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UNDERSTANDING SALT TOLERANCE MECHANISMS IN WHEAT GENOTYPES BY EXPLORING ANTIOXIDANT ENZYMES

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The activities of antioxidant enzymes were analyzed in six wheat genotypes under different concentrations of NaCl (0, 100 and 200 mM). Plants were harvested after either 15 or 30 days of salt stress. The most salt tolerant genotype (SARC-1) maintained lower Na^+ and higher relative growth rate (RGR), shoot fresh weight (SFW), shoot-root ratio, and K^+:Na^+ ratio, compared to the most salt sensitive genotypes (S-9189 and S-9476). Superoxide dismutase (SOD) and catalase (CAT) increased significantly in SARC-1 and SARC-2 with increasing salt stress, while there was no difference in S-9189 and S-9476. Additionally, glutathione reductase (GR) activity was decreased in salt sensitive (S-9189 and S-9476) than salt tolerant (SARC-1) genotypes. Under salt stress conditions a negative relationship between SOD and leaf Na^+, and a positive between SOD and shoot fresh weight (SFW), were observed. The higher efficiency of antioxidant enzymes of tolerant genotypes could be considered as one of the factors involved in salt tolerance of wheat.
Keywords: Salt tolerance, oxidative stress, antioxidants, wheat

INTRODUCTION

Wheat is a staple food crop around the world which is grown on both good soils and under drier conditions, such as in the Mediterranean region (Jacobsen *et al.,* 2012). In Pakistan wheat is cultivated on 8.5 million ha (Hashmi and Shafiullah, 2003). Among abiotic stresses, salinity is the major constraint to wheat production in Pakistan as 6.7 million ha is affected by salinity to varying extent which equals about 1/3 of total cultivated area in the country (Khan, 1998).

In addition to osmotic and ionic effects (Ahmad *et al*., 2012; Arshad *et al*., 2012 and Saqib *et al*., 2012), salinity may lead to the excessive production of reactive oxygen species (ROS) that is being considered as a main stress factor (Khan *et al.,* 2010; D'Souza and Devaraj, 2010). ROS is a group of oxygen containing molecules having free electrons like hydroxyl radical (HO^-), superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The ROS are extremely cytotoxic and can disrupt a number of biomolecules like proteins, nucleic acids and lipids (Azevedo *et al.,* 2006; Parvaiz and Satyawati, 2008). ROS causes the peroxidation of essential amino acids by reacting with unsaturated fatty acids in plasmalemma or intracellular organelles, and cellular leak and desiccation of the cell (Costa *et al.,* 2005). Especially OH^- can destruct DNA, lipids, chlorophyll, protein and other macromolecules, that adversely affect the plant physiological process necessary for proper growth and yield (Gapinska *et al.,* 2008; Davenport *et al.,* 2005). ROS also play a role as key regulators of growth, development and defense pathways, however the mechanism by which these are controlled is not known (Breusegem *et al.,* 2008).

ROS are counteracted by a group of scavenging antioxidant enzymes that neutralize ROS like superoxide dismutase (SOD) catalyses the dismutation of O_2^- radical to H_2O_2 and O_2 (Li *et al.,* 2010; Asada, 2006; Fedoroff, 2006). The H_2O_2 produced, which is also cytotoxic for plants, is subsequently catalyzed by enzymes such as APX, GR, CAT (Farhoudi *et al.,* 2012; Venkatesan and Sridevi, 2009; Radic *et al.,* 2006). Under normal growth conditions, there is equilibrium between generation and scavenging of ROS (Keshavkant *et al.,* 2012). However, when the plants are exposed to adverse environmental conditions, the scavenging system lags behind resulting in an excess of ROS. Vital molecules are attacked and cell metabolism is disturbed, causing cell death (Weisany *et al.,* 2012).

