population (Sharma *et al.*, 2025). About 222.52 million hectares of land are used for wheat cultivation globally, making it a significant contributor to nutrition and global food security (USDA). Wheat offers approximately 24% of the caloric intake (Gupta *et al*., 2024) and 55% of the carbohydrate requirements of the human diet, and it shows its key role in supporting food security for the global population (Acevedo *et al.*, 2018). Wheat has an extraordinary significance in Pakistan due to its socioeconomic importance. It is cultivated on more than 9 million hectares and yielded 27.3 million tons of grains between 2023-24. It contributes 1.9% to the national GDP and has 7.8% value addition to agriculture (Wheat Policy Analysis, Government of Pakistan, 2023–24). Increase in per-acre yield has resulted in a 0.9% increase in annual wheat yield during the past decade (Shah *et al.*, 2024). Despite global advancements in wheat productivity, Pakistan is still facing challenges in acquiring sustainable productivity growth because of increasing climatic variations (Irum *et al.*, 2023). Increasing wheat productivity is therefore vital for national food security of Pakistan and global cereal supply chains.

Soil salinity is one of the most serious abioic stresses affecting crop productivity. It affects nearly 20% of the global irrigated agricultural land and it is continuously expanding due to traditional irrigation practices, poor drainage, and rising groundwater table (Rehman *et al*., 2025).

Growth and development in plants is affected by ion toxicity, osmotic stress, nutrient imbalance, and oxidative stress caused by salinity and these factors lead to reduced rate of photosynthesis and stunted growth of the plants which ultimately results in the substandard yield. (Nadeem *et al.,* 2020; EL Sabagh *et al.,* 2021). Cellular homeostasis is affected by the presence of ions such as Na^+ and Cl^- as they disrupt the enzyme function, while water uptake and turgor are disturbed due to osmotic stress (Naz and Bano 2013). Oxidative stress induced by salinity promotes excessive production of reactive oxygen species (ROS), which causes damage to proteins, cellular membranes and nucleic acids (Naz *et al.,* 2014; Masood *et al*., 2025). Effects of salinity are further aggravated by drought and heat, which are directly associated with climate change.

In order to develop climate resilient crops in the current climate change scenario, it is important to understand the biochemical, physiological, and molecular mechanisms present in plants as these mechanisms are responsible for the plant's tolerance to salinity stress. Understanding the salinity tolerance in wheat is therefore crucial for developing resilient cultivars capable of sustaining yield under saline environments. Investigations of the genotypic variations, antioxidant defense systems, and stress-adaptive mechanisms in wheat are of prime importance for breeding programs which are focused on the increased tolerance of plants for salinity stress. During current study, agronomic, physiological, and biochemical responses of fourteen Pakistani wheat varieties in response to salinity stress were evaluated and we tried to identify the efficiency of resistant and susceptible varieties in terms of their responses to oxidative stress and protection of photosynthetic pigments which can result in better growth.

Genetically diverse wheat varieties were used in this study. These varieties are the result of continuous selection and breeding for decades and developed for rainfed and irrigated regions like Punjab and Sindh. *Inqilab 91* and *Auqab 2000* have shown a reduction in ion homeostasis and biomass reduction under salinity stress (Khalid and Hameed 2017; Irshad *et al.,* 2022), while *Sehar-2006, Pakistan 2013,* and *Ujala 2016* have demonstrated higher yield retention under salinity stress (Nadeem *et al.,* 2020; Iqra *et al.,* 2020). Varieties like *Pasban* and *Bhakkar 2000*, developed for sodic soils, provide positive controls for Na^+ exclusion (Abbasi *et al.,* 2003; Khalid and Hameed 2017). Along with these, we used some high-yielding varieties, including *Galaxy 2013* and *Akbar 2019* and tested all these varieties under salinity stress and identified their responses towards salinity. It is the need of time to understand the behavior of high-yielding varieties along with other valuable genotypes, which can help with sustainable food security under accelerated climate change and a rapidly increasing population.

This research identifies oxidative stress response mechanisms of diverse wheat varieties and advances our understanding of salinity-induced vulnerabilities and tolerances in Pakistani varieties, offering new foundations for breeding the resilient wheat lines to mitigate global food security challenges posed by soil salinization.

MATERIALS AND METHODS

Plant Material Experimental Design: The experiment was conducted under natural environmental conditions at COMSATS University Islamabad. Seeds of fourteen wheat (*Triticum aestivum* L.) varieties— *Galaxy 2013, Inqilab 91, Chakwal 86, Sehar-2006, Margalla 99, Pasban, Suleman 96, Pakistan 2013, Ujala 2016, Auqab 2000, Bhakkar 2000, Akbar-2019, Pakistan 81 and Chakwal 97* — were obtained from National Agricultural Research Center (NARC), Islamabad. Pot experiment was conducted at the COMSATS University Islamabad during the wheat season to evaluate the salinity tolerance of 14 Pakistani wheat varieties. Soil used for filling the pots had these properties: pH 7.9±0.2, ECe 1.0-1.2 dS m^{-1} and sandy clay loam texture. Soil was air-dried and passed through a 2 mm sieve and thoroughly mixed for uniformity. Plastic pots (diameter: 22-25 cm) were filled with 3 kg of this soil and placed under 0 mM externally applied NaCl (normal conditions).

Seeds were immersion in 70% ethanol for 1 min, followed by 10% (v/v) sodium hypochlorite for 10 min for surface sterilization, and were rinsed three times with distilled water. Five healthy seeds were sown per plastic pot containing soil with above given properties. Three plantlets were kept in each pot after germination and extra plantlets were discarded.

CRD design with factorial arrangement was used for these experiments. Pots were placed randomly, and plants were maintained under natural light with a photoperiod of approximately 12 h, daytime temperature of 24-30°C, and relative humidity of 60-75%. After 30 days of growth under non-stress conditions. Salinity stress was imposed by applying a 150 mM NaCl solution in one step while control plants received the same volume of distilled water. Salinity stress was uniformly applied to the designated pots in three replicates with two pots per replicate. One-step method of salinity application has been reported in a number of studies (Hussain *et al.,* 2021; Dargiri *et al.,* 2025).

Sampling: Five seeds were sown per pot and thinned to three uniform plants per pot. Each experimental unit (replicate) of the three replicates consisted of two pots containing three plants each, and all observations and harvests were taken on a whole-pot (pooled three plants) basis. Salinity stress was initiated 30 days after sowing and maintained for 10 days until

final sampling on day 40. After 10 days of salinity exposure, samples were collected for biochemical and enzymatic analyses. Fresh weights and other growth parameters were recorded, and samples were stored at -20°C until enzyme assays and biochemical assays were performed.

