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Differential Response of Salt-Sensitive and Salt-Tolerant *Brassica juncea* L. Genotypes to N Application: Enhancement of N-Metabolism and Anti-Oxidative Properties in the Salt-Tolerant Type

Manzer H. Siddiqui[1,2*] • Firoz Mohammad[1] • Mohd. Nasir Khan[1] • Mohd. Naeem[1] • Mohd. Masroor A. Khan[1]

[1] Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh 202002, U.P., India
[2] Department of Botany & Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

Corresponding author: * manzerhs@yahoo.co.in

ABSTRACT

Different strategies in response to the application of nutrients are required to overcome the adverse effects of NaCl stress. The objective of the present study was to determine if different added levels of nitrogen (N) in growth medium could alleviate the adverse effects of salt stress on plant N-metabolism and the antioxidative system. Two-week-old plants of salt-sensitive (cv. 'Chuutki') and salt-tolerant (cv. 'Radha') genotypes of *Brassica juncea* L. Czern. & Coss. were treated with: (i) 0 mM NaCl + 0 mg N kg^{-1} sand (control), (ii) 90 mM NaCl +30 mg N kg^{-1} sand, (iii) 90 mM NaCl + 60 mg N kg^{-1} sand, (iv) 90 mM NaCl + 90 mg N kg^{-1} sand and (v) 90 mM NaCl + 120 mg N kg^{-1} sand. Under salinity stress, the salt-tolerant genotype exhibited maximum value for growth attributes [shoot length (SL) $plant^{-1}$, area (*A*) $leaf^{-1}$, and leaf area index (LAI)], sulphur assimilation enzyme [ATP-sulphurylase (ATP-S)], N-assimilation enzymes [nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GOGAT)], and antioxidative enzymes [superoxide dismutase (SOD), ascorbate peroxidise (APX) and glutathione reductase (GR)] and content of antioxidant [glutathione (GSH) and ascorbate (ASC)] at 60 mg N kg^{-1} sand than the salt-sensitive genotype. These results suggest that the salt-tolerant genotype may have better N-metabolism, antioxidative system and protection from reactive oxygen species (ROS) with N application under salt stress.

Keywords: antioxidants, ATP-sulphurylase, nitrogen assimilation, reactive oxygen species, sulphur assimilation
Abbreviations: ***A***, area $leaf^{-1}$; **ATPS**, adenosine triphosphate-sulphurylase; **ASC**, ascorbate; **APX**, ascorbate peroxidise; **GOGAT**, glutamate synthase; **GS**, glutamine synthetase; **GR**, glutathione reductase, **GSH**, glutathione; **LAI**, leaf area index; **NR**, nitrate reductase; **NiR**, nitrite reductase; **SL**, shoot length $plant^{-1}$; **SOD**, superoxide dismutase

INTRODUCTION

A high concentration of salts in soil is an enormous constraint for World Agriculture because it is an important limiting factor for crop productivity and impedes the use of previously uncultivated land. The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed (Flowers and Yeo 1995). Salinity impairs seed germination, and produces physiological disorders which lead to a series of metabolic changes to ion toxicity, mineral distribution, respiration rate, osmolytic synthesis, seed germination, osmotic adjustment, enzymes activity and photosynthesis, including activities of many enzymes related to nitrogen (N) assimilation like glutamine synthetase (GS) and glutamate synthase (GOGAT) the efficiency of which on salt stress depends on species (Amonkar and Karmarkar 1995; Dubey 1997; Khan and Srivastva 1998; Marschner 2002; Khan *et al*. 2007; Siddiqui *et al*. 2008a).

The mechanism of salt tolerance is based on genetic variation at intra- and inter specific levels and physiological and biochemical characteristics of the plant (Ashraf and McNeilly 2004; Misra and Dwivedi 2004; Khan *et al.* 2007; Siddiqui *et al.* 2009). Therefore, it is very important to understand physiological and biochemical mechanisms in the response of plants to salt stress to improve plant tolerance to salinity.

N is an essential plant macronutrient required in the largest quantities (1-3% on a dry weight basis) by plants and is most limiting where maximal biomass production is desired (Salisbury and Ross 1992; Hell and Hillebrand 2001). N assimilation plays an important role in plant metabolism. It is used for generating glutamine (Gln) that is the precursor of various amino acid syntheses (Bagh *et al*. 2004). Plant growth depends on an adequate supply of N in order to form amino acids, protein, nucleic acid, enzymes, plant growth regulators, chlorophyll, vitamins and other cellular constituents necessary for development. N metabolism is a complex process and varies with species (Garg *et al.* 1993; Mansour 2000). N and sulphur (S) assimilation is an essential pathway for offsetting salinity. N nutrition has a strong regulatory influence on S assimilation and *vice versa* (Fismes *et al.* 2000; Koprivova *et al.* 2000; Siddiqui 2005), and both are involved in protein synthesis (Ceccotti 1996). Reuveny *et al.* (1980), Smith (1980) and Brunold and Suter (1984) reported that N-deficient *Lemna minor* and cultured tobacco cells exhibited decreased activities of ATP-Sulphurylase (ATP-S; EC: 2.7.7.4), adenosine 5′ phosphosulphate reductase (APR; SC: 1.8.4.9) and *O*-accetylserine (thiol) lyase (OASTL; EC: 2.5.1.47). They were restored when nitrate or ammonia were resupplied. Glutathione (GSH) synthesis is regulated by the N and S supply of plants because GSH contains three moles of N per mole of S and also GSH biosynthesis is regulated by the amino acids cysteine, glutamate and glycine (Kopriva and Rennenberg 2004). Glutamic acid is the initial product of the GS-GOGAT pathway of N assimilation (Geßler *et al.* 1998). N metabolism is a complex phenomenon and varies from species to species (Mansour 2000). The improvement of N-use efficiency, particularly in *Brassica*, is a major goal of plant improve-

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ment under salt stress; such improved plants would make better use of the N-fertilizer supplied, they would also produce higher yields by alleviating the adverse effect of salt stress.

The selection of sensitive and tolerant genotypes of *Brassica juncea* L. was described in our earlier paper under salt stress (Siddiqui *et al.* 2009). Although N plays a central role in plant metabolism, scant information is available regarding the effect of NaCl on N metabolism and stress-induced changes in free radicals and antioxidative defence system in plants in the presence of N. Therefore, in the present study, we have made an attempt to find out a range of N levels that were either limiting or in excess and also find out the relationship among the activities of N assimilation enzymes and ATP-S and analyse the N potential for scavenging free radicals by antioxidative system under salinity.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were purchased from Himedia Pvt. Ltd. (Mumbai, India) unless stated otherwise.