Membranes are the primary sites of salt injury to cells and organelles (Kumar *et al.,* 2009). It has been frequently reported that the enhanced activities of antioxidant enzymes are responsible for salt stress tolerance. In rice, the salt-tolerant varieties have higher SOD activity and lower lipid peroxidation than salt-sensitive varieties (Kumar *et al.,* 2009). In tomato and citrus, elevated activities of SOD, APX and CAT were responsible for improved salt tolerance (Chookhampaeng *et al.,* 2008; Mittova *et al.,* 2004). Further supporting evidence on the involvement of antioxidant enzymes in salt tolerance has been provided by transgenic plants with a reduced or an increased expression of antioxidant enzymes (D'Souza and Devaraj, 2010).

In previous studies SARC-1, SARC-2, SARC-3, SARC-4 genotypes were found to be relatively salt-tolerant whereas S-9189 and S-9476 were described as salt-sensitive (Saqib *et al.,* 2006, 2012; Qureshi *et al.,* 2003). Therefore, these genotypes differing in salt-tolerance were used for comparison of antioxidant activity; this information will help us comprehend the biochemical and physiological mechanisms responsible for salt stress tolerance of plants. Based on these facts, the objective of our current experiment was to examine the role of antioxidant enzymes activity in salt tolerance in wheat genotypes.

MATERIALS AND METHODS

***Plant material and growth conditions*:** Six wheat (*Triticum aestivum* L.) genotypes SARC-1, SARC-2, SARC-3 and SARC-4 (obtained from Saline Agriculture Research Centre, University of Agriculture, Faisalabad), and S-9189 and S-9476 (obtained from Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad) were used in this study. The experiment was conducted under control conditions in a growth chamber (Light intensity of 400 µmol m^{-2} s^{-1} for 15 h, day and night temperatures 25 and 18ºC, relative humidity (RH) 60-65%). Healthy seeds of each genotype were surface sterilized with 1% sodium hypochlorite solution and sown in polythene lined iron trays having two inches layer of acid washed quartz sand. Seedlings, after germination were irrigated with Hoagland's nutrient solution (Hoagland and Arnon, 1950). Uniform seedlings at two leaf stage were randomly transferred to Hoagland's solution. NaCl salinity (0, 100 and 200 mM) was developed by stepwise addition of NaCl after one week of transplanting, adding a third part of total salt for three consecutive days. Aeration was given with air pumps for 8 hours a day, pH 6.0-6.5. After every 10 days interval nutrient solution was changed. Leaf samples were taken after 15 and 30 days of salt treatment and stored in refrigerator for analysis.

***Growth Parameters*:** To assess the effect of NaCl on plant growth, 15 and 30 day old seedlings were collected and shoot (SFW) and root fresh weights (RFW), shoot (SL) and root lengths (RL) were measured. Relative growth rate (RGR) was calculated according to Hoffmann and Poorter (2002).

***Leaf sap analysis*:** The youngest fully expanded 2-3 leaves of wheat genotypes were detached after 15 and 30 days of salt treatment, quickly rinsed with distilled water, dried with tissue paper and stored in Eppendorf tubes at freezing temperature. Frozen leaf samples were thawed and crushed using a stainless steel rod with tapered end. The sap was collected in Eppendorf tubes by Gilson pipette and centrifuged at 6500 x g for 10 min (Gorham, 1984). The leaf sap was diluted as required by adding distilled water. Sodium and potassium was determined using Sherwood 410 Flame photometer with the help of standard solutions using reagent grade salts of NaCl and KCl.

Determination of Antioxidant Enzymes

***Enzyme extraction*:** About 0.5g leaf sample was homogenized in ice cold 0.1 M potassium phosphate buffer having 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 15000 x g. The supernatant was saved and used for the activity of enzymes (Esfandiari *et al.,* 2007).

***Enzyme assay*:** Superoxide dismutase (SOD) activity was determined according to Sen Gupta *et al.,* (1993) by noting the decrease in absorbance of the reaction mixture [0.05 ml enzyme extract, 0.1 ml (200 mM) methionine, 0.01 ml (2.25 mM) nitro-blue tetrazolium NBT, 0.1 ml (3 mM) EDTA, 1.5 ml (100 mM) potassium phosphate buffer and 1 ml distilled water] at 560 nm. The reaction mixture having no enzyme extract acted as control. The reaction was started by adding riboflavin (0.1 ml, 60 µM) and placing the tubes under 15 W fluorescent lamp. After fifteen minutes, reaction was stopped by switching off the lamp and absorbance was measured on UV spectrophotometer.