Growth parameters: Morphological parameters, including shoot and root lengths, were measured for all treatment groups and compared with the control. Fresh biomass of roots and shoots was recorded at the time of harvest, ten days after the onset of salinity stress.

Photosynthetic pigments: Total chlorophyll contents were determined by taking 0.05 g of the plant leaves and dissolving them in 10 mL of dimethyl sulfoxide (DMSO) in Falcon tubes. The solution was incubated at 65^0C for 4 h.

Absorbance was measured at 470 nm, 645 nm, and 665 nm, and then total chlorophyll, chlorophyll a, and chlorophyll b were measured (Ronen and Galun 1984).

$$Chlorophyll\ a\ (mg/g\ FW) = 12.7(A663) - 2.69(A645)$$
$$Chlorophyll\ b\ (mg/g\ FW) = 22.9(A645) - 4.68(A663)$$

MDA determination: For the measurement of MDA content, 0.5 g of leaves were ground in 5 mL trichloroacetic acid (TCA) and were centrifuged at *10,000 rpm* for 20 min. 2 mL of supernatant was collected in Falcon tubes, and 2 mL of 0.6% 2-thiobarbituric acid was then added. Solution was heated for 30 min at 95 ^{0}C in an incubator. Cooled on ice right after removing from the water bath to stop the reaction. Absorbance was measured at 450 nm, 532 nm, and 600 nm (Heath and Packer, 1968).

MDA (µmol/g FW) = (6.45×(A532-A600)-0.56×A450)

The molar extinction coefficient of the MDA-TBA adduct at 532 nm (ε_{532}) = 155 $mM^{-1}cm^{-1}$.

SOD activity: The SOD activity was assessed by using 0.2 g of wheat leaves and grinding them in 4 mL of 50 mM sodium phosphate buffer (pH 7.0) with 1% polyvinyl pyrrolidone (PVP). This mixture was centrifuged for 10 min at *4000 rpm*, keeping the temperature at 4 ^{0}C. Supernatant was preserved, and the pellet was discarded. A reaction mixture containing 75 µM nitro blue tetrazolium (NBT), 13 mM methionine and 0.1 mM ethylene diamine tetra acetic acid disodium salt dehydrate (Na_2EDTA) was made, out of which 2 mL was added to 0.5 mL enzyme extract. Then, 0.5 mL from a solution of 2 µM riboflavin diluted in phosphate buffer (pH 7.8) was added. One set of test tubes was put in the dark while the other was put in light to initiate the reaction at 30 ^{0}C for 1 h. The absorbance of both sets was recorded at 560 nm and SOD activity was measured in terms of units/gFW (Demirezen Yilmaz and Uruç Parlak, 2011).

POD activity: POD activity was determined spectrophotometrically by measuring the oxidation of guaiacol at 470 nm, by using the method of Zhang and Kirkham (1994). 3 mL of reaction mixture consisted of 50 mM sodium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2 and 100 µL enzyme extract (prepared as for SOD activity). The increase in absorbance was recorded between 0 and 3 min and POD activity was measured in terms of change in absorbance/min/gFW.

Catalase activity: Catalase activity was assessed by using 0.2 g of wheat leaves that were homogenized in 4 mL of 50 mM phosphate buffer mix and centrifuged at *10,000 rpm* for 10 min, keeping the temperature at 4 ^{0}C as in case of SOD and POD activity. The supernatant was preserved, and the pellet was discarded. 0.2 mL of the enzyme extract was added to 0.5 mL of 0.05 M phosphate buffer (pH 7). 0.1 mL of 3% H_2O_2 was added to the reaction mixture, and the first reading was taken immediately at 240 nm. The second reading was recorded at 3 min and catalase activity was measured as µmol/min/gFW (Taggar *et al.,* 2012).

APX Activity: APX activity was assessed by using the Talaat and Todorova (2022) method. 0.1g of leaf samples (0.1 g) were ground with 2 mL of 0.1 M phosphate buffer (pH 7.4), which had 1% polyvinyl pyrrolidine (PVP) and 1 mM Na_2EDTA. Centrifugation was done at *10,000 rpm* at 25 °C for 10 min. Supernatant was then used for the determination of APX activity. APX activity was measured as decline in absorbance at 290 nm due to ascorbate oxidation after 3 min and APX activity was measured as µmol/min/g FW.

Statistical Analysis: The experiments were performed in three replicates by using CRD under factorial arrangement. The data was collected and this data was tabulated in Microsoft (MS) Excel. Means and standard deviations were calculated by using MS Excel. Statistical analysis was done by two-way ANOVA and Tukey's HSD test at 95% level of significance. This was done by using Statistix 8.1All pairwise means were compared. The alphabetical letters on the top of the bars in each graph represent significant differences between means at $p<0.05$. Microsoft Excel was used for Pearson correlation analysis. Correlation coefficients(r) ranged between -1 and +1, while value of 1 was set for self-correlations. It showed the correlation between antioxidant defense mechanisms, oxidative damage, growth inhibition, and photosynthetic efficiency.

RESULTS

Plant Growth: The physical characteristics of the plants were assessed to investigate the effects of salinity on plant growth. The roots were the most affected part of the plants under salinity compared to normal conditions. Salinity caused a 10% to 32% reduction in root length in different varieties. Salinity caused a reduction of 7% to 31% in root fresh weight compared to the control plants (Table 1). The highest reduction in root length and root weight was observed in *Pakistan 81*, followed by *Sehar 2006*, *Chakwal 97*, *Suleman 96*, and *Inqilab 91*. On the other side, *Chakwal-86*, *Galaxy-2013*, and *Akbar-2019* showed least reduction in growth and biomass, showing their tolerance for salinity stress.

The plant shoots also showed significant growth inhibition under salinity stress. Shoot length decreased by 7% to 29%, while shoot biomass decreased by 3% to 27% (Table 1) in the plants under salinity stress compared to plants under control conditions. The highest reduction in shoot length and shoot weight was observed in *Pakistan 81, Inqilab 91, Chakwal 97,* and *Suleman 96*. However, *Chakwal 86, Pakistan 2013, Galaxy 2013*, and *Akbar 2019* showed less reduction in shoot length and shoot weight, which shows their tolerance for salinity stress.