Plant materials and growth conditions

Seeds of two genotypes of mustard (*Brassica juncea* L. Czern and Coss.) 'Chuutki' (salt-sensitive) and 'Radha' (salt-tolerant) were selected for the study in our earlier experiment (Siddiqui *et al.* 2009). The experiment was conducted in a net-house under natural environmental conditions during the rabi/winter of 2007-2008. The earthen pots of 30 cm diameter, lined with polythene bags (to avoid contamination), were filled with sand, washed with acid, followed by tap and distilled water. The pots were arranged in a simple randomized design with a single factor and 4 replicates. Before sowing, seeds were surface sterilized with 1% sodium hypochlorite for 10 min, then vigorously rinsed with double distilled water (DDW) and sown in sand filled pots supplied with Raukura's nutrient solution. After 2 weeks of sowing, thinning was done and three healthy plants of uniform size were maintained in each pot. When the plants were at the stage of 2-3 true leaves, NaCl solution was added to the pots with experimental *Brassica* plants to attain the final concentration. A half dose of N was applied basally at the time of sowing, and the remaining half dose was given as a spray treatment after 1 week of NaCl treatment. The experimental pots were irrigated daily with DDW (100-200 cm^3) to keep the sand moist. The following treatments were applied: (i) $NaCl_0$ mM +N_0 mg kg^{-1} sand (control), (ii) $NaCl_{90}$ mM +N_{30} mg kg^{-1} sand, (iii) $NaCl_{90}$ mM +N_{60} mg kg^{-1} sand, (iv) $NaCl_{90}$ mM +N_{90} mg kg^{-1} sand, (v) $NaCl_{90}$ mM +N_{120} mg kg^{-1} sand. The concentration of NaCl was selected on the basis of earlier findings (e.g. Siddiqui *et al.* 2009). Each pot was given 400 mL of nutrient solution at every two days. The salts used to make up the nutrient solution were as follows: Macronutrient stock solution (g L^{-1}): $Mg(NO_3)_2 \cdot 6H_2O$, 4.94; $Ca(NO_3)_2 \cdot 4H_2O$, 16.78; NH_4NO_3, 8.48; KNO_3, 2.28. Macronutrient stock solution (g L^{-1}): KH_2PO_4, 2.67; K_2HPO_4, 1.64; K_2SO_4, 6.62; Na_2SO_4, 0.60; NaCl, 0.33. Micronutrient supplement (mg L^{-1}): H_3BO_3, 128.80; $CuCl_2 \cdot 2H_2O$, 4.84; $MnCl_2 \cdot 4H_2O$, 81.10; $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$, 0.83; $ZnCl_2$, 23.45; $FeC_6H_5O_7 \cdot 5H_2O$, 809.84. The dilute solution which was applied to the plants was prepared by mixing 200 mL of each of the macronutrient stock solution with 100 mL of the micronutrient supplement and diluting to 4.5 l with DDW. The pH was maintained at 6.0 by adding 0.2 N H_2SO_4 or 0.2 N KOH solutions.

Sampling was done after 50 days of sowing. The performance of 'Chuutki' and 'Radha' was assessed in terms of growth characteristics and physiological and biological parameters.

Measurement of growth characteristics

The effect of salinity on growth parameters was studied in terms of shoot length (SL plant−1), leaf area (*A* leaf−1) and leaf area index (LAI). LAI was determined by the following formula as used by Watson (1958):

$$\text{LAI} = \text{LAI} = \frac{\text{Leaf area}}{\text{Ground area}}$$

The leaf area was obtained directly with the help of graph paper. The area of 3 leaves (upper, middle and lower) of each plant of the sample (consisting of five plants) was determined.

Determination of physiological and biochemical parameters

1. Nitrate reductase (NR) activity

NR (E.C.1.6.6.1) activity was estimated by the intact tissue method of Jaworski (1971). Fresh leaf samples were weighed and transferred to plastic vials. To each vial, 2.5 ml phosphate buffer (pH 7.5), 0.2 M potassium nitrate and 5% isopropanol (Sisco Research Laboratories Pvt. Ltd. Mumbai) solutions were added. Each vial was incubated for 2 h in the dark at 30°C. To the incubated mixture, 1% sulphanilamide and 0.2% *N*-1-naphthylethylene-diamine dihydrochloride (Himedia) were added. The reaction mixture was kept for 20 min for colour development. The absorbance was read spectrophotometrically (Spekol-1500, Analytikjena, Germany) at 540 nm and was compared with that of the calibration curve. The activity of NR was expressed as n mol NO_2 $h^{-1}g^{-1}$ leaf FW.

2. Nitrite reductase (NiR) activity

NiR (E.C. 1.7.7.1) was assayed from the rate of disappearance of nitrite from the reaction mixture as described by Sawhney and Naik (1973). The assay mixture in a final volume of 2 mL contained the following: 100 µmol phosphate buffer (pH 7.5); 0.4 µmol methyl viologen (Sigma-Aldrich, Pvt. Ltd., India); 1.0 µmol $NaNO_2$; 0.4 µmol H_2O; and 0.4 mL of the enzyme extract. The reaction was started with 0.01 mL of sodium dithionite solution prepared in 0.29 M $NaHCO_3$ solution. The assay mixture without dithionate served as the control. After incubation for 30 min at 30°C, the reaction was stopped by shaking the tubes vigorously to oxidize completely the methyl viologen as indicated by the disappearance of blue colour. The amount of residual nitrite in a suitable aliquot (0.1 mL) of reaction mixture was then determined spectrophotometrically according to the procedure of Nicholas and Nanson (1957) as described above. The NiR activity was expressed as µmol NO_2 utilized h^{-1} g^{-1} FW.

3. Glutamine synthetase (GS) activity

GS (E.C. 6.3.1.2) was determined by measuring the amount of γ-glutamyl hydroxamate produced as described by Washitani and Sato (1977). The standard assay mixture (1 mL) for transferase activities of GS consisted of 100 µmol Tris HCl buffer (pH 8.0); 10 µmol glutamine; 60 µmol hydroxylamine hydrochloride (neutralized with NaOH, pH 7.0); 1 µmol ADP; 20 µmol sodium hydrogen arsenate; 1 µmol $MnCl_2$; and 0.3 mL enzyme extract. The reaction was started by adding glutamine. Glutamine was omitted in the control. After incubation for 30 min at 37°C, the γ-glutamyl hydroxamate formed was determined by adding 2 mL of the $FeCl_3$ reagent [equal volumes of 0.37 M $FeCl_3$, 0.67 M HCl, 0.2 M trichloroacetic acid] and measuring the absorbance at 540 nm. The activity of GS was expressed as µmol γ-glutamyl hydroxymate released h^{-1} g^{-1} FW.

4. Glutamate synthase (GOGAT) activity

GOGAT (E.C. 2.6.1.53) was assayed spectrophotometrically following the oxidation of NADH at 340 nm (Boland *et al.* 1978). The reaction mixture (2.5 mL) contained 160 µmol Tris-HCl buffer (pH 7.5); 10 µmol α-ketoglutarate (pH 6.8–7.0, neutralized with Na_2CO_3); 0.4 µmol NADH; 10 µmol glutamine; and 0.15 µmol enzyme extract. GOGAT activity was expressed as µmol NADH oxidized h^{-1} g^{-1} FW.

5. ATP-sulphurylase (ATP-S) activity

Fresh tissue was rapidly ground at 4°C in a buffer consisting of 10 mM Na_2EDTA, 20 mM Tris-HCl (pH 8.0), 2 mM DDT and ap-

proximately 10.07 g/mL insoluble PVP, using 1:4 (w/v) tissue to buffer ratio. The homogenate was strained through gauge and centrifuge at 20,000 × *g* for 10 min at 4°C. The supernatant was used for the *in vitro* ATP-S (EC 2.7.7.4) assays. ATP-S activity was measured using molybdate-dependent formation of pyrophosphate. The reaction was started by adding 0.1 mL of crude extract to 0.5 mL of the reaction mixture, which contained 7 mM $MgCl_2$, 5 mM Na_2MoO_4, 2 mM Na_2ATP, and 0.032 U/mL of sulphate-free inorganic pyrophosphate (Sigma-Aldrich) in 80 mM Tris-HCl buffer (pH 8.0). Another aliquot from the same extract was added to the same reaction mixture except that Na_2MoO_4 was absent. Incubations were carried out side by side at 37°C for 15 min after which phosphate was determined spectrophotometrically. The ATP-sulphurylase dependant formation of pyrophosphate was estimated from the difference between the two figures (Lappartient and Touraine 1996).

6. Catalase (CAT) activity

CAT (E.C. 1.11.1.6) was measured as described by Aebi (1984). The decomposition of hydrogen peroxide (H_2O_2) was monitored by the decrease in absorbance at 240 nm. For the assay a 50 mM phosphate buffer (pH 7.8) and 10 mM H_2O_2 was used.

7. Superoxide dismutase (SOD) activity

Activity of SOD (E.C. 1.15.1.1) was determined by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) according to the methods of Giannopolitis and Ries (1977). The reaction solution (3 mL) contained 50 µmol NBT, 1.3 µmol riboflavin, 13 mmol methionine, 75 nmol EDTA, 50 mmol phosphate buffer (pH 7.8) and 20 to 50 µL enzyme extract. The reaction solution was irradiated under a bank of fluorescent lights at 75 µmol m^{-2} s^{-1} for 15 min. The absorbance at 560 nm was read against the blank (non-irradiated reaction solution) with a spectrophotometer. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.