Catalase (CAT) activity was assayed by measuring the decomposition of H_2O_2 (Aebi 1984). The reaction mixture (3 ml) comprising of potassium phosphate buffer (1.5 ml, 100 mM, pH=7), H_2O_2 (0.5 ml, 75 mM), enzyme extract (0.05 ml) and distilled water to make up the volume. H_2O_2 was added to start the reaction and absorbance was measured at 240 nm for 1 minute.

Glutathione reductase (GR) activity was assayed according to Sairam *et al.* (2002). The reaction mixture containing potassium phosphate buffer (0.2 M, pH=7.5), EDTA (0.1 mM), DTNB (0.5 ml, 3 mM), NADPH (0.1 ml, 2 mM), enzyme extract (0.1 ml) and distilled water to make up a final volume of 2.9 mL. Reaction was initiated by adding 0.1 mL of 2 mM GSSG and increase in absorbance at 412 nm over a period of 5 min on a spectrophotometer.

The leaf protein content was assayed by the Bradford method (Bradford, 1976).

***Statistical analysis*:** The experiment was conducted using a completely randomized design (CRD) with three replicates. Each treatment was analyzed and a standard error (SE) was calculated; data are expressed as mean ± SE replicates. Results were analyzed by analysis of variance with "Statistix 8.1" (www.statistix.com) (Steel and Torrie, 1997).

RESULTS

Salt stress significantly inhibited shoot fresh weight (SFW), relative growth rate (RGR) and shoot-root ratio of all wheat genotypes (Table 1-2, Fig. 1). The reduction in SFW of the most tolerant SARC-1 was 21 and 38% at 100 and 200 mM NaCl, respectively at first harvest (15 days of salt stress). Prolonged salt stress up to 30 days significantly increased

Table 1. Shoot fresh weight (SFW, g) at increasing salinity in different wheat genotypes. Each value is an average of three replicates ±SE. Values in the ( ) are the % reduction from control

Genotypes	0 mM NaCl		100 mM NaCl		200 mM NaCl	
	15d salinity	30d salinity	15 d salinity	30d salinity	15 d salinity	30d salinity
SARC-1	1.11 ± 0.04	4.91 ± 0.65	0.88 ± 0.12 (21)	3.54 ± 0.34 (28)	0.69 ± 0.08 (38)	1.67 ± 0.09 (66)
SARC-2	0.99 ± 0.06	6.16 ± 0.44	0.70 ± 0.07 (29)	3.85 ± 0.13 (38)	0.38 ± 0.09 (61)	0.68 ± 0.02 (89)
SARC-3	1.42 ± 0.07	7.86 ± 0.87	0.82 ± 0.13 (42)	3.86 ± 0.16 (51)	0.58 ± 0.05 (59)	0.76 ± 0.06 (90)
SARC-4	1.20 ± 0.09	7.12 ± 0.48	0.84 ± 0.08 (30)	4.24 ± 0.47 (40)	0.34 ± 0.03 (72)	0.82 ± 0.03 (88)
S-9189	1.40 ± 0.11	6.98 ± 0.57	0.81 ± 0.10 (36)	3.89 ± 0.26 (44)	0.38 ± 0.03 (73)	0.42 ± 0.42 (94)
S-9476	1.24 ± 0.19	5.49 ± 0.32	0.71 ± 0.08 (40)	2.72 ± 0.14 (50)	0.32 ± 0.03 (74)	0.33 ± 0.15 (93)

Table 2. Shoot-root ratio at increasing salinity in different wheat genotypes. Each value is an average of three replicates ± SE. Values in the ( ) are the % reduction from control