It was found that tolerant varieties maintained better growth under salinity stress due to more effective responses of their physiological and biochemical systems. This allowed them to develop normally in the presence of salinity stress. However, the susceptible varieties had less effective response systems and due to this, salinity had higher effects on the growth and development of these plants.

Photosynthetic Pigments: The chlorophyll content displayed the extent to which the photosynthetic efficiency of plants was affected under stress. We observed a significant reduction in photosynthetic pigments under salinity stress. A considerable decrease of up to 38% was observed in total chlorophyll contents under stress condition compared to control. However, tolerant varieties had less affected chlorophyll contents, which showed a loss of up to 10% under stress (Table 2).

Most affected wheat varieties were *Pakistan 81*, *Auqab 2000*, *Chakwal 97*, *Suleman 96*, and *Ujala 2016*, while *Chakwal 86*, *Akbar 2019*, and *Pakistan 2013* showed a relatively less reduction in chlorophyll contents under stress. A significant decrease in chlorophyll resulted in decreased photosynthetic efficiency and physical growth of plants under salinity stress in comparison with the plants under normal conditions. These effects are clearly visible in Table 2, which demonstrate the effects of salinity on physical health and photosynthetic efficiency of plants. This shows that there are systems in stress-tolerant varieties, which maintain the photosynthetic pigments under stress conditions and hence try to maintain the overall health of plants.

Activity of Antioxidant Enzymes and Lipid Peroxidation

Catalase Activity: Most of the wheat varieties showed a significant increase in the activity of calatalse under salinity stress. An increase of 6% to 48% was observed in different wheat varieties under salinity stress (Fig. 1). The varieties with highest increase in Catalase activity were *Chakwal 86*, *Pakistan 2013*, and *Galaxy 2013*. Some varieties which displayed less increase in Catalase activity were *Pakistan 81*, *Inqilab 91*, *Suleman 96*, and *Ujala 2016*. The increase in activity of catalase was higher in stress-tolerant varieties. Catalase activity helped the tolerant varieties in dealing with stress conditions.

SOD Activity: Wheat plants showed a significant increase in SOD activity under stress. An increase of 7% to 19% in the activity of SOD was observed under salinity stress compared to the control condition in the varieties under study (Fig. 2). The highest increase was observed in *Chakwal 86*, *Galaxy 2013* and *Akbar 2019*. On the other hand, *Chakwal 97*, *Ujala 2016* and *Pakistan 81* had a little increase in the activity of SOD under salinity stress compared to control conditions.

APX Activity: A 2-29% increase in the activity of APX was observed under saline conditions as compared to the control (Fig. 3). The increase was less than 5% in *Chakwal 97, Bhakkar 2000*, *Ujala 2016*, *Suleman 96*, and *Margalla 99*. A higher increase in APX activity was observed in *Chakwal 86* and *Galaxy 2013*.

POD Activity: Figure 4 shows the impact of salinity on POD activity. Plants under oxidative stress induced by salinity showed a significant increase in POD activity. An increase of 11% to 45% was observed in the wheat varieties under study. Varieties with the highest increase in POD activity were *Chakwal 86* and *Galaxy 2013*, but less increase in POD activity was shown by *Sehar 2006*, *Inqilab 91*, and *Pakistan 81*.

MDA Concentration: MDA contents depict the extent of lipid peroxidation in response to oxidative stress. We observed a significant increase in MDA concentration in response to salinity stress, compared to the control conditions (Fig. 5). 28%-62% increase in MDA content was observed in different varieties. The increase was higher in the case of *Pakistan 81, Inqilab 91, Margalla 99*, *Suleman 96*, *Ujala 2016*, and *Sehar 2006*. These varieties had a little increase in antioxidant

enzyme activity when plants were under salinity stress. Increase in the MDA content was less in *Chakwal 86*, *Galaxy 2013*, *Pakistan 2013*, and *Akbar 2019* compared to other varieties under salinity stress.

Correlation Analysis: To assess the correlation between antioxidant defense mechanisms, oxidative damage, growth inhibition, and photosynthetic efficiency, the Pearson correlation analysis (Fig. 6) was used. The percentage difference between mean values of all the assessed varieties under salinity stress and control conditions was used for correlation analysis. Percentage differences in the activity of antioxidant enzymes, including APX, Catalase, SOD and POD, exhibited moderate to strong positive intercorrelations (r=0.38 to 0.78), indicating the upregulation under oxidative stress caused by salinity. However, these enzymes showed consistent negative correlations with difference in growth parameters (root weight, root length, shoot length, shoot weight; r=-0.36 to -0.83) and difference in photosynthetic pigments (total chlorophyll, chlorophyll a and chlorophyll b contents; r=-0.50 to -0.83), which indicates that increased enzymatic activity corelated with lesser change in biomass and chlorophyll content due to salinity stress. Differences in MDA content showed a negative correlation with the change in antioxidant enzyme (r=-0.36 to -0.76) yet change in MDA contents positively correlated with reduction in growth (r=0.54-0.83) and chlorophyll levels (r=0.60), which shows that a higher increase in MDA content correlated with highly affected growth and photosynthetic pigments. Growth parameters were strongly positively interlinked (r=0.66-0.86), as well as the chlorophyll contents (r ≥ 0.999), underscoring overall plant vigor trade-offs under differential salinity exposure.