8. Ascorbate peroxidase (APX) activity

APX (E.C. 1.11.1.11) was determined and extracted as described by Foyer and Halliwell (1976) The reaction mixture contained 50 mM potassium phosphate (pH 7.8), 0.1 mM EDTA, 0.1 mM H_2O_2 and 0.5 mM ascorbate. The H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm (absorbance co-efficient at 2.8 mM cm^{-1}).

9. Glutathione reductase (GR) activity

GR (E.C. 1.6.4.2) activity was assayed as described by Foyer and Halliwell (1976), with minor modifications. The assay mixture consisted of 50 µL of the enzyme extract, 100 mM phosphate buffer (pH 7.8), 0.1 µM EDTA, 0.05 mM NADPH, and 3.0 mM oxidized glutathione in a total volume of 1.0 mL. NADPH oxidation rate was monitored by reading the absorbance at 340 nm at the moment of H_2O_2 addition and 1 min later. The difference in absorbance (A_{340}) was divided by the NADPH molar extinction coefficient (6.22 mM^{-1} cm^{-1}).

10. Glutathione (GSH) content

Total glutathione was determined in homogenates spectrophotometrically as 340 nm using glutathione reductase, 5, 5-dithio-*bis*-nitrogbenzoic acid (DTNB) and NADPH. Oxidized glutathione (GSSG) was assayed by the same method (GSG+GSSG) in the presence by 2-vinyl pyridine and GSH content was calculated as a difference between total glutathione and GSSG (Anderson 1985).

11. Ascorbate content

Ascorbate content was determined according to the method of Foyer *et al.* (1983) with some modifications. 0.1 g of leaves was ground in liquid N and 1 ml of 2.5 M perchloric acid was added. The crude extract was centrifuged at 2°C for 10 min at 10,000 × *g*, and the supernatant was neutralized with saturated Na_2CO_3 using methyl orange as the indicator. The reduced ascorbate was assayed spectrophotometrically at 265 nm in 1 M NaH_2PO_4 buffer, pH 5.6, with 1 U ascorbate oxidase. The total ascorbate was assayed after incubation in the presence of 30 mM DTT.

Statistical analysis

Each pot was treated as one replicate and all the treatments were repeated four times. The data was analyzed statistically with SPSS-11 statistical software (SPSS Inc., Chicago, IL, USA). Means were statistically compared by Duncan's Multiple Range Test (DMRT) at $P < 0.05$.

RESULTS

Effects of N on morphology of salt exposed plants

Data revealed that *Brassica* genotypes 'Chuutki' and 'Radha' responded differently to salinity at four levels of N application. The tolerant genotype, 'Radha' showed a decrease of SL, *A* and LAI by 8.8, 11.4 and 6.94% respectively over the respective controls at $NaCl_{90}$ mM + N_{60} mg while it was 11.9, 17.4 and 14.7% in 'Chuutki', a sensitive cultivar (**Table 1**). However, the value for *A* in 'Radha' was at par with that of treatment $NaCl_{90}$ mM +N_{90} mg and the value for 'Chuutki' was at par with that of $NaCl_{90}$ mM +N_{30} mg for LAI and FW (**Table 1**).

Effect of NaCl on physiological and biochemical parameters

Salinity significantly inhibited the activities of N assimilating enzymes (NR, NiR, GOGAT and GS) and also S-assimilation enzyme (ATP-S) in both genotypes as compared with non-saline plant under different levels of N (**Fig. 1**). However, the decline in the enzymes activity was maximum in the sensitive genotype as compared with 'Radha'. The percent reduction in NR activity was found minimum in 'Chuutki' (9.0%) and 'Radha' (6.6%) with the $NaCl_{90}$ +N_{60} treatment compared with the respective controls (**Fig. 1**). Similarly, the percent decline in NiR, GOGAT, and GS activity was minimum in 'Chuutki' (15.6, 14.1 and 12.7%, respectively) and 'Radha' (14.9, 5.8 and 8.4%, respectively) with the $NaCl_{90}$ +N_{60} treatment when compared with the respective controls (**Fig. 1**). However, the degree of efficiency of $NaCl_{90}$ +N_{60} treatment in alleviating the adverse effect of salt stress in N-assimilation enzymes was found to be maximum in 'Radha' (**Fig. 1**). ATP-S activity was found maximum at $NaCl_{90}$ +N_{60} under salt stress in both geno-

Table 1 Effect of nitrogen application on the activities of SL plant $^{-1}$, A leaf^{-1} and LAI of Chuutki and Radha genotypes of *Brassica juncea* L. under salt stress (means of four replicates).

Treatment	Chuutki	Radha
SL plant^{-1} (cm)		
$NaCl_0$+N_0	61.56 ± 0.33 a	75.64 ± 0.64 a
$NaCl_{90}$+N_{30}	47.37 ± 1.16 c	63.73 ± 1.07 c
$NaCl_{90}$+N_{60}	54.22 ± 1.53 b	68.99 ± 1.76 b
$NaCl_{90}$+N_{90}	42.37 ± 1.62 d	59.96 ± 0.86 cd
$NaCl_{90}$+N_{120}	38.47 ± 1.46 d	55.92 ± 2.42 d
A leaf^{-1} (cm^2)		
$NaCl_0$+N_0	29.40 ± 0.46 a	34.89 ± 0.91 a
$NaCl_{90}$+N_{30}	22.56 ± 0.94 c	27.22 ± 0.52 c
$NaCl_{90}$+N_{60}	25.54 ± 0.49 b	30.92 ± 1.18 b
$NaCl_{90}$+N_{90}	22.48 ± 0.70 c	28.62 ± 0.84 bc
$NaCl_{90}$+N_{120}	20.33 ± 0.55 d	20.82 ± 0.80 d
LAI		
$NaCl_0$+N_0	3.66 ± 0.06 a	3.89 ± 0.09 a
$NaCl_{90}$+N_{30}	2.95 ± 0.03 bc	3.26 ± 0.06 c
$NaCl_{90}$+N_{60}	3.12 ± 0.06 b	3.62 ± 0.07 b
$NaCl_{90}$+N_{90}	2.87 ± 0.03 cd	3.14 ± 0.07 cd
$NaCl_{90}$+N_{120}	2.76 ± 0.10 d	2.99 ± 0.06 d

Means followed by the same letter in the column do not differ statistically at $P < 0.05$ (Duncan's multiple range test)