Genotypes	0 mM NaCl		100 mM NaCl		200 mM NaCl	
	15 d	30 d	15 d	30d	15 d	30 d
SARC-1	2.41 ± 0.42	1.64 ± 0.13	2.09 ± 0.25 (13)	1.24 ± 0.14 (24)	1.51 ± 0.25 (37)	1.13 ± 0.10 (31)
SARC-2	1.93 ± 0.23	1.85 ± 0.05	1.65 ± 0.28 (14)	0.95 ± 0.05 (49)	0.96 ± 0.06 (50)	0.55 ± 0.09 (70)
SARC-3	1.97 ± 0.23	1.77 ± 0.09	1.52 ± 0.13 (23)	0.97 ± 0.02 (45)	0.96 ± 0.09 (57)	0.48 ± 0.05 (73)
SARC-4	1.86 ± 0.10	1.78 ± 0.11	1.53 ± 0.29 (18)	0.92 ± 0.05 (48)	0.94 ± 0.10 (49)	0.76 ± 0.05 (57)
S-9189	2.50 ± 0.24	1.75 ± 0.14	1.96 ± 0.43 (22)	1.04 ± 0.06 (41)	0.90 ± 0.15 (64)	0.78 ± 0.08 (56)
S-9476	2.16 ± 0.32	1.35 ± 0.14	1.44 ± 0.15 (33)	0.98 ± 0.17 (28)	0.96 ± 0.20 (55)	0.79 ± 0.10 (42)

the reduction in SFW of SARC-1 with 28 and 66% reduction at 100 and 200 mM NaCl, respectively (Table 1).

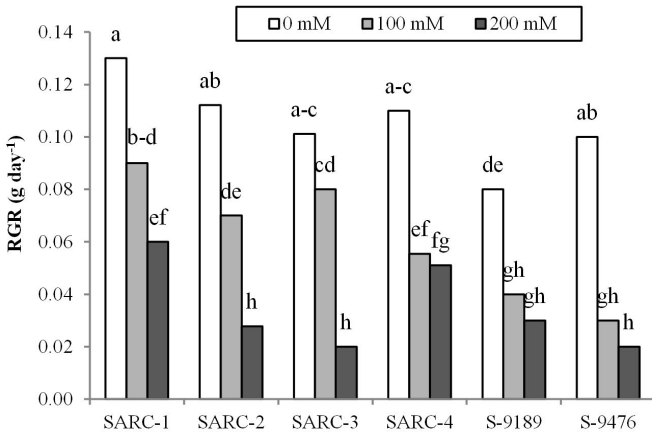

Figure 1. Relative growth rate (RGR) at increasing salinity (100 and 200 mM NaCl) in different wheat genotypes. Values sharing same letters represent Non-significance.

SFW of sensitive wheat genotypes was reduced drastically with 50 and 93% reduction in S-9476 at 100 and 200 mM NaCl respectively, after 30 days of salt stress.

The reduction in RGR of tolerant genotypes (SARC-1 and SARC-2) was less compared to the sensitive genotypes (S-9189 and S-9476) showing the maximum reduction at 100 and 200 mM NaCl stress (Fig.1). SARC-1 had the best RGR under non-saline (0 mM NaCl) and both stressful conditions.

Shoot-root ratio of all the genotypes decreased significantly with increasing salt stress as well as duration of exposure to salt (15 and 30 days) (Table 2). The salt tolerant genotype (SARC-1) had less reduction (13 and 37% compared to control) at 100 and 200 mM NaCl respectively, while 33 and 55% reduction was observed in salt sensitive (S-9476) at the two salt concentrations. The reduction was more pronounced after 30 days (SARC-1 24 and 31%, and S-9476 28 and 42%, at 100 and 200 mM NaCl respectively.

All six genotypes exhibited a significant increase in leaf Na^+ and decrease in leaf K^+ concentration with increasing salt (NaCl) concentration in the growth medium (Table 3-4). However, the increase in leaf Na^+ content was lower in SARC-1 than the other genotypes. As a result, K^+:Na^+ was

Table 3. Leaf Na^+ content (mol m^{-3}) at increasing salinity in different wheat genotypes. Each value is an average of three replicates ± SE