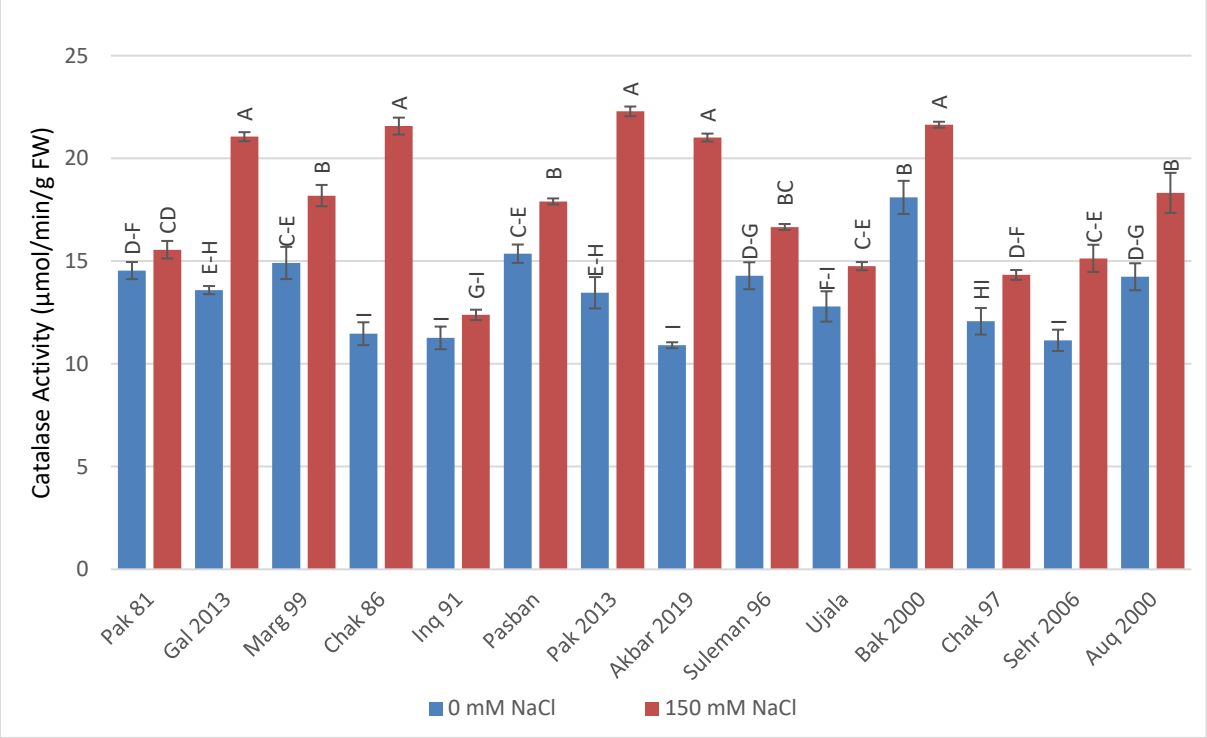

Figure 1: Effects of salinity stress (150 mM NaCl) on Catalase activity of different wheat varieties. Means of three replicates ± SD (standard deviation) is presented. Means±SD with different letters differ significantly from each other at p<0.05 according to Tukey's HSD test.

Table 1: Effects of salinity stress (150 mM NaCl) on growth indicators of different wheat varieties

VARIETY	Root Length			Root Weight			Shoot Length			Shoot Weight		
	0 mM NaCl	150 mM NaCl	% Change	0 mM NaCl	150 mM NaCl	% Change	0 mM NaCl	150 mM NaCl	% Change	0 mM NaCl	150 mM NaCl	% Change
Pakistan 81	22.4 ± 0.73^{B-E}	11.5 ± 0.93^{MN}	51.5	0.31 ± 0.0049^{G-I}	0.16±0.0111^{M}	50.7	55.4±0.96^{D-F}	32.5±0.93^{J}	58.7	3.37±0.037^{HI}	2.2±0.058^{L}	65.5
Galaxy 2013	23.5 ± 0.77^{B-E}	14.6 ± 0.77^{G-L}	62.1	0.29 ± 0.0046^{D-I}	0.19±0.0057^{J-L}	63.7	53.8±0.71^{AB}	36.6±0.93^{D-G}	68	2.91±0.014^{AB}	2±0.034^{C-E}	68.6
Margalla 99	19.8 ± 0.67^{D-G}	11.8 ± 0.05^{K-M}	59.8	0.33 ± 0.0181^{E-I}	0.19±0.0095^{LM}	58.7	43.1±0.37^{CD}	30.8±1.14^{I}	71.5	2.69±0.071^{B}	1.89±0.02^{F-H}	70.3
Chakwal 86	23.9 ± 0.33^{B-E}	17.5 ± 0.82^{E-J}	73.1	0.19 ± 0.0087^{D-I}	0.14±0.0074^{H-J}	74.1	60.9±1.02^{A-C}	42.8±0.67^{DE}	70.3	3.73±0.006^{CD}	2.43±0.063^{D-F}	65
Inqilab 91	24.8 ± 0.25^{D-H}	17 ± 0.62^{MN}	68.6	0.36 ± 0.0054^{B-E}	0.23±0.0068^{J-L}	64.3	51.4±0.25^{E-G}	34.7±1.03^{J}	67.4	2.88±0.062^{HI}	2.05±0.038^{L}	71.1
Pasban	16.5 ± 0.57^{AB}	12.7 ± 0.53^{E-J}	77	0.28 ± 0.0077^{B-D}	0.21±0.0037^{H-K}	75.3	50.3±0.22^{D-F}	39±0.6^{HI}	77.5	3.32±0.048^{C}	2.35±0.049^{HI}	70.7
Pakistan 2013	19.3 ± 0.37^{A-D}	15.3 ± 0.8^{F-K}	79.1	0.25 ± 0.0135^{B-E}	0.21±0.0114^{F-I}	83.4	49.6±0.57^{A}	47.3±4.92^{DE}	95.4	2.72±0.022^{A}	2.61±0.065^{C}	95.9
Akbar 2019	19.1 ± 0.54^{A}	14.2 ± 0.49^{B-E}	74.2	0.24 ± 0.0102^{AB}	0.18±0.0073^{C-H}	76.9	45.9±0.26^{AB}	35.1±0.76^{D-G}	76.5	3.14±0.024^{C}	2.14±0.02^{G-I}	68.1
Suleman 96	23.3 ± 0.33^{K-M}	13.8 ± 0.53^{NO}	59.3	0.27 ± 0.004^{BC}	0.18±0.0061^{LM}	66.1	40.5±0.93^{AB}	27.6±0.77^{E-G}	68	2.6±0.013^{AB}	1.41±0.052^{J}	54.3
Ujala	23.5 ± 1.14^{A-C}	14 ± 1.68^{H-L}	59.6	0.34 ± 0.0106^{A}	0.24±0.0076^{C-I}	70.2	51.2±1.18^{AB}	36.1±0.24^{FG}	70.6	3.42±0.056^{B}	2.44±0.311^{I}	71.2
Bhakkar 2000	25.4 ± 1.1^{C-E}	11.3 ± 1.76^{J-M}	44.3	0.26 ± 0.0034^{B-F}	0.12±0.0074^{LM}	46.7	52±1.37^{D-G}	31±0.58^{I}	59.6	2.97±0.01^{D-G}	1.53±0.049^{K}	51.6
Chakwal 97	21.1 ± 0.61^{C-F}	13.7 ± 0.95^{LM}	64.9	0.26 ± 0.0118^{C-G}	0.16±0.011^{LM}	59.3	49.7±0.9^{AB}	34.1±0.76^{GH}	68.7	2.59±0.066^{HI}	1.77±0.062^{L}	68.3
Sehar 2006	19.8 ± 0.74^{I-M}	15.2 ± 0.45^{O}	76.7	0.14 ± 0.0029^{AB}	0.1±0.0069^{I-K}	72.8	44.5±0.76^{BC}	34.5±0.77^{D-G}	77.7	2.63±0.013^{C}	1.96±0.02^{J}	74.6
Auqab 2000	17.5 ± 0.76^{E-I}	10.6 ± 0.71^{LM}	60.5	0.28 ± 0.0057^{B-D}	0.18±0.0092^{K-M}	63.9	48.7±0.92^{D-G}	30.3±0.21^{I}	62.3	2.67±0.115^{E-H}	1.62±0.026^{JK}	60.8

