

## ASSESSMENT OF ANTIOXIDANTS ACTIVITY IN SEVEN WHEAT GENOTYPES GROWN UNDER NaCl SALINITY

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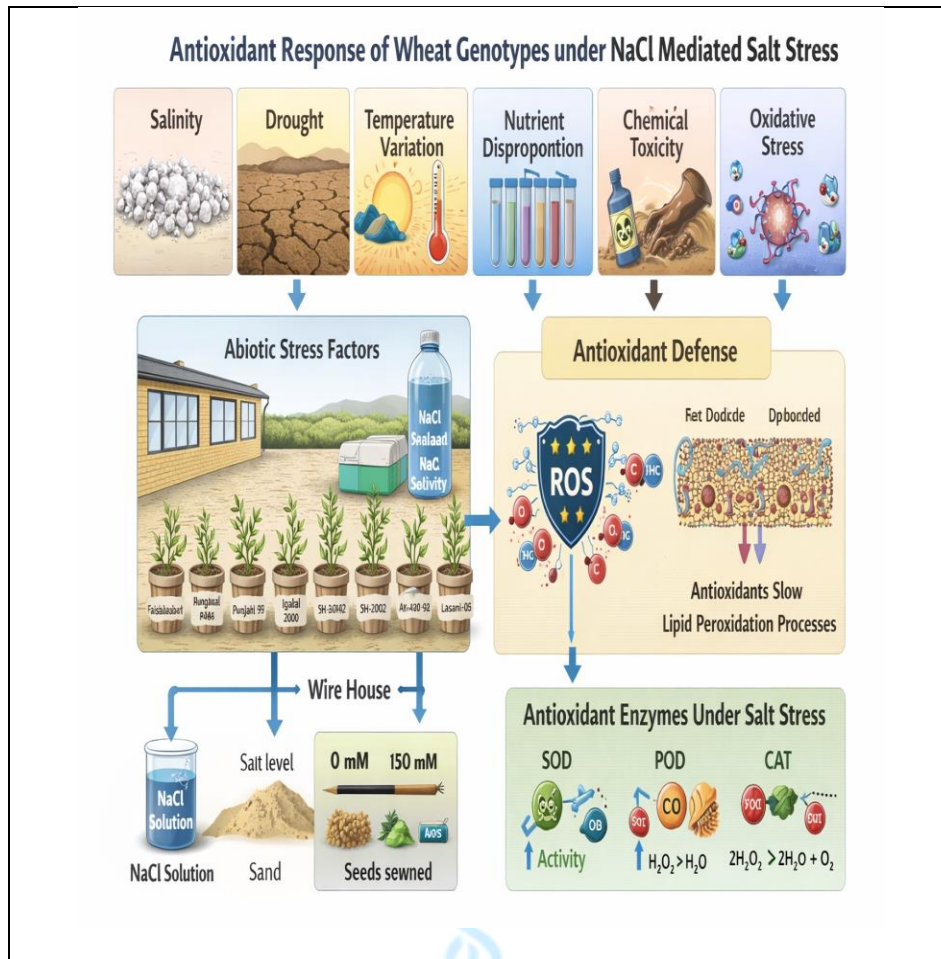
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### Abstract

Salinity, drought, temperature variation, nutrient disproportion, chemical toxicity and oxidative stress are the very important environmental problems that effect crop productivity in the whole world. A sand culture experiment was carried out in a wire house of old Botanical Garden, University of Agriculture, Faisalabad. Seven genotypes of wheat were collected from Ayub Agricultural Research Institute, Faisalabad that was Parwaz 94, Faisalabad-83, Punjab-96, Iqbal 2000, SH- 2002, AS- 2002 and Lasani- 08. Seeds were sown in plastic pots containing 10 kg of thoroughly washed sand and irrigated with full strength Hoagland's nutrient solution. The experiment was laid out in completely randomized design (CRD) factorial with 3 replications. Two salt levels i.e. 0 and 150 mM L<sup>-1</sup> were applied to establish salt stress conditions. After germination, the seedlings were treated with salinity. Antioxidants are the substances that counteract oxidative stress in plants subjected to some abiotic menace due to up regulation of reactive oxygen species. Antioxidants slow down lipid per-oxidation processes and thus shield biological membranes. It is believed that plants with high antioxidant levels have better confrontation to reactive oxygen species. In present studies we checked the effect of salinity on production of antioxidant species in wheat. The main objective of this study was to check the activity of antioxidants under NaCl stress. In current study the antioxidant (SOD, POD, CAT) activity was enhanced under NaCl stress.