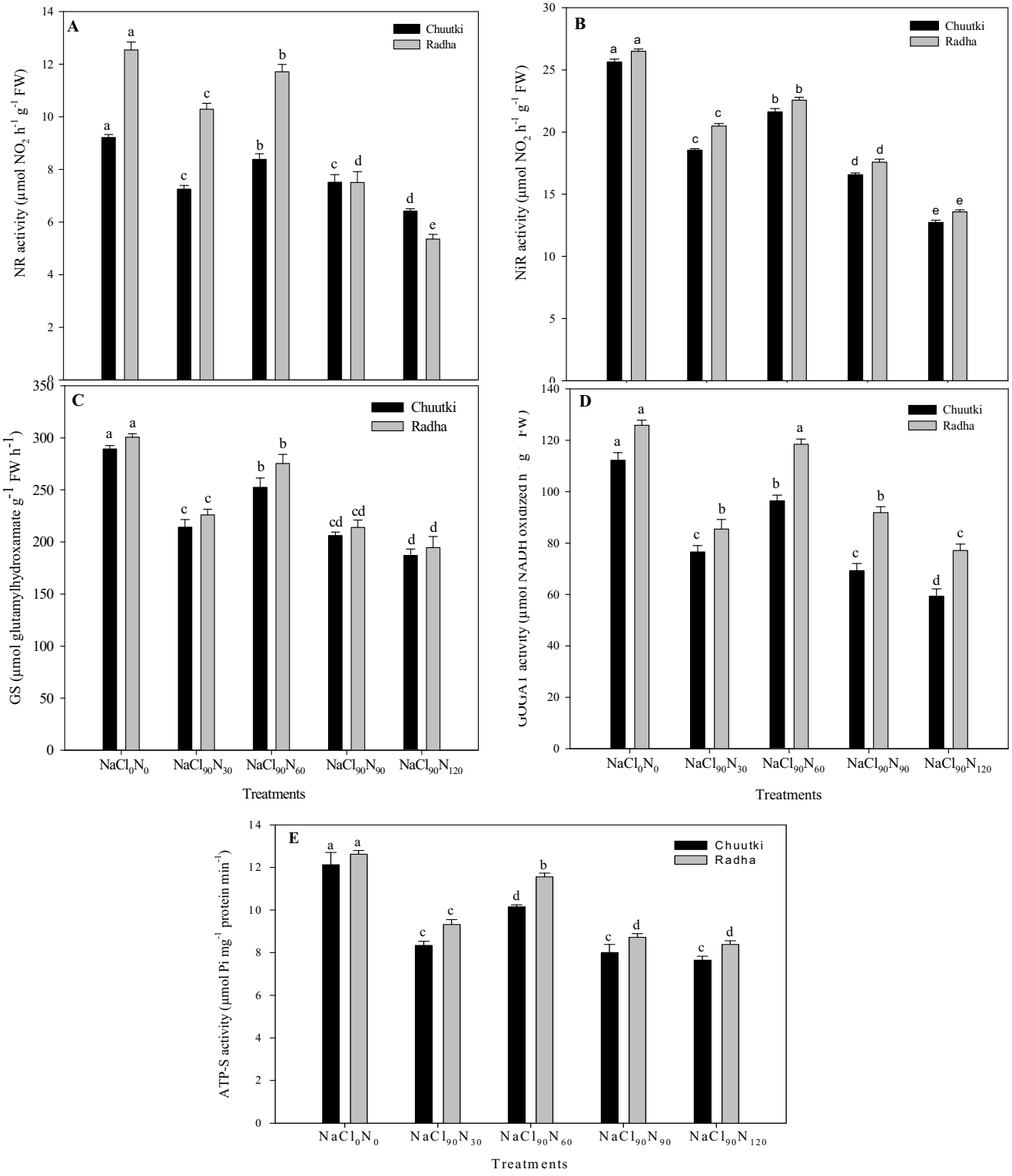

Fig. 1 Effect of nitrogen application on the activities of NR (**A**), NiR (**B**), GS (**C**), GOGAT (**D**) and ATP-S (**E**) of salt-sensitive and salt-tolerant genotypes of *Brassica juncea* L. under salt stress.

types. However, the maximum ATP-S activity was found in 'Radha' compared to 'Chuutki'. The minimum reduction of ATP-S activity was observed in 'Chuutki' (16.3%) and 'Radha' (8.4%) at $NaCl_{90}$ +N_{60} under salinity over the respective controls (**Fig. 1**).

The production of H_2O_2 was maximum in the sensitive genotype compared with 'Radha'. The production of H_2O_2 in the sensitive genotype increased by 67.1%, while the increase in H_2O_2 production in 'Radha' was 65.7%, at 60 mg N kg^{-1} sand in comparison to respective controls (**Fig. 2**). Under salt stress, SOD activity increased with increasing levels of N in 'Chuutki' as well as 'Radha' (**Fig. 2**). The enzyme activity increased by 39.3% in 'Chuutki', but the increase was 50.0% in 'Radha' with 60 mg N kg^{-1} sand, in comparison to the respective controls. The enhancement of CAT activity was maximum in 'Radha' compared with 'Chuutki' (**Fig. 2**). CAT activity was enhanced by 41.5% in 'Chuutki' and 43.3% in 'Radha' at 30 mg N kg^{-1} sand when compared with respective controls under salt stress. However, the lowest enhancement was recorded at $NaCl_{90}$ mM +N_{60} mg kg^{-1} sand in both genotypes (**Fig. 2**). In general, APX activity increased with increasing levels of N in both genotypes under salt stress (**Fig. 2**). The level in APX activity was 42.8% in 'Chuutki', where the increase was 66.7% in 'Radha' with 60 mg N kg^{-1} sand, when compared to the respective controls under salt stress (**Fig. 2**). Similarly, GR

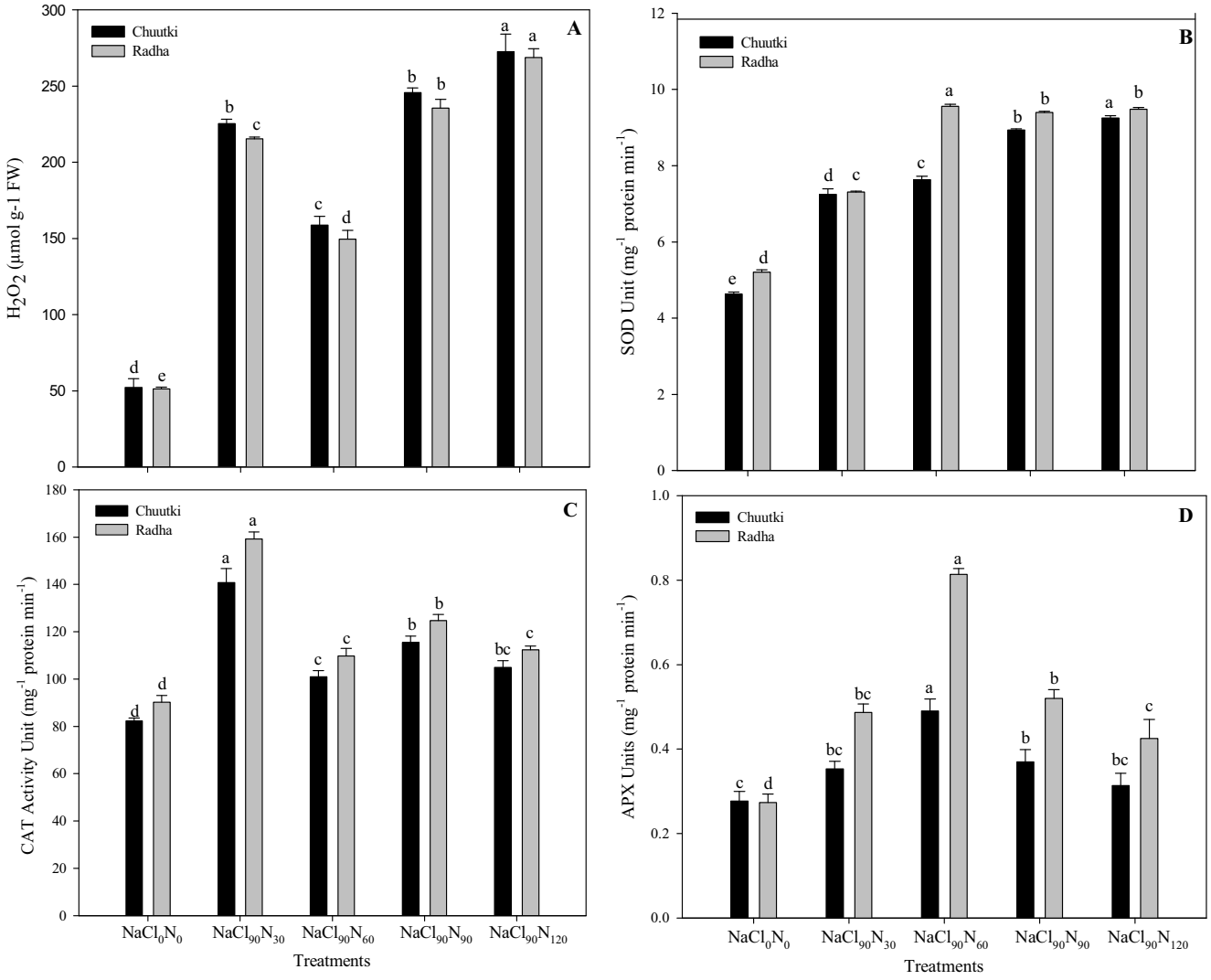

Fig. 2 Effect of nitrogen application on the activities of H_2O_2 (**A**), SOD (**B**), CAT (**C**) and APX (**D**) of salt-sensitive and salt-tolerant genotypes of *Brassica juncea* L. under salt stress.

activity was increased with increasing levels of N under salt stress (**Fig. 3**). GR activity was enhanced by 52.2% in 'Chuutki' and by 62.5% in 'Radha' at 60 mg N kg^{-1} sand under salt stress, in comparison to the respective controls. The efficiency of the $NaCl_{90}$ +N_{60} treatment in alleviating the ill effect of salt stress in APX and GR activity was maximum in 'Radha' compared to 'Chuutki' (**Figs. 2, 3**).