Means±SD with different letters differ significantly from each other at p<0.05 according to Tukey's HSD test

Table 2: Effects of salinity stress (150 mM NaCl) on chlorophyll contents of different wheat varieties

VARIETY	Chlorophyll a			Chlorophyll b			Total Chlorophyll		
	0 mM NaCl	**150 mM NaCl**	**% Change**	**0 mM NaCl**	**150 mM NaCl**	**% Change**	**0 mM NaCl**	**150 mM NaCl**	**% Change**
Pakistan 81	9.26±0.24^{A}	5.68±0.54^{F-H}	61.4	4.92±0.13^{A}	3.02±0.29^{F-H}	61.3	14.18±0.37^{A}	8.7±0.83^{F-H}	61.4
Galaxy 2013	9.07±0.29^{A}	6.64±0.28^{C-F}	73.2	4.82±0.15^{A}	3.53±0.15^{C-F}	73.3	13.89±0.44^{A}	10.17±0.43^{C-F}	73.2
Margalla 99	6.88±0.1^{C-F}	5.37±0.1^{GH}	78	3.67±0.05^{C-F}	2.86±0.06^{GH}	77.8	10.55±0.15^{C-F}	8.22±0.15^{GH}	77.9
Chakwal 86	6.99±0.48^{C-F}	6.32±0.14^{D-G}	90.3	3.71±0.26^{C-E}	3.35±0.07^{D-G}	90.4	10.7±0.74^{C-E}	9.67±0.2^{D-G}	90.4
Inqilab 91	7.6±0.47^{BC}	5.24±0.47^{GH}	68.9	4.04±0.26^{BC}	2.79±0.25^{GH}	69.1	11.64±0.73^{BC}	8.03±0.72^{GH}	69
Pasban	8.8±0.36^{AB}	6.64±0.28^{C-F}	75.4	4.68±0.19^{AB}	3.53±0.15^{C-F}	75.5	13.48±0.55^{AB}	10.17±0.43^{C-F}	75.5
Pakistan 2013	8.92±0.1^{A}	7.25±0.08^{CD}	81.3	4.74±0.05^{A}	3.86±0.04^{CD}	81.5	13.66±0.15^{A}	11.11±0.12^{CD}	81.4
Akbar 2019	8.9±0.12^{A}	7.62±0.31^{BC}	85.6	4.73±0.07^{A}	4.05±0.17^{BC}	85.6	13.63±0.19^{A}	11.67±0.49^{BC}	85.6
Suleman 96	8.63±0.34^{AB}	5.67±0.29^{F-H}	65.7	4.59±0.18^{AB}	3.02±0.15^{F-H}	65.7	13.22±0.52^{AB}	8.68±0.44^{F-H}	65.7
Ujala	8.59±0.1^{AB}	5.7±0.07^{F-H}	66.4	4.57±0.05^{AB}	3.03±0.03^{F-H}	66.4	13.16±0.15^{AB}	8.74±0.1^{F-H}	66.4
Bhakkar 2000	9.06±0.28^{A}	6.39±0.63^{C-G}	70.5	4.82±0.15^{A}	3.39±0.33^{C-G}	70.4	13.88±0.43^{A}	9.78±0.96^{C-G}	70.5
Chakwal 97	8.83±0.25^{AB}	5.82±0.22^{E-H}	65.9	4.69±0.13^{AB}	3.09±0.12^{E-H}	65.9	13.52±0.38^{AB}	8.91±0.34^{E-H}	65.9
Sehar 2006	8.72±0.39^{AB}	6.12±0.47^{D-G}	70.2	4.64±0.21^{AB}	3.24±0.25^{D-G}	70	13.36±0.6^{AB}	9.36±0.71^{D-G}	70.1
Auqab 2000	7.15±0.13^{CD}	4.66±0.36^{H}	65.1	3.8±0.07^{CD}	2.47±0.19^{H}	65	10.95±0.2^{CD}	7.13±0.55^{H}	65.1

Means±SD with different letters differ significantly from each other at p<0.05 according to Tukey's HSD test