Genotype	0 mM NaCl		100 mM NaCl		200 mM NaCl	
	15 d	30 d	15 d	30 d	15 d	30 d
SARC-1	27 ± 2.64	40 ± 1.67	54 ± 4.87	59 ± 3.95	104 ± 6.87	109 ± 9.50
SARC-2	28 ± 1.96	39 ± 2.94	60 ± 5.16	69 ± 6.21	115 ± 9.68	131 ± 9.59
SARC-3	28 ± 1.89	38 ± 1.16	56 ± 5.23	67 ± 5.73	114 ± 10.23	129 ± 10.62
SARC-4	30 ± 2.89	41 ± 1.74	62 ± 4.89	72 ± 4.90	121 ± 11.16	134 ± 8.79
S-9189	46 ± 2.07	50 ± 1.31	107 ± 8.69	113 ± 7.62	158 ± 10.82	180 ± 12.23
S-9476	45 ± 3.12	48 ± 1.04	105 ± 9.19	111 ± 8.37	163 ± 11.17	173 ± 12.74

Table 4. Leaf K^+ content (mol m^{-3}) at increasing salinity in different wheat genotypes. Each value is an average of three replicates ± SE

Genotype	0 mM NaCl		100 mM NaCl		200 mM NaCl	
	15 d	30 d	15 d	30 d	15 d	30 d
SARC-1	90 ± 1.24	198 ± 3.08	82 ± 1.19	186 ± 4.97	75 ± 1.34	122 ± 2.84
SARC-2	90 ± 2.81	179 ± 1.22	85 ± 2.77	170 ± 2.82	77 ± 2.95	127 ± 2.74
SARC-3	94 ± 2.68	175 ± 2.29	88 ± 2.09	169 ± 3.58	79 ± 1.48	123 ± 3.65
SARC-4	90 ± 1.41	180 ± 2.73	89 ± 1.77	169 ± 2.07	80 ± 2.96	125 ± 2.48
S-9189	170 ± 1.72	193 ± 3.19	125 ± 5.26	159 ± 3.72	73 ± 1.93	106 ± 2.03
S-9476	176 ± 2.13	195 ± 4.81	129 ± 2.50	153 ± 4.78	61 ± 1.04	112 ± 1.93

also higher in the most salt tolerant genotype (SARC-1) as compared to salt sensitive genotypes (Table 5) at high salt stress.

Superoxide dismutase (SOD) activity had similar pattern in all six genotypes. In SARC-1, with the increased NaCl concentration, SOD activity increased significantly at 100 and 200 in comparison with the 0 mM NaCl (Fig 2).

Catalase (CAT) showed a similar pattern in all six genotypes (Fig 2). In SARC-1, CAT activity significantly increased at 200 in comparison with 0 mM NaCl.

GR activity decreased significantly when adding NaCl (100 and 200 mM) in comparison with 0 mM NaCl in all genotypes. A significant decrease in GR was observed in SARC-1 as compared to other genotypes (Fig 2).

DISCUSSION

Salt stress severely affected the growth of all six wheat genotypes after 15 and 30 days of salinity (Table 1 and 2). SARC-1 showed significantly higher values of SWF and shoot-root ratio than other genotypes, and the difference was most pronounced at the highest salt level. Munns and Tester (2008) presented two phase model of plant growth under salinity stress. In the first phase, plant respond rapidly to increase in external osmotic pressure and in the second phase the slower response to accumulation of Na^+ in leaves. The decrease in growth of wheat genotypes might also be due to oxidative stress caused by excessive (Parvaiz and Satyawati, 2008).

A negative relationship (r^2=0.684) was observed between shoot fresh weight (SFW) and leaf Na^+ concentration (Fig. 3). The K^+: Na^+ ratio decreased significantly due to increased Na^+ concentration in salt sensitive wheat genotypes with the addition of NaCl in the nutrient solution. The lower K^+ concentration in plant tissues salt stress (Table 4) might be due to antagonism Na^+ and K^+ at the roots uptake sites and lower transport of K^+ into the xylem (Willenborg *et al.,* 2004). The tolerant genotypes (SARC-1 and SARC-2) maintained substantially greater shoot biomass (Tab. 1), shoot-root ratio (Tab. 2) and RGR (Fig. 1), K^+: Na^+

Table 5. K^+/Na^+ at increasing salinity in different wheat genotypes. Each value is an average of three replicates ± SE