Accumulation of GSH and ASA increased with increasing levels of N treatment under salt stress (**Fig. 3**). GSH content increased by 48.5% in 'Chuutki' and by 48.1% in 'Radha' when compared to respective controls. Similarly, ASC content was enhanced by 18.7% in 'Chuutki' and 16% in 'Radha' over respective controls (**Fig. 3**).

DISCUSSION

Effect on morphological attributes

Salt stress triggered the lowering of SL, *A*, and LAI in 'Chuutki' and 'Radha'. This is in accordance with our previous findings in linseed (Khan *et al.* 2007) and in mustard (Siddiqui *et al.* 2009). As suggested by Xie *et al.* (2008) in cotton, Lee *et al.* (2001) in rice, Ashraf (2002) in cotton and Jebara *et al.* (2006) in common bean, inhibition of plant growth under saline conditions may be due to the generation of reactive oxygen species (ROS), *viz*. $\cdot O_2^-$ and H_2O_2. The increased H_2O_2 concentration led to lipid peroxidation, causing membrane damage and leakage of electrolytes (Khan *et al.* 2007; Siddiqui *et al.* 2009). In this present study, suppression in SL, *A* and LAI and induction in H_2O_2 were partially alleviated by applying N under saline medium. Therefore, it could be a possible reason for the decreased levels of ROS, such as $\cdot O_2^-$ and H_2O_2 in leaves of N-fed plants of 'Chuutki' and 'Radha' (**Fig. 2**). Among the treatments, 60 mg N kg^{-1} sand was found to be more effective in alleviating the effect of salt stress. This may be explained on the basis of their roles, as many N-containing compounds such as amino acids, amides, actoine (1,4,4,6-terahydro-2-methyl-4-carboxylpyrimidene), proteins and quartenary ammonium compounds, have been proven to benefit several enzyme activities in plants (Greenway and Munns 1980).

Effect on physiological and biochemical parameters of salt exposed plants

Salt stress lead to a significant decrease in activities of NR, NiR, GOGAT and GS with increasing levels of N in 'Chuutki' and 'Radha' (**Fig. 1**). This in accordance with the findings of Garg *et al.* (1993) in mustard, Meloni *et al.* (2004) in *Prosopis alba*, Nathawat *et al.* (2005) in mustard and Siddiqui *et al.* (2008a) in *Brassica juncea* L. In this sense, salinity seems to interfere with NO_3^- uptake in plants (Grattan and Grieve 1994; Khan and Srivastava 1998) which alters the ammonia-assimilating enzymes, amino acid synthesis, increased activity of hydrolyzing enzymes, such as RNase, DNase, protease and several others, leading to the degradation of macromolecules (Dubey and Pessarakli (1994), Mansour (2000), Nathawat *et al.* (2005), and Siddiqui *et al.* (2008a). Interestingly, the extent of reduction in N-assimilation enzymes' activity was found minimum with

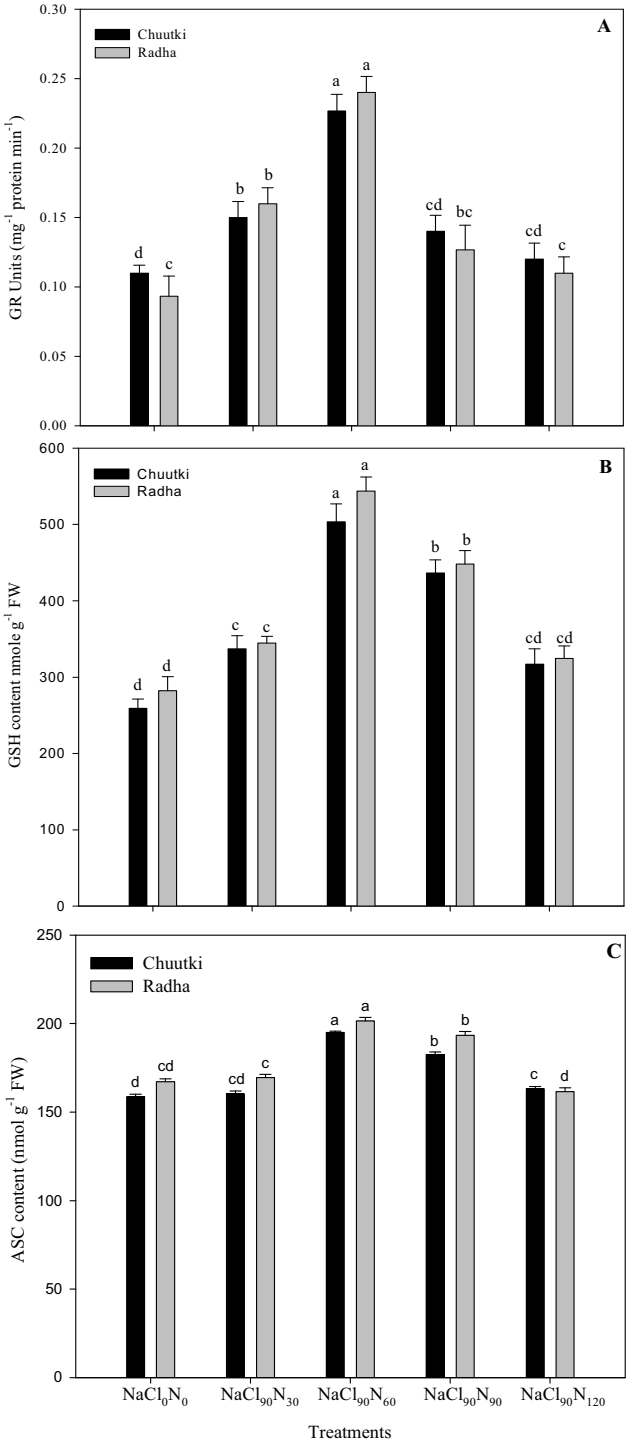

Fig. 3 Effect of nitrogen application on GR activity (**A**), GSH content (**B**) and ASC content (**C**) of salt-sensitive and salt-tolerant genotypes of *Brassica juncea* L. under salt stress.

60 mg kg^{-1} sand in 'Chuutki' and 'Radha' (**Fig. 1**). Moreover, the extent of reduction was minimum in 'Radha' (**Fig. 1**). In this sense, NR activity appears to limit nitrate assimilation (Campbell 1999), cytosolic nitrate protect the NR enzyme against the action of proteases and/or inhibitors besides triggering the *de novo* synthesis of NR protection by induction of NR gene expression (Campbell 1999; Meloni *et al.* 2004). Leustek *et al.* (2000) and Brunold *et al.* (2003) reported that cysteine is the initial product of sulphate assimilation in plants, and its immediate metabolite glutathione (GSH) are both cellular constituents that originate from the three most important pathways of plants i.e., photosynthesis, N assimilation, and sulphate assimilation. Thus we may postulate that N application was found to be effective to overcome salt stress by improving the activities of NR, NiR, GS and GOGAT in 'Chuutki' and 'Radha' under salt stress.

ATP-S catalyses the first reaction in the assimilation of inorganic sulphate. Many authors have established regulatory interactions between sulphate and nitrate assimilation in plants (Brunold 1993; Takahashi and Saito 1996; Kim *et al.* 1999; Koprivova *et al.* 2000). Siddiqui *et al.* (2009) reported that rate of ATP-S activity was suppressed under NaCl stress. In the present study, the rate of ATP-S activity decreased at all levels of N, but maximum alleviating effect of salt stress was recorded at 60 mg N kg^{-1} sand under salt stress in both genotypes (**Fig. 1**). The enhancement of ATP-S activity might have helped in the restoration of S-metabolism. Ernst (1998), Ruiz and Blumwald (2002) and Kopriva and Rennenberg (2004) reported that ATP-S catalyses the first step of S-assimilation which is an essential pathway being a source of reduced S for various cellular processes and for the synthesis of the S-containing amino acids, cysteine and methione, the important tripeptide glutathione, a major factor in plant stress defence.