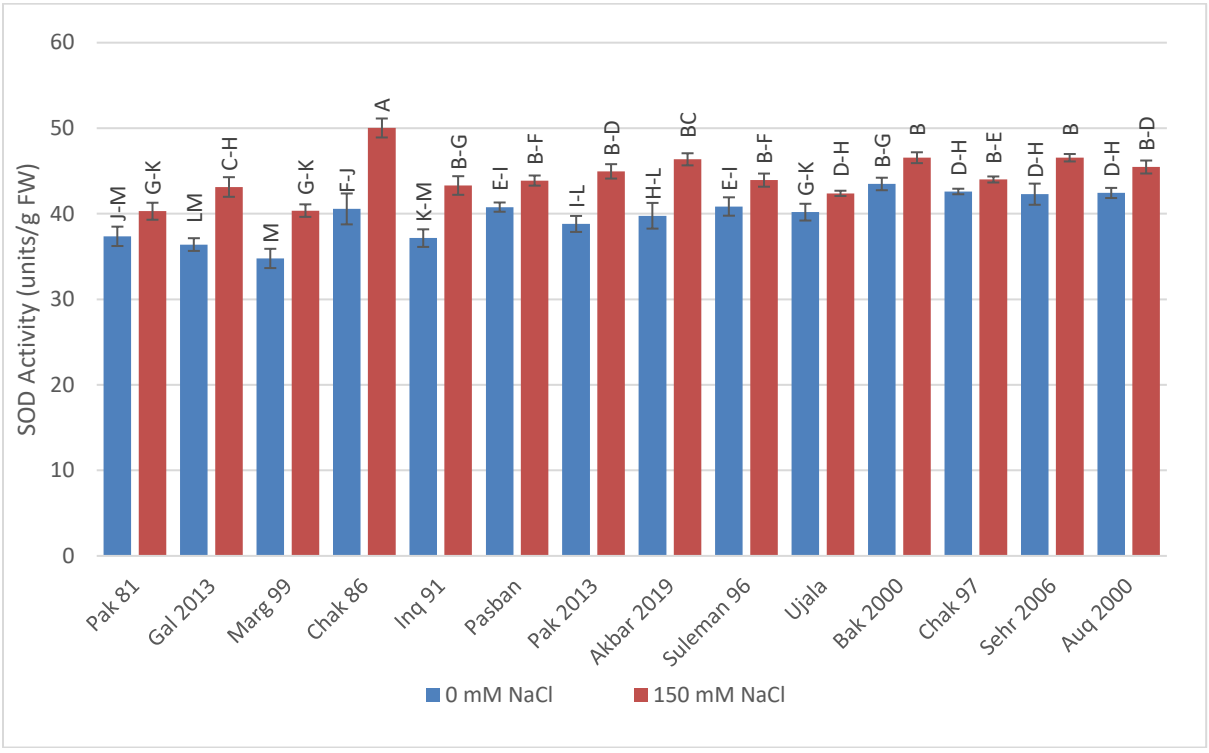

Figure 2: Effects of salinity stress (150 mM NaCl) on SOD activity of different wheat varieties. Means of three replicates ± SD (standard deviation) is presented. Means±SD with different letters differ significantly from each other at p<0.05 according to Tukey's HSD test.

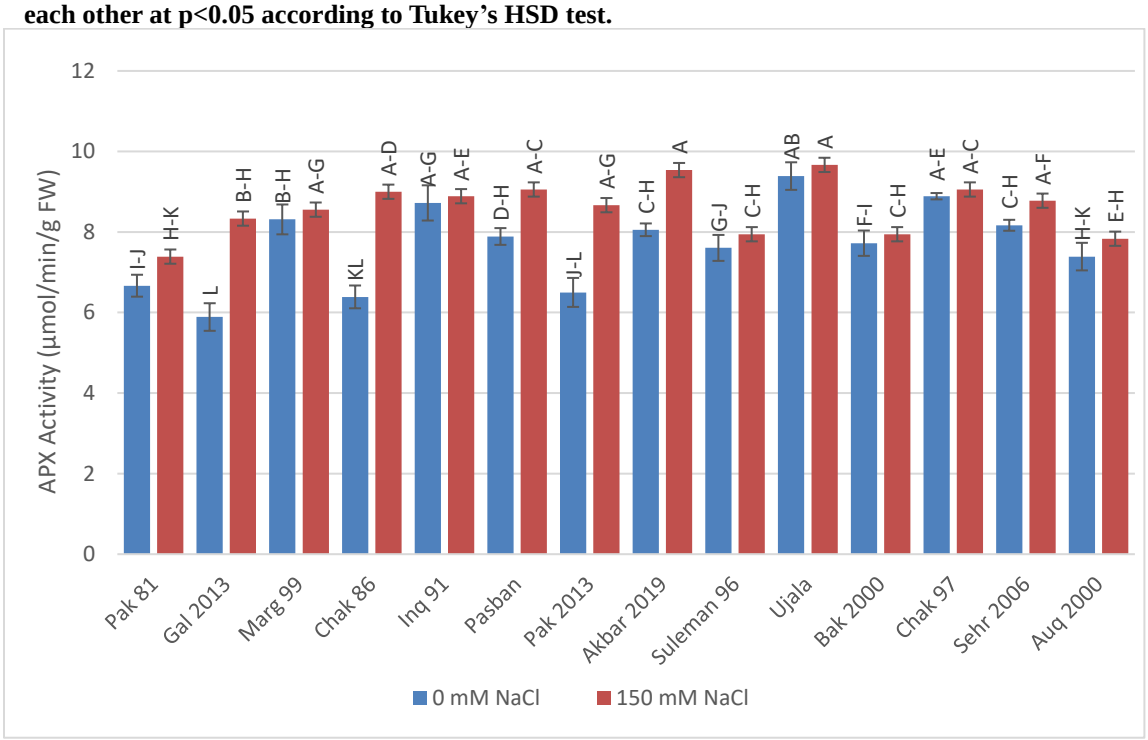

Figure 3: Effects of salinity stress (150 mM NaCl) on APX activity of different wheat varieties. Means±SD with different letters differ significantly from each other at p<0.05 according to Tukey's HSD test

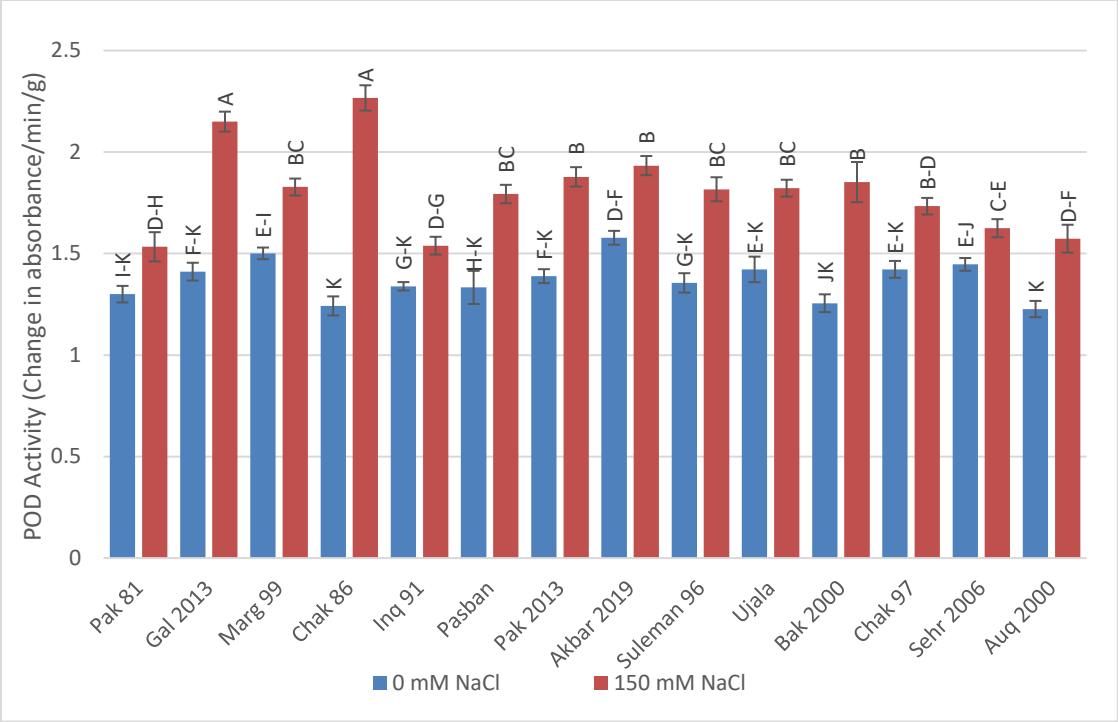

Figure 4: Effects of salinity stress (150 mM NaCl) on POD activity of different wheat varieties. Means of three replicates ± SD (standard deviation) are presented. Means±SD with different letters differ significantly from each other at p<0.05 according to Tukey's HSD test.