Genotype	0 mM NaCl		100 mM NaCl		200 mM NaCl	
	15 d	30 d	15 d	30 d	15 d	30 d
SARC-1	3.35 ± 0.08	4.96 ± 0.06	1.52 ± 0.06	3.15 ± 0.06	0.72 ± 0.04	1.12 ± 0.03
SARC-2	3.21 ± 0.05	4.58 ± 0.09	1.41 ± 0.05	2.47 ± 0.07	0.67 ± 0.03	0.97 ± 0.06
SARC-3	3.37 ± 0.06	4.60 ± 0.08	1.58 ± 0.1	2.52 ± 0.04	0.69 ± 0.07	0.96 ± 0.03
SARC-4	3.18 ± 0.04	4.40 ± 0.10	1.44 ± 0.04	2.35 ± 0.05	0.66 ± 0.05	0.93 ± 0.05
S-9189	3.69 ± 0.06	3.86 ± 0.04	1.17 ± 0.07	1.41 ± 0.03	0.46 ± 0.05	0.59 ± 0.07
S-9476	3.91 ± 0.07	4.07 ± 0.07	1.23 ± 0.05	1.38 ± 0.04	0.38 ± 0.08	0.65 ± 0.04

ratio and lower Na^+ tissue concentration (Table 5) than the salt sensitive genotypes (S-9189 and S-9476).

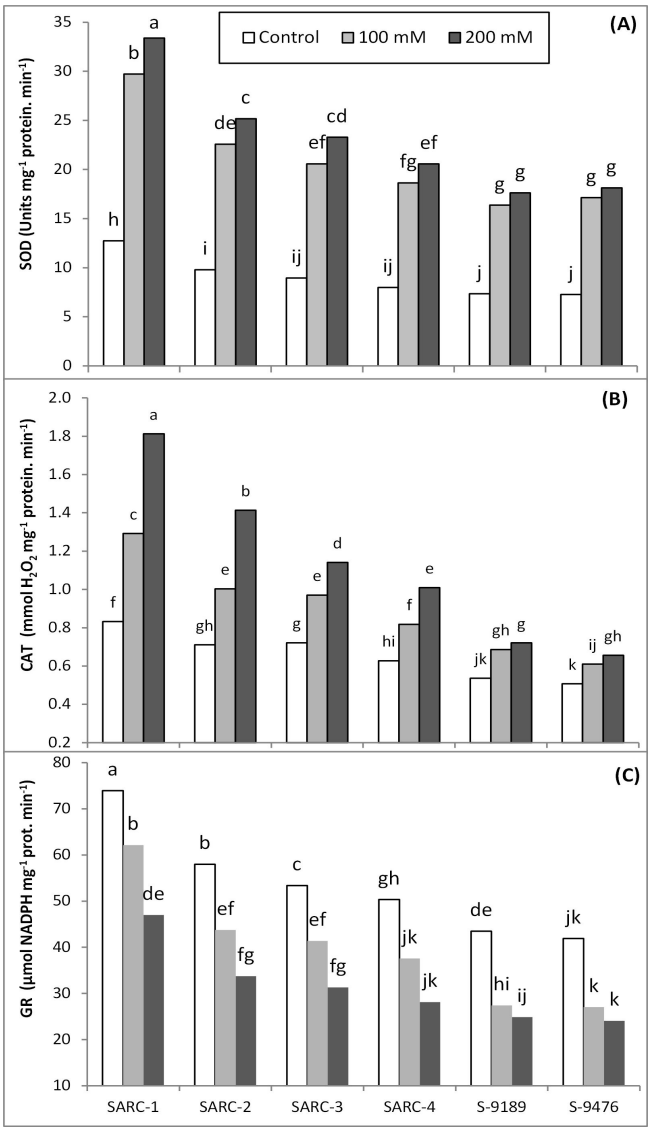

Figure 2. Antioxidant enzymes activity at increasing salinity (100 and 200 mM NaCl) in different wheat genotypes after 30 days of exposure. Values sharing same letters represent Non-significance.