The formation of ROS is induced by salt stress in plant cells metabolically (Elstner and Oswald 1994; Foyer and Noctor 2000). Because of the presence of ROS in cells, disruption of membrane stability, protein, lipids and DNA occurs. Salinity generates H_2O_2 which is a major by-product of β-oxidation and contributor of the ROS that leads to aleurone programmed cell death (Bethke and Jones 2001; Fath *et al.* 2001). From the foregoing it is clear that there is a need to limit the production of ROS to detoxify ROS once formed, and to repair damage caused by ROS. The level of H_2O_2 was higher in the sensitive and lower in the tolerant genotype under salt stress, which implies that the generation of H_2O_2 was quenched by the efficient antioxidant mechanism of the 'Radha' (**Fig. 2**). In both genotypes, the level of H_2O_2 was minimum at 60 mg N kg^{-1} sand (**Fig. 2**), which is indicative of lower oxidation stress imposed by NaCl in soil at this level and also may be N-induced regulatory and stimulatory influence on protein, sucrose, proline and glycinebetaine synthesis; hence, these solutes may play a role in osmotic adjustment (Rhodes and Hanson 1993; Siddiqui *et al.* 2009). These are helpful for maintaining cellular pH, detoxification of cells, and scavenging of free radicals (Drolet *et al.* 1986; Rabe 1990; Rhodes and Hanson 1993; Dubey 1997; Mansour 1998). Even, N nutrition had a strong regulatory influence on S assimilation and *vice versa* (Fismes *et al.* 2000; Siddiqui 2008b) by increasing the activity of ATP-S (**Fig. 1**) N also reduces Na^+ accumulation (Siddiqui *et al.* 2008a).

The scavenging of ROS is associated with the enzymes involved in antioxidative processes of cells, particularly SOD, CAT, APX, and GR (Dajic 2006). SOD activity increased with increasing levels of N under salt stress, but maximum activity was recorded with 'Radha' in comparison to 'Chuutki' (**Figs. 2, 3**). We infer that maximum SOD activity in both genotypes under salt stress could increase the ability to scavenge $O_2^{\cdot-}$ radicals which could cause membrane damage (Scalet *et al.* 1995; Agarwal and Pandey 2004). In the 'Radha', SOD activity was maximum because it could be more efficient in alleviating the adverse effect of NaCl stress by increasing N-metabolism under N application than the sensitive genotype (**Fig. 1**). Siddiqui *et al.* (2008a) reported that N-fed *Brassica* genotypes exhibited maximum activity of NR, a key enzyme of N metabolism, and also recorded maximum activity in 'Radha' than 'Chuutki' under salt stress.

Under salt stress, CAT activity was maximum at 30 mg N kg^{-1} sand and minimum at 60 mg kg^{-1} sand (**Fig. 2**) in both genotypes. The differences in the magnitude of CAT activity under different levels of N might be due to a greater formation of ROS at all levels of N except for 60 mg kg^{-1} sand under NaCl stress (**Fig. 2**). The rate of CAT activity was found to be higher in the sensitive than in the tolerant genotype. This may suggest that a higher rate of production of ROS in the sensitive than in the tolerant genotype (Bhattacharjee 1997/98; Rios-Gonzalez *et al.* 2002).

An efficient antioxidant system is often correlated with the alleviation of oxidative damage and improved tolerance to salt stress (Shalata and Tal 1998; Hasegawa *et al.* 2000; Shalata *et al.* 2001; Amor *et al.* 2006). The ASC-GSH cycle has a well established role in the defense system (Noctor and Foyer 1998; Hoque *et al.* 2007). In this cycle detoxification of H_2O_2 takes place by APX, whereas reduced ASC acts as a substrate for APX and reduced GSH act as an electron donor for dehydroascorbate reductase to regenerate ASC through the reduction of dehydroascorbate via the Halliwell-Asada pathway (Noctor and Foyer 1998; Asada 1999). In the present study, the rate of APX activity in both genotypes was greater than CAT activity (**Fig. 2**). It might be due to APX-scavenged H_2O_2 when CAT activity is depressed at 60 mg N kg^{-1} sand and the endogenous level of H_2O_2 is enhanced (Nakano and Asada 1981; Hoque *et al.* 2007). This indicates that increased H_2O_2 was detoxified by APX. In many studies, the rate of APX activity was suppressed by NaCl, whereas the result obtained in this study revealed that the application of 60 mg N kg^{-1} sand led to a considerable increase in APX activity under salt stress (**Fig. 2**). It may be explained on the basis of its roles, as many N-containing compounds such as amino acids, amides, proteins and quaternary ammonium compounds, have been proven benefit for several enzyme activities in plants (Greenway and Munns 1980; Grumet *et al.* 1985; Jacoby 1999; Mansour 2000; Siddiqui *et al.* 2008a). Hoque *et al.* (2007) in tobacco demonstrated that proline and glycinebetaine enhanced the activity of APX. The rate-limiting step in photorespiration is the re-assimilation of ammonia catalysed by chloroplastic GS (Wallsgrove *et al.* 1987). When rice plants were transformed with the GS2 gene, they accumulated about 1.5 times more GS2 than control plants and had increased photorespiration capacity and enhanced salt tolerance (Hoshida *et al.* 2000). This result is consistent with these findings and we postulate that the application of N in the present study was more effective in enhancing salt tolerance in plants.

Rios-Gonzalez *et al.* (2002) in maize and sunflower and Medici *et al.* (2004) in *Hordeum vulgare* L. *and Arabidopsis thaliana* L. reported that GR activity increased in ammonium because of increases in growth rate, photosynthesis and metabolic activity, which would require an enhancement of the scavenging of ROS. Increased GR activity may also maintain a high ratio of GSH/GSSG, which is required for the regulation of ascorbate threshold level and activation of several CO_2-fixing enzymes (Foyer and Halliwel 1976; Noctor and Foyer 1998). The data presented in this experiment revealed that GR activity increased with increasing N concentration (**Fig. 3**). An increased rate of GR activity in 'Radha' under N application (**Fig. 3**) suggested that it is more efficient in alleviating the ill effect of salt stress than 'Chuutki'.

GSH, the tripeptide γ-glutamylcysteinyl-glycine, is a key non-enzymatic antioxidant in plant cells that scavenges ROS either directly or indirectly by participating in the ASC-GSH cycle (Noctor and Foyer 1998; Polle 2001). GSH also plays a protective role in salt tolerance by maintaining the redox state (Shalata *et al.* 2001). In addition, GSH plays a role in the regulation of inter-organ sulphur allocation (Lappartient and Touraine 1996) and gene expression (Baier and Dietz 1997). In the present experiment, GSH content decreased with increasing levels of N, but maximum content was recorded at 60 mg N kg^{-1} sand in both genotypes. However, the maximum GSH content was recorded in the tolerant genotype (**Fig. 3**). Maximum content of GSH in 'Radha' under salt stress suggests that it is more efficient in protecting against salt stress than 'Chuutki'. The degree of alleviation of the adverse effect of salt stress in plants supplemented with N may be that N nutrition has a strong regulatory influence on S assimilation and *vice versa* (Fismes *et al.* 2000; Siddiqui *et al.* 2008b). Leustek *et al.* (2000) and Brunold *et al.* (2003) reported that glutathione is originated from three pathways of plants i.e. photosynthesis, nitrogen assimilation and sulphur assimilation. According to Kopriva and Rennenberg 2004, GSH synthesis in plant cells is induced by the N and S supply because GSH contain three moles of N per mole of S and is also regulated by amino acid, cysteine, glutamate and glycine.