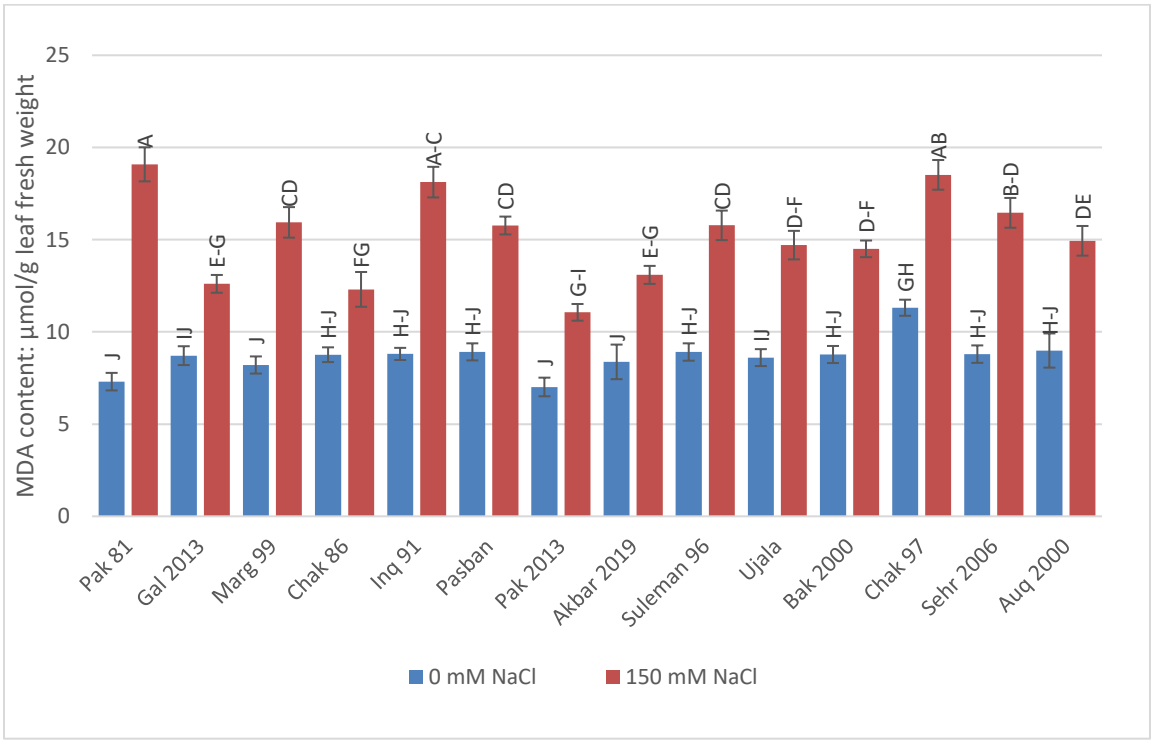

Figure 5: Effects of salinity stress (150 mM NaCl) on MDA content of different wheat varieties. Means of three replicates ± SD (standard deviation) is presented. Means±SD with different letters differ significantly from each other at p<0.05 according to Tukey's HSD test.

	APX	Catalase	POD	SOD	MDA	Root Length	Root Weight	Shoot Length	Shoot Weight	Chlorophyll a	Chlorophyll b	Total Chlorophyll
APX	1.00											
Catalase	0.78	1.00										
POD	0.65	0.48	1.00									
SOD	0.69	0.69	0.38	1.00								
MDA	-0.60	-0.76	-0.73	-0.36	1.00							
Root Length	-0.67	-0.81	-0.70	-0.62	0.83	1.00						
Root Weight	-0.77	-0.76	-0.42	-0.68	0.54	0.67	1.00					
Shoot Length	-0.57	-0.76	-0.56	-0.34	0.83	0.66	0.54	1.00				
Shoot Weight	-0.80	-0.83	-0.61	-0.71	0.64	0.85	0.86	0.70	1.00			
Chlorophyll a	-0.67	-0.83	-0.50	-0.78	0.60	0.82	0.86	0.63	0.88	1.00		
Chlorophyll b	-0.67	-0.83	-0.50	-0.78	0.60	0.82	0.86	0.62	0.88	1.00	1.00	
Total Chlorophyll	-0.67	-0.83	-0.50	-0.78	0.60	0.82	0.86	0.63	0.88	1.00	1.00	1.00

Figure 6: Pearson correlation matrix depicting the relationships among key biochemical and morphological parameters in plants subjected to salinity stress versus normal (control) conditions. Rows and columns represent APX, Catalase, POD, SOD, MDA, root length, root weight, shoot length, shoot weight, chlorophyll a, chlorophyll b, and total chlorophyll. Correlation coefficients (r) range from -1 (strong negative, red) to +1 (strong positive, blue), with diagonal values set to 1 for self-correlations. The analysis is based on differences observed between salinity-treated and normal conditions, illustrating stress-induced physiological dynamics.

DISCUSSION

Up to 50% of global yield potential of cereal crops can be affected by different kinds of environmental stresses. Salinity is one of the most important abiotic stress in the salinity affected areas (Acquaah 2009). Salinity can have multidimensional effects on plant physiology. The most important physiological effects of salinity include ion toxicity, osmotic imbalance and oxidative damage. These factors collectively disrupt cellular homeostasis and metabolic functions in the plants under stress (Arif *et al.*, 2020). Detrimental effects of salinity also affect the water uptake system and nutrient balance of the plants under stress which leads to reduced photosynthetic efficiency and reduced growth and development in the susceptible plants (Turan *et al.*, 2009; Shoukat *et al.*, 2023). As a result, plants exposed to salinity often exhibit poor germination, stunted vegetative growth and weak seedling establishment, which ultimately results in higher yield losses, especially in susceptible plants (Balasubramaniam *et al.*, 2023; Ghafoor *et al.* 2025). During this study, different kinds of responses by different varieties were observed in terms of growth indicators, oxidative stress responses and photosynthetic pigments. *Chakwal-86*, *Galaxy-2013*, and *Akbar-2019* showed better survival properties in terms of growth indicators under salinity stress while *Sehar 2006*, *Chakwal 97*, *Suleman 96*, and *Inqilab 91* showed susceptibility towards oxidative stress. These differences underscore the role of genetic diversity in salinity response of the wheat varieties under study.