SOD activity increased with the increasing salt concentration. Tolerant genotype SARC-1 showed maximum SOD activity both in salt stressed and non-saline conditions, whereas the lowest SOD activity was in S-9189 and S-9476 (Fig. 2). The higher activity of SOD in SARC-1 resulted in proper scavenging of ROS especially superoxide ($O_2^{\cdot-}$) leading to decreased oxidative damage and hence greater biomass production (Fig. 3). Plants that survive during the early stages of salt stress and produce more biomass will probably produce more grain yield. A low activity of SOD results in higher level of $O_2^{\cdot-}$ which disturbs vital biomolecules (Parvaiz and Satyawati, 2008; Esfandiari *et al.,* 2011). SOD inactivated other antioxidant enzymes including catalases required for scavenging of H_2O_2 (Fig. 2) (Oueslati *et al.,* 2010) and peroxidases (Yasar *et al.,* 2008). These enzymes work in series as SOD dismutases $O_2^{\bullet-}$ to H_2O_2, in the next step CAT neutralizes H_2O_2. In the salt sensitive genotypes, SOD activity was low to scavenge superoxide radical ($O_2^{\cdot-}$). Scavenging of this dangerous radical was not done properly, hence attacking vital biomolecules and damaging cell membranes. Elevated levels of SOD activity were closely related with decrease in oxidative damage (Esfandiari *et al.,* 2007; Oueslati *et al.,* 2010; Aghaleh and Niknam, 2009; Koca *et al.,* 2007; Koskeroglu and Tuna, 2008 and Zhao *et al.,* 2007).

SARC-1 had significantly higher CAT activity at 100 and 200 compared to the 0 mM NaCl and other genotypes, but with no increase in the sensitive S-9189 and S-9476. CAT activity in SARC-1 increased 1.7 and 2.3 fold compared to control at 100 and 200 mM NaCl, respectively, whereas it was 1.3 fold in S-9189 and S-9476. CAT is an important antioxidant enzyme that catalyses the conversion of H_2O_2 to H_2O in the peroxisomes (Aghaleh and Niknam, 2009; Venkatesan and Sridevi, 2009). In peroxisomes β-oxidation of fatty acids and photorespiration produces H_2O_2, (Weisany *et al.,* 2012). To scavenge H_2O_2 elevated levels of CAT are required to reduce H_2O_2 level in cells which in turn increases CO_2 fixation and membrane stability (Shao *et al.* 2005 a, b, c; Esfandiari *et al.,* 2007; Oueslati *et al.,* 2010; Afzal *et al*., 2011).

GR activity was reduced in all genotypes in response to NaCl treatment (Fig. 2). Results indicate that in SARC-1even if GR activity decreased with the increased salt stress, GR level was higher as compared to the other genotypes. There was a considerable difference between GR activity of the genotypes. GR catalyzes conversion of glutathione from oxidized (GSSG) to reduced (GSH) form (Koca *et al.,* 2007; Azevedo *et al.,* 2006). Consequently, in S-9189 and S-9476 GR activity was sensitively influenced by salt stress and lower levels of GR resulted in inability to scavenge ROS. Yazici *et al.,* (2007), Reza *et al.,* (2006) and Telesiñski *et al.,* (2008) previously found the same results in purslane (*Portulaca oleracea* L.), barley and beans (*Phaseolus vulgaris* L.), which strengthens the differentiation between the salt sensitive and tolerant genotypes. In the halophyte quinoa key traits seem to be an efficient control of Na^+ sequestration in leaf vacuoles, xylem Na^+ loading, higher ROS tolerance, better K^+ retention, and an efficient control over stomatal development and aperture (Adolf *et al.,* 2012).