CONCLUSIONS

From the results of our study, it can be concluded that enhanced level of N assimilation enzymes (NR, NiR, GOGAT, GS) and S assimilation enzyme (ATP-S) of N-fed plants of both genotypes and a parallel increase in the activity of enzymes involved in the ASC-GSH cycle may be responsible for the enhanced SL and *A* under salt stress. These results indicate that the application of N reversed the inhibitory effect of salt stress and adjusted the plants to perform normally via detoxifying the ROS which was brought about by the induction of N metabolism and the antioxidant system. Taken together, the results suggest that N-assimilation enzymes, ATP-S and antioxidant protection activity of the 'Radha' against salt stress are stronger than those of 'Chuutki' because of the superior ability of the former to increase the enzyme activity of N-assimilation and antioxidant system.

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REFERENCES

Aebi H (1984) Catalase *in vitro*. *Methods in Enzymology* **105**, 121-126

Agarwal S, Pandey V (2004) Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. *Biologia Plantarum* **48**, 555-560

Amonkar DV, Karmarkar SM (1995) Nitrogen uptake and assimilation in halophytes. In: Srivastava HS, Singh RP (Eds) *Nitrogen Nutrition in Higher Plants*, Associated Publ. Co. New Delhi, pp 431-445

Amor NB, Jeménez A, Megdiche W, Lundqvist, M, Sevilla F, Abdelly C (2006) Response of antioxidant systems to NaCl stress in the halophyte *Cakile maritime*. *Physiologia Plantarum* **126**, 446-457

Anderson ME (1985) Determination of glutathione and glutathione disulfide in biological samples. *Methods in Enzymology* **113**, 548-555

Asada K (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 601-639

Ashraf M (2002) Salt tolerance of cotton: some new advances. *Critical Reviews in Plant Sciences* **21**, 1-30

Ashraf M, McNeilly T (2004) Salinity tolerance in *Brassica* oilseeds. *Critical Reviews in Plant Sciences* **23**, 157-174

Bagh K, Hiraoki T, Thorpe TA, Vogel HJ (2004) Nitrogen-15 NMR studies of nitrogen metabolism in *Picea glauca* buds. *Plant Physiology and Biochemistry* **42**, 803-809

Baier M, Dietz KJ (1997) The plant 2-Cys peroxiredoxin BAS1 is a nuclear-encoded chloroplast protein: its expressional regulation, phylogenetic origin, and implications for its specific physiological function in plants. *The Plant Journal* **12**, 179-190

Bethke PC, Jones RL (2001) Cell death of barley aleurone protoplasts is mediated by reactive oxygen species. *The Plant Journal* **25**, 19-29

Bhattacharjee S (1997/98) Membrane lipid peroxidation, free radical scavengers and ethylene evolution in *Amaranthus* as affected by lead and cadmium. *Biologia Plantarum* **40**, 131-135

Boland MJ, Fordyce AM, Greenwood RM (1978) Enzymes of nitrogen metabolism in legume nodules: A comparative study. *Australian Journal of Plant Physiology* **5**, 553-559

Brunold C (1993) Regulation interaction between sulphate and nitrate assimilation. In: Rennenberg H, Brunold C, De Kok LJ, Stulen I (Eds) *Sulfur Nutrition and Sulfur Assimilation in Higher Plants*, SPB Academic Publishing, The Hague, The Netherlands, pp 61-75

Brunold C, Suter M (1984) Regulation of sulphate assimilation by nitrogen nutrition in the duckweed *Lemma minor* L. *Plant Physiology* **76**, 579-583

Brunold C, Von Ballmoos P, Hesse H, Fell D, Kopriva S (2003) Interactions between sulfur, nitrogen and carbon metabolisms. In: Davidian JC, Grill D, De Kok LJ, Stulen I, Hawkesford MJ, Schnug E, Rennenberg H (Eds) *Sulfur Transport and Assimilation in Plants Regulation, Interaction, and Signaling*, Backhuys Publishers, The Netherlands, pp 45-56

Campbell WH (1999) Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. *Annual Review of Plant*