Salinity has also been reported to induce oxidative stress caused by ROS across diverse crop species, including wheat, barley, and rice. ROS also act as signaling molecules under stress conditions (Kumar *et al.*, 2020; Pastuszak *et al.*, 2020). The plants respond to this oxidative stress by using different kinds of enzymatic and nonenzymatic defense mechanisms. Increase in the activity of key antioxidant enzymes, for instance Catalase, POD, SOD and APX has already been reported in plants under oxidative stress (Muthukumarasamy *et al.*, 2000; Jaleel *et al.*, 2007; Datir *et al.*, 2020; Naz *et al.*, 2022). All tested wheat varieties showed elevated antioxidant enzyme activities in response to salinity stress, though the extent of induction varied considerably among varieties. *Chakwal-86* variety showed the highest activity of antioxidant enzymes in response to salinity stress and was better able to protect itself under stress conditions. In addition to *Chakwal-86*, *Galaxy-2013* and *Akbar-2019* also exhibited a higher increase in antioxidant enzyme activities under salinity stress compared to other varieties, suggesting a more efficient antioxidative defense system in these varieties. It also means that these wheat varieties were able to keep ROS within limits, where it acted as signaling agent and helped the plants instead of causing damage. Catalase exhibited the greatest variation, ranging between 6% to 48% in its activity under salinity stress, followed by POD (11% to 45%), which also showed a significant difference compared with the control. Although APX (2%-29%) and SOD activities (7%-10%) increased significantly under salinity, their enhancement was less

pronounced than that of Catalase and POD. In contrast to *Chakwal 86, Akbar 2019* and *Galaxy 2013* the varieties with highly affected growth, including *Pakistan-81, Inqilab-91, Sehar-2000, Suleman-96*, and *Ujala 2016* exhibited only a minimal increase in antioxidant enzyme activities under salt stress compared with control conditions, showing a weaker oxidative stress response.

Oxidative stress also triggers lipid peroxidation in plants, which can be measured by measuring MDA content, which is known as an indicator of membrane damage (Natasha *et al.,* 2022; Hussein *et al.,* 2023). In this study, we found that MDA levels were significantly higher in all wheat varieties under salinity stress. However, the tolerant varieties exhibited only a slight increase in MDA, whereas the susceptible ones showed a pronounced rise, indicating greater oxidative damage and reduced membrane stability. Higher MDA contents of up to 62% were observed in *Pakistan 81*, *Margalla 99* under salinity, which indicates the increased vulnerability of the membranes in these varieties. while less increase in MDA of up to 28% was observed in case of *Chakwal 86, Galaxy 2013*, *Pakistan 2013* showing the stability of membranes in these varieties under stress. Chlorophyll content is known as a reliable indicator of the physiological and photosynthetic status of plants under stress conditions. Consistent with already published reports, salinity stress has been shown to significantly decrease photosynthetic pigment levels in different plant species including wheat (Arain *et al.,* 2021; EL Sabagh *et al.,* 2021; Akram *et al*., 2025). Higher reduction in photosynthetic pigments was observed in case *Pakistan 81*, *Auqab 2000*, *Chakwal 97*, *Suleman 96*, and *Ujala 2016* with up to 38% reduction in total chlorophyll contents under salinity, which shows the reduced photosynthetic efficiency of these plants which resulted in their reduced growth under stress. *Chakwal 86, Akbar 2019*, and *Pakistan 2013* showed less reduction in photosynthetic pigments under stress conditions, with a minimum reduction of up to 10%. This indicates that these varieties were able to efficiently use the light energy for photosynthesis and sustained growth. *Chakwal-86*, *Galaxy-2013*, and *Akbar-2019* maintained comparatively higher pigment levels, showing greater tolerance to salinity-induced oxidative damage. The effects of salinity stress varied significantly among the studied wheat varieties in the current pot-based experiments. *Chakwal-86*, *Akbar-2019* and *Galaxy-2013* showed the least reduction in growth and physiological parameters under salinity stress. This shows a higher degree of tolerance of these varieties towards salinity stress. On the contrary, *Pakistan-81, Inqilab-91, Suleman-96, Ujala 2016* and *Sehar-2006* exhibited higher growth inhibition and physiological damage under salinity stress.

Overall, the tolerant varieties showed enhanced activity of antioxidant enzymes, which contributed to the prevention against oxidative damage in these varieties and maintained better vegetative growth of these plants under salinity stress. However, the susceptible varieties showed lower activity of antioxidant enzymes, followed by higher lipid peroxidation and significant loss of photosynthetic pigments.

These findings suggest that stronger antioxidant defense mechanisms play a major role in alleviating the detrimental effects of salinity and hence support the physiological stability in tolerant wheat genotypes. Wheat varieties identified of salinity tolerance (*Chakwal-86*, *Akbar-2019*, and *Galaxy-2013*) may therefore serve as valuable genetic resource for future breeding programs aimed at developing salinity tolerant wheat varieties.

Conclusion: This study offers valuable insights into the differential salinity responses of Pakistani wheat varieties, highlighting antioxidant defences and physiological adaptations. However, it is limited by the absence of direct measurements such as ion concentrations (e.g., Na^+/K^+ ratios), osmolyte accumulation (e.g., proline or glycine betaine), or gene expression profiles that could provide deeper mechanistic understanding. Future research should expand to multi-location field trials to assess the interactions with other abiotic and biotic factors. Incorporating advanced genomic tools, such as identifying QTLs or transcription factors for enhanced salinity tolerance, could accelerate breeding efforts.

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Authors Contribution: R.K., R.N. and MSG conceived and designed the study. M.S.G. performed the experiments. Data analysis was conducted by M.S.G., A.N., H.Y., M.S., and R.K. M.S.G. and R.N. drafted, revised the manuscript, with input from all authors.

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