***Conclusion*:** The results obtained support the evidence that higher efficiency of antioxidant enzymes of tolerant

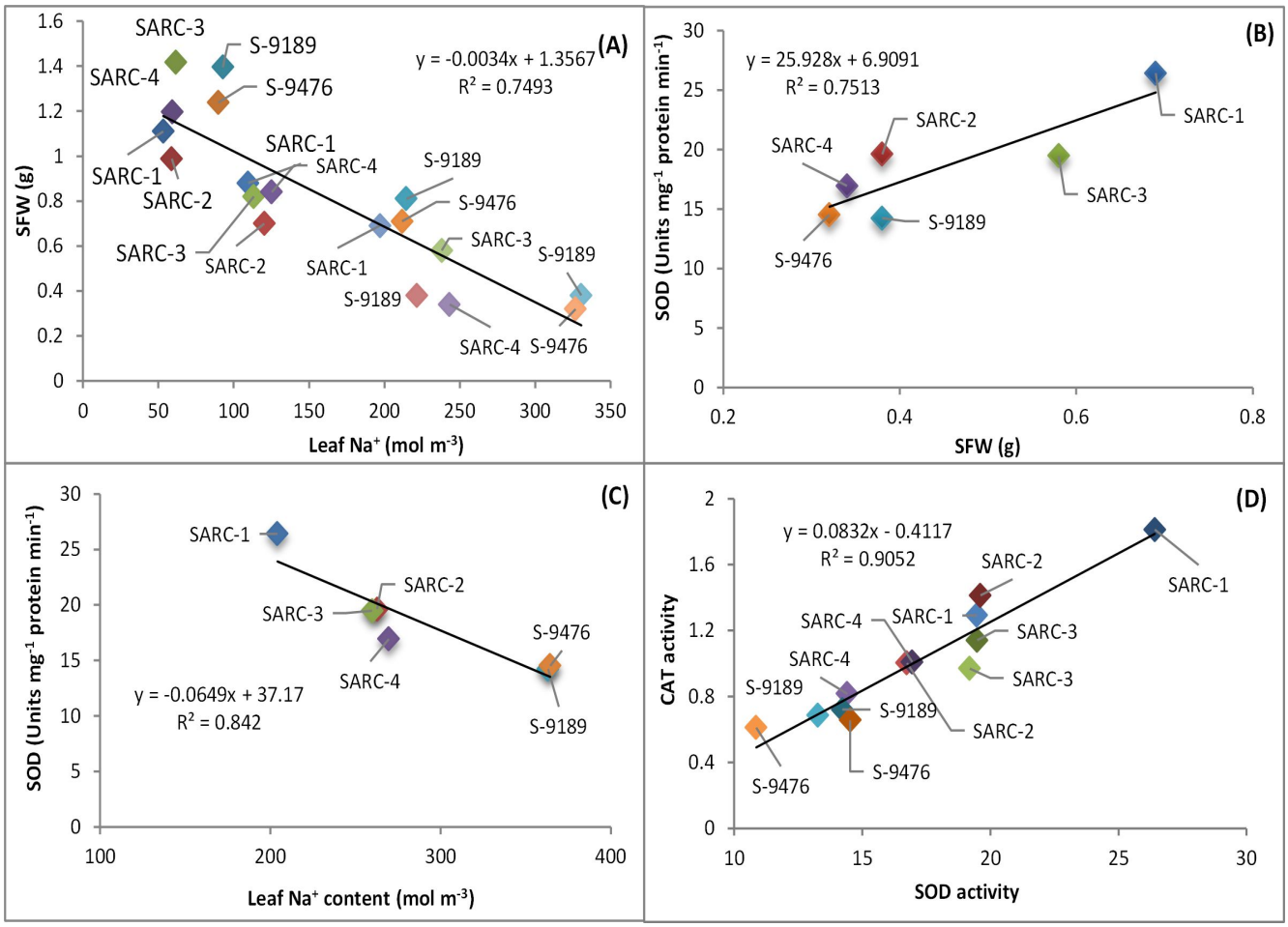

Figure 3. Correlation between different parameters under salt stress after 30 days exposure to NaCl between (A) Na and SFW (B) SFW and SOD at 200 mM NaCl (C) Leaf Na content and SOD at 200 mM NaCl (D) SOD and CAT at 100 and 200 mM NaCl. All values are means of three replicates. Values sharing same letters represent Non-significance.

genotypes could be considered as one of the factors responsible for its tolerance to salt stress. Therefore, it is suggested that antioxidant enzyme activities increasing SOD, CAT and decreasing GR could be considered an important biochemical marker for salt tolerance in wheat germplasm.

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