Physiology and Plant Molecular Biology **50**, 277-303
Ceccotti SP (1996) Plant nutrient sulphur – a review of nutrient balance, environmental impact and fertilizers. *Fertilizer Research* **43**, 117-125
Dajic Z (2006) Salinity and tolerance mechanisms of plants. In: Madhava Rao KV, Radhavendra AS, Jannardhan Reddy K (Eds) *Physiology and Molecular Biology of Stress Tolerance in Plants*, Springer-Verlag, The Netherlands, pp 41-100
Drolet G, Dumbroff BG, Legge RL, Thompson JE (1986) Radical scavenging properties of polyamines. *Phytochemistry* **35**, 367-371
Dubey RS (1997) Photosynthesis in plants under stressful conditions. In: Pessarakli M (Ed) *Handbook of Photosynthesis*, Marcel Dekker, New York, pp 859-875
Dubey RS, Pessarakli M (1994) Physiological mechanisms of nitrogen absorption and assimilation in plants under stressful conditions. In: Pessarakli M (Ed) *Handbook of Plant and Crop Physiology*, Marcel Dekker, Inc., New York, pp 605-625
Elstner EF, Oswald W (1994) Mechanism of oxygen activation during plant stress. In: *Oxygen and Environmental Stress in Plants*, *Proceedings of the Royal Society of Edinburgh* **102b**, pp 131-154
Ernst WHO (1998) Sulfur metabolism in higher plants: potential for phytoremediation. *Biodegradation* **9**, 311-318
Fath A, Bethke PC, Jones RL (2001) Enzymes that metabolize reactive oxygen species in barley aleurone cells are down-regulated prior to gibberellic acid-induced programmed cell death in barley aleurone. *Plant Physiology* **126**, 156-166
Fismes J, Vong PC, Guckert A, Frossard E (2000) Influence of sulphur on apparent N-use efficiency, yield and quality of oilseed rape (*Brassica napus* L.) grown on a calcareous soil. *European Journal of Agronomy* **12**, 127-141
Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants: where next? *Australian Journal of Plant Physiology* **22**, 875-884
Foyer C, Rowell J, Walker D (1983) Measurements of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination. *Planta* **157**, 239-244
Foyer CH, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* **133**, 21-25
Foyer CH, Noctor G (2000) Oxygen processing in photosynthesis: regulation and signaling. *New Phytologist* **146**, 359-388
Garg BK, Byas SP, Kathju S, Lahiri AN, Mali PC, Sharma PC (1993) Salinity-fertility interaction on growth, mineral composition and nitrogen metabolism of Indian mustard. *Journal of Plant Nutrition* **16**, 1637-1650
Geßler A, Schneider S, Weber P, Hanemann U, Rennenberg H (1998) Soluble N compounds in trees exposed to high loads of N: a comparison between the roots of Norway spruce (*Picea abies* (L.) Karst) and beech (*Fagus sylvatica*) trees grown under field conditions. *New Phytologist* **138**, 385-399
Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology* **59**, 309-314
Grattan SR, Grieve CM (1994) Mineral nutrient acquisition and response by plants grown in saline environments. In: Pessaraki M (Ed) *Handbook of Plant and Crop Stress*, Marcel Dekker, New York, pp 203-226
Greenway H, Munns R (1980) Mechanism of salt tolerance in non-halophytes. *Annual Review of Plant Physiology* **31**, 149-190
Grumet R, Isleib TG, Hanson AD (1985) Genetic control of glycinebetaine level in barley. *Crop Science* **25**, 618-622
Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**, 463-499
Hell R, Hillebrand H (2001) Plant concept for mineral acquisition and allocation. *Current Opinion in Biotechnology* **12**, 161-168
Hoque MA, Banu MNA, Okuma E, Amako K, Nakamura Y, Shimoishi Y, Murata Y (2007) Exogenous proline and glycinebetaine increase NaCl-induced ascorbate–glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco bright yellow-2 suspension-cultured cells. *Journal of Plant Physiology* **164**, 1457-1468
Hoshida H, Tanaka Y, Hibino T, Hayashi Y, Tanaka A, Takabe T, Takabe T (2000) Enhanced tolerance to salt stress in transgenic rice that overexpresses chloroplast glutamine synthase. *Plant Molecular Biology* **43**, 103-111
Jacoby B (1999) Mechanism involved in salt tolerance of plants. In: Pessarakli M (Ed) *Handbook of Plant and Crop Stress* (2nd Edn), Marcel Dekker, New York, pp 97-123
Jaworski EG (1971) Nitrate reductase assay in intact plant tissues. *Biochemical and Biophysical Research Communications* **43**, 1274-1279
Jebara M, Harzalli-Jebara S, Payré H, Aouani ME, Drevon JJ (2006) Influence of salinity and abscisic acid on the O_2 uptake by N_2-fixing nodules of common bean. *Biologia Plantarum* **50**, 717-721
Khan MN, Siddiqui MH, Mohammad F, Khan MMA, Naeem M (2007) Salinity induced changes in growth, enzyme activities, photosynthesis, proline accumulation and yield in linseed genotypes. *World Journal of Agricultural Sciences* **3**, 685-695
Khan MG, Srivastava HS (1998) Changes in growth and nitrogen assimilation in maize plants induced by NaCl and growth regulators. *Biologia Plantarum* **41**, 93-99
Kim H, Hirai MY, Hayashi H, Chino M, Naito S, Fujiwara T (1999) Role of *O*-acetyl-L-serine in the coordinated regulation of the expression of a soybean seed storage-protein gene by sulfur and nitrogen nutrition. *Planta* **209**, 282-289
Kopriva S, Rennenberg H (2004) Control of sulphate assimilation and glutathione synthesis: interaction with N and C metabolism. *Journal of Experimental Botany* **55**, 1831-1842
Koprivova A, Suter M, den Camp RO, Brunold C, Kopriva S (2000) Regulation of Sulfate assimilation by nitrogen in *Arabidopsis*. *Plant Physiology* **122**, 737-746
Lappartient AG, Touraine B (1996) Demand-driven control of root ATP sulfurylase activity and SO_2-uptake in intact canola. The role of phloem-translocated glutathione. *Plant Physiology* **111**, 147-157
Lee DH, Kim YS, Lee CB (2001) The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *Journal of Plant Physiology* **58**, 737-745
Leustek T, Martin MN, Bick JA, Davies JP (2000) Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**, 141-165
Mansour MMF (1998) Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. *Plant Physiology and Biochemistry* **36**, 767-772
Mansour MMF (2000) Nitrogen containing compounds and adaptation of plants to salinity stress. *Biologia Plantarum* **43**, 491-500
Marschner H (2002) *Mineral Nutrition of Higher Plants* (2nd Edn), Academic Press, London, 889 pp
Medici LO, Azevedo RA, Smith RJ, Lea PJ (2004) The influence of nitrogen supply on antioxidant enzymes in plant roots. *Functional Plant Biology* **31**, 1-9
Meloni DA, Gulotta MR, Martinez CA, Oliva MA (2004) The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba*. *Brazilian Journal of Plant Physiology* **16**, 39-46
Misra N, Dwivedi UN (2004) Genotype differences in salinity tolerance in green gram cultivars. *Plant Science* **166**, 1135-1142
Nakano G, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidise in spinach chloroplasts. *Plant and Cell Physiology* **22**, 867-880
Nathawat NS, Kuhad MS, Goswami CL, Patel Al, Kumar R (2005) Nitrogen-metabolism enzymes: effect of nitrogen sources and saline irrigation. *Journal of Plant Nutrition* **28**, 1089-1101
Nicholas DJD, Nanson A (1957) Determination of nitrate and nitrite. In: Colowich CP, Kaplan NO (Eds) *Methods in Enzymology* (Vol 3), Academic Press, New York, pp 981-984
Noctor G, Foyer CH (1998) Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**, 249-279
Polle A (2001) Dissecting the superoxide dismutase-ascorbate-glutathione-pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis. *Plant Physiology* **126**, 445-462
Rabe B (1990) Stress physiology: the function significance of the accumulation of nitrogen containing compounds. *Journal of Horticultural Science* **65**, 231-243
Reuveny Z, Dougall DK, Trinity PM (1980) Regulatory coupling of nitrate and sulfate assimilation pathways in cultured tobacco cells. *Proceedings of the National Academy of Sciences USA* **77**, 6670-6672
Rhodes D, Hanson AD (1993) Quaternary ammonium and tertiary sulfonium compound in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**, 357-384
Rios-Gonzalez K, Erdei L, Lips SH (2002) The activity of antioxidant enzymes in maize and sunflower seedlings as affected by salinity and different nitrogen sources. *Plant Science* **162**, 923-930
Ruiz JM, Blumwald E (2002) Salinity-induced glutathione synthesis in *Brassica napus*. *Planta* **214**, 965-969
Salisbury FB, Ross CW (1992) *Plant Physiology* (4th Edn), Wadsworth Publishing Company, Belmont, USA, 682 pp
Sawhney SK, Naik MS (1973) Effect of chloramphenicol and cycloheximide on the synthesis of nitrate reductase and nitrite reductase in rice leaves. *Biochemical and Biophysical Research Communications* **51**, 67-73
Scalet M, Federice R, Guido MC, Manes F (1995) Peroxidase activity and polyamine changes in response to ozone and simulated acid in Aleppo pine needles. *Environmental and Experimental Botany* **35**, 417-425
Shalata A, Tal M (1998) The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. *Physiologia Plantarum* **104**, 169-174
Shalata A, Mittova V, Volokita M, Guy M, Tal M (2001) Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersiconpennellii* to salt-dependant oxidative stress: the root antioxidant system. *Physiologia Plantarum* **112**, 487-494
Siddiqui MH (2005) Study of the effect of N, P and S application on the performance of rapeseed-mustard. PhD thesis, Aligarh Muslim University, Aligarh, India, 125 pp
Siddiqui MH, Khan MN, Mohammad F, Khan MMA (2008a) Role of nitrogen and gibberellins (GA_3) in the regulation of enzyme activities and in osmoprotectant accumulation in *Brassica juncea* L. under salt stress. *Journal of*

Agronomy and Crop Science **194**, 214-224

Siddiqui MH, Mohammad F, Khan MN, Khan MMA (2008b) Cumulative effect of soil and foliar application of N, P and S on growth, physio-biochemical parameters, yield attributes and fatty acid composition in oil of erucic acid-free rapeseed-mustard genotypes. *Journal of Plant Nutrition* **31**, 1284-1298

Siddiqui MH, Mohammad F, Khan MN (2009) Morphological and physio-biochemical characterization of *Brassica juncea* L. Czern. & Coss. genotypes under salt stress. *Journal of Plant Interactions* **4**, 67-80

Slocum RD, Kaur-Sawhney R, Galston AW (1984) The physiology and biochemistry of polyamines in plants. *Archives of Biochemistry and Biophysics* **35**, 283-303

Smith IK (1980) Regulation of sulfate assimilation in tobacco cells: effect of nitrogen and sulfur nutrition on sulfate permease and *O*-acetylserine sulfhydryllase. *Plant Physiology* **66**, 877-883

Takahashi H, Saito K (1996) Subcellular localization of spinach cysteine synthase isoforms and regulation of their gene expression by nitrogen and sulfur. *Plant Physiology* **112**, 273-280

Wallsgrove RM, Turner JC, Hall NP, Kendall AC, Bright SWJ (1987) Barley mutants lacking chloroplast glutamine synthetase-biochemical and genetic analysis. *Plant Physiology* **83**, 155-158

Washitani I, Sato S (1977) Studies on the function of proplastids in the matabolism of *in vitro* cultured tobacco cells II. Glutamine synthetase/glutamate synthetase pathway. *Plant and Cell Physiology* **18**, 505-512

Watson DJ (1958) The dependence of net assimilation rate on leaf area index. *Annals of Botany* **22**, 37-54

Xie Z, Duan L, Tian X, Wang B, Eneji AE, Li Z (2008) Coronatine alleviates salinity stress in cotton by improving the antioxidative defense system and radical-scavenging activity. *Journal of Plant Physiology* **165**, 375-384