Graphical abstract for assessment of antioxidants activity in seven Wheat genotypes grown under NaCl salinity

## INTRODUCTION

Wheat is one of the major cereals in the world and is the main sources of protein as well as calories. For basic calories and protein, approximately 85% and 82% of the global population depends on wheat respectively [1]. Moreover, it is used in the making of many wheat products, like leavened bread and steamed breads, pasta, cakes, biscuits, couscous, noodles and beer [2]. Wheat is cultivated in tropical and subtropical regions and under both irrigated and rain-fed cultivation. However, crop production is severely affected by unfavorable environmental stresses [3].

The adaptation to salinity could be classified in three mechanisms: lenience to osmotic stress (not particular to salinity and coupled with water stress);

$\text{Na}^+$  elimination from leaf blades ( $\text{Na}^+$  is mount up in the root shielding the leaves from toxic levels of salt) and tissue tolerance to assemble  $\text{Na}^+$  (the ion is compartmentalized at intracellular and also at cellular level to avoid toxic substances within the cytoplasm [4]). Various growth factors like root length, shoot length, dry mass of root and shoot are used for the determination of salinity of plants [5]. Different environmental biotic and abiotic factors (Such as light, temperature and heavy metals) change the content and composition of organic solutes, although a little is known about organic solutes accumulation in response to salinity [6]. Increased concentrations of any kind of secondary metabolites

and organic solutes are involved in osmotic balance [7].

There are different salt resistance methods in various halophyte plant species, including ion homeostasis, osmoregulation, antioxidant and hormonal regulation [8]. Some compatible solutes play vital role in the plant defensive mechanisms; for example, sugars are compatible solutes which accumulate in plant tissues and resulted to abiotic stresses, such as, water deficiency, temperatures fluctuation and salt stress. The sugars accumulation plays an important role in osmoregulation and energy conservation [8]. The main objective of the study was to determine the hazardous effects of salt stress on some antioxidant enzyme activities in wheat genotypes and also to find out the wheat genotype with better production of antioxidants under salt stress conditions.

#### Materials and methods

The research work presented in this manuscript was conducted to evaluate the effects of NaCl salinity on antioxidative enzyme activity in seven wheat genotypes.

#### Study area

A sand culture experiment was carried out in wire house of old Botanical Garden, University of Agriculture, Faisalabad.

#### Wheat lines

The seven wheat genotypes were Parwaz 94, Faisalabad-83, Punjab-96, Iqbal 2000, SH- 2002, AS-2002 and Lasani- 08.

#### Seed source

Seven wheat genotypes were collected from Ayub research center Faisalabad.

#### Experiment

Seeds were sown in plastic pots containing 10 kg of thoroughly washed sand and irrigated with full strength Hoagland's nutrient solution. The experiment was laid out in completely randomized design (CRD) factorial with 3 replications. Two salt levels i.e. 0 and 150 mM L<sup>-1</sup> were applied to establish salt stress conditions. The seeds of 7 wheat cultivars i.e. Parwaz 94, Faisalabad-83, Punjab-96, Iqbal-2000, SH-2002, AS 2002 and Lasani-08 were obtained from Ayub research center. After germination, the

seedlings were treated with salinity. Hoagland's nutrient solution was given in order to provide nutrients to the plants

#### Collection of Data

The data for antioxidant activity were studied. Thereafter, two plants from each pot were harvested and leaves were preserved as fresh material for analysis i.e determination of activity of antioxidants.

#### Enzyme extraction

To extract antioxidant enzymes, fresh leaves (0.5 g) were ground using a tissue grinder in 5 mL of 50 mM cooled phosphate buffer (pH 7.8) placed in an ice bath. The homogenate was centrifuged at 15000 rpm for 20 min at 4<sup>o</sup>c. The supernatant was used for assays of activities of enzymes.

#### Superoxide dismutase: (SOD)

The activity of SOD was determined by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) following the method of [9]. The reaction solution (3mL) contained 50 micro molar NBT, 1.3 micro molar riboflavin, 13 mM methionine, 75 nM EDTA, 50 mM phosphate buffer (pH 7.8) and 20 to 50 micro liter enzyme extract. Test tubes containing the reaction solution were irradiated under light (15 fluorescent lamps) at 78 micro mol m<sup>-2</sup> s<sup>-1</sup> for 15 min. The absorbance of irradiated solution at 560 nm was read using a spectrophotometer. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.

#### Catalase and peroxidase

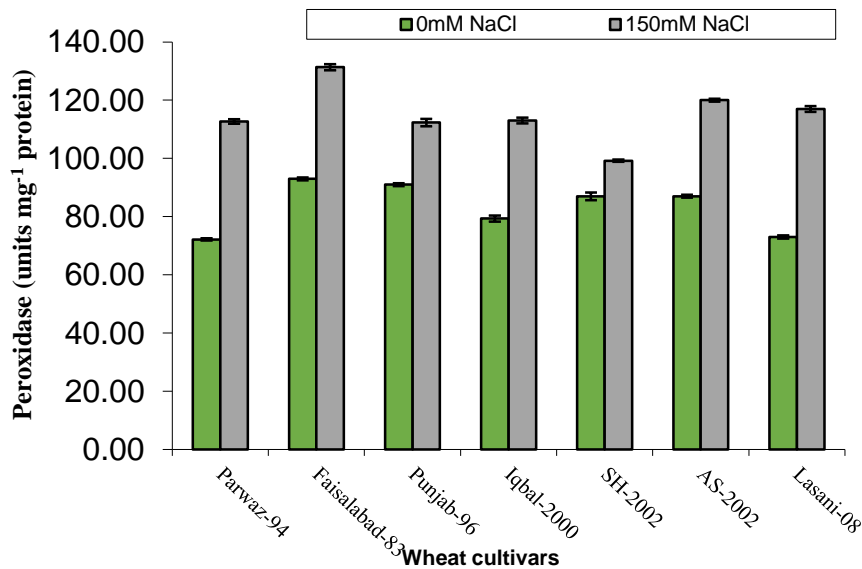
Activities of CAT and POD were measured using the method of [10] with some modification. The CAT reaction solution (3 mL) contained 50 mM. Phosphate buffer (PH 7), 5.9 mM H<sub>2</sub>O<sub>2</sub>, and 0.1 mL enzyme extract. Then reaction was initiated by attracting enzyme extract. Changes in absorbance of the reaction solution at 240 nm were read every 20 sec. one unit CAT activity was defined as an absorbance change of 0.01 units per min. The POD reaction solution (3 mL) contained 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM H<sub>2</sub>O<sub>2</sub>, and 0.1mL enzyme extract. Changes in absorbance of the reaction solution at 470 nm were

determined every 20 s. One unit, POD activity was defined as an absorbance change of 0.01 units per min [11]. The activity of each enzyme was expressed on protein basis. Protein weight concentration of the extract was measured by the method of [12].

**Statistical analysis**

The data was recorded and analyzed statistically and treatment means will be compared by least significant differences (LSD) test at 5% probability level.

**Results**



**Fig. 1: Peroxidase activity of seven wheat (*Triticum aestivum* L.) genotypes when 55 days old plants were grown under non-saline and saline conditions. Peroxidase (POD) (Units mg<sup>-1</sup> protein)**

Analysis of variance of data for POD activity of 7 wheat genotypes is presented in (Fig 1). Imposition of salt stress (150 Mm NaCl) caused an increase in POD activity of all 7 wheat genotypes. Maximum

increase of POD activity with respect to their controls was observed in all wheat genotypes except in SH-2002 (Fig 1).

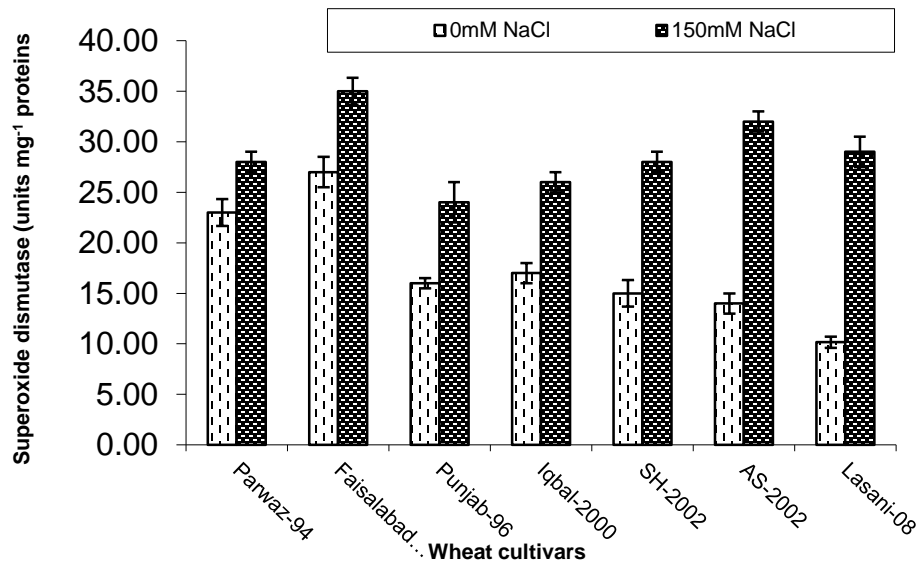


Fig. 2: Superoxide dismutase activity of seven wheat (*Triticum aestivum* L.) genotypes when 55 days old plants were grown under non-saline and saline conditions.

Superoxide dismutase ( units mg<sup>-1</sup> proteins)  
Analysis of data for Superoxide dismutase activity of 7 wheat genotypes is presented in (Fig 2). There is an increase in Superoxide dismutase activity in all of 7 wheat genotypes due to the application of salt stress.

All the wheat genotypes differ with respect to Superoxide dismutase activity (Fig 2). Maximum increase of Super oxide dismutase activity was observed in AS-2002 and Lasani-08. However minimum increase was observed in Parwaz-94.

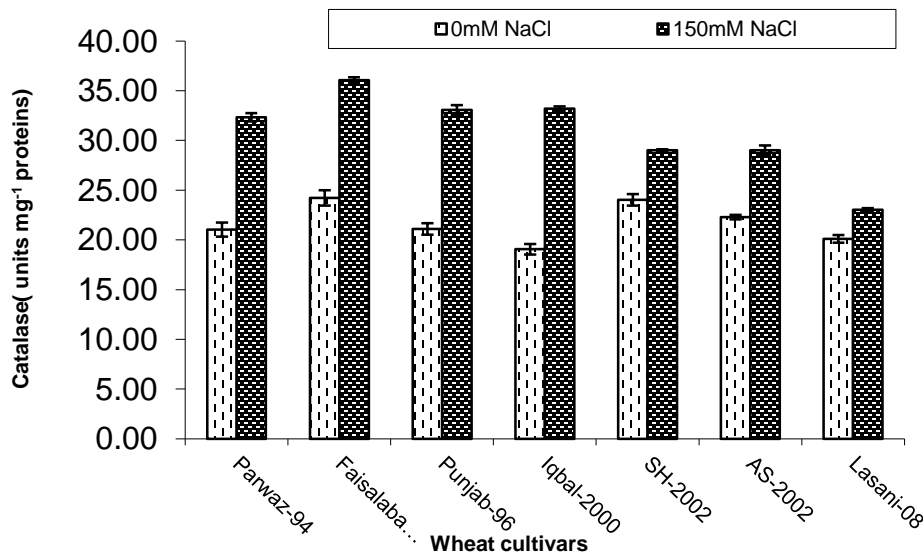


Fig. 3: Catalase activity of seven wheat (*Triticum aestivum* L.) genotypes when 55 days old plants were grown under non-saline and saline conditions.  
Catalase (units mg<sup>-1</sup> proteins)

Data for Catalase activity of 7 wheat genotypes is presented in (Fig 3). Application of salt stress (150 Mm NaCl) caused an enhancement in catalase activity of all 7 wheat genotypes. Maximum increase was observed in cv. Iqbal-2000 followed by cvs. Faisalabad-83, Parwaz-94 and Punjab-96 but minimum increase in catalase activity was observed in cv Lasani-08. However SH-2002 and AS-2002 showed similar behavior under saline and non-saline conditions (Fig. 3).

### Discussion

Antioxidants are the substances that counteract oxidative stress in plants subjected to some abiotic menace due to up regulation of reactive oxygen species. Antioxidants inhibits lipid peroxidation processes and thus protects biological membranes. It is believed that plants with high antioxidant levels, whether induced or constitutive, have better resistance to ROS [13]. In present studies the antioxidant (SOD, POD, CAT) activity was enhanced under salinity stress. [14] noted the activity of catalase in two wheat genotypes in relation to salt tolerance. It was observed that salt-stress caused an increase in activity of catalase in both wheat cultivars. Cultivar Lu-26 was higher in activity of catalase. A significant relationship between the activity of catalase and plant growth was found which appraised in terms of dry biomass. However, contrarily, [15] found a non-significant relationship between growth and the activities of SOD, CAT and POD in five cultivars of turnip under saline conditions. [16] conducted an experiment to evaluate the Effect of salt stress on antioxidant activity and seedling growth of three canola cultivars. They found that the activity of CAT and POD was enhanced under salinity stress and these results were in line with those found in present research.

In present study, there was also a significant increase in activity of catalase in all wheat cultivars. Maximum increase was observed in cv. Punjab-96 followed by cvs. Faisalabad-83, Parwaz-94 and Iqbal-2000 but minimum increase in catalase activity was observed in cv Lasani-08. However SH-2002 and AS-2002 showed similar behavior under saline and non-saline conditions. POD activity was increased in 7 wheat cultivars except SH-2002. However maximum increased in SOD activity was observed in cvs. AS-

2002 and lasani-08 and minimum was in Parwaz-94. Same was observed in wheat [17], [18], [19], [20], Peeper [21] Kochia [22], Pumpkin [23].

### Conclusion

Under saline stress the antioxidant activity is enhanced and each variety has ability to perform better under salt stress conditions.

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