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Variation in Salt Tolerance of Wheat Cultivars: Role of Glycinebetaine and Ethylene

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ABSTRACT

Four wheat (*Triticum aestivum* L.) cultivars 711, PBW343, 3765 and WH542 were screened for studying variations in glycinebetaine (GB) content and plant dry mass under 100 mmol L^{-1} NaCl stress. A tolerance index was calculated using plant dry mass data to select salt-tolerant and salt-sensitive types and find association between tolerance index and GB content. Tolerance index has been used as a good criterion to select the tolerant types under high salinity stress. Further, physiological differences in salt-tolerant cultivar 711 and salt-sensitive cultivar WH542 were examined. The salt-tolerant cultivar exhibited greater GB content, which was found correlative with ethylene. The cultivar also showed higher nitrogen (N) content and nitrate reductase activity, reduced glutathione and higher redox state resulting in maximal protection of plant dry mass than the salt-sensitive type. Thus, the content of GB may be considered as important physiological criteria for selecting salt-tolerant wheat types.

Key Words: glutathione, nitrogen assimilation, salt stress

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Salt stress is one of the most widespread abiotic stresses and constitutes the most important factor in limiting plant distribution and productivity (Nazar *et al.*, 2011a). According to the Food and Agriculture Organization (FAO) over 6% of the world's land is affected by salinity, and this covers over 400 Mha of the world land area. Salinity occurs through natural or human-induced processes that result in the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth.

The deleterious effects of salt stress on plant growth and metabolism are associated with low osmotic potential of soil solution (osmotic stress), nutritional imbalance or specific ion toxicity (salt stress) (Munns and Tester, 2008). However, the effectiveness of salt stress differs greatly with species and cultivars (Ghoulam *et al.*, 2002). Salt stress induces a number of changes in metabolic processes of plant including nitrogen (N) assimilation. It has been reported that the negative effect of salt stress on plant N assimilation is strictly related to salt-induced modification in nitrate reductase (NR) activity (Debouba *et al.*, 2007; Nazar *et al.*, 2011b). In order to counteract the salt-induced adverse effects, one of the defense mechanisms that plants operate includes the up-regulation of antioxidant system such as increased synthesis of reduced glutathione (GSH): a low molecular weight antioxidant. GSH plays essential roles within plant metabolism and capacity of the antioxidant system (Nazar *et al.*, 2011b).

The use of physiological and biochemical criteria has been recommended to achieve a rapid and simple screening of highly salt-tolerant individuals (Ashraf, 2004). Classical methods of screening for salt tolerance are based on the plant yield and are very costly and time consuming. Typical physiological markers include growth rate or yield (Greenway, 1962), plant survival at high salinity (Sayed, 1985), seed germination rate (von Well and Fossey, 1998), leaf or root elongation rate (Cramer and Quarrie, 2002), plant height (Nobel and Rogers, 1992), leaf area (Franco *et al.*, 1993) and relative growth rate (Cramer *et al.*, 1990). The biochemical markers are leaf injury and reduction of CO_2 assimilation (James *et al.*, 2002), loss of chlorophyll and damage to the photosynthetic apparatus (KrishnaRaj *et al.*, 1993), Na^+ exclusion (Garcia *et al.*, 1995) and Cl^- exclusion (Rogers and Nobel, 1992). Besides compatible osmolytes generally found in higher plants that include low molecular weight sugars, organic acids, polyols, and N containing compounds such as amino acids, amides, imino acids, proteins and quaternary ammonium compounds and soluble sugar also act as biochemical markers (Ashraf and Tufail, 1995).

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Plants containing efficient antioxidant system have shown considerable resistance to oxidative damage caused by salt-induced reactive oxygen species (Syeed *et al.*, 2010). Plants also accommodate the ionic balance in the cell by accumulating low-molecular mass osmolytes because these compounds do not interfere with normal biochemical reactions (Zhifang and Loescher, 2003) and instead, they replace water in biochemical reactions. These osmolytes maintain osmotic balance and support continued water influx (Hasegawa *et al.*, 2000). To adapt to diverse environments, plants accumulate compatible solutes that are nontoxic at high concentrations (Chen and Murata, 2002). Among these osmolytes, proline and glycinebetaine (GB) are considered to play a key role in salinity tolerance by maintaining the osmotic pressure in a cell (Rajasekaran *et al.*, 1998). GB is considered as one of the most effective osmoprotectants owing to its features that enable its interaction with both the hydrophobic and hydrophilic domains of macromolecules without perturbing the cellular functions (Sakamoto and Murata, 2002). It has been reported that GB protects the cells from stresses by stabilizing the quaternary structures of antioxidant enzymes, biomembranes and oxygen-evolving photosystem II (PSII) complex (Robinson and Jones, 1986). Recently, it has been reported that ethylene plays a role in salt tolerance. Study of Cao *et al.* (2007) has shown that ethylene-insensitive mutants *etr1-1* and *ein2-1* of *Arabidopsis thaliana* were more sensitive to salt stress signifying the requirement of ethylene signaling for plant salt tolerance. It may be emphasized that formation of ethylene from *S*-adenosyl-L-methionine (SAM) results in the release of methyl groups that take part in the synthesis of GB (Fig. 1). Thus, ethylene together with GB might also involve in salt tolerance.

The present study was undertaken to screen four wheat (*Triticum aestivum* L.) cultivars for GB content and plant dry mass under 100 mmol L^{-1} NaCl and select salt-tolerant and salt-sensitive types on the basis of tolerance index calculated from dry mass. Moreover, physiological basis of the difference in these salt-tolerant and salt-sensitive types was evaluated.

MATERIALS AND METHODS

Plant material and growth conditions

Healthy seeds of wheat (*Triticum aestivum* L.) cultivars 711, PBW343, 3765 and WH542 were surface sterilized with 0.01 g L^{-1} mercuric chloride solution followed by repeated washings with double distilled water and were sown in 300 mL plastic pots filled with

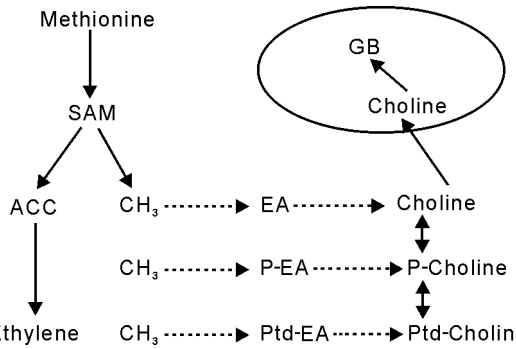

Fig. 1 Synthesis of glycinebetaine (GB) in relation to ethylene. GB is synthesized from choline which in turn is synthesized by three sequential methylation of N-terminus of ethanolamine (EA), phospho-ethanolamine (P-EA) and phosphatidyl-ethanolamine (Ptd-EA). Methyl group is donated at each step of the choline synthesis and *S*-adenosyl-L-methionine (SAM) donates methyl group at each step. SAM donates methyl group to choline in the process of ethylene formation. ACC = 1-aminocyclopropane 1-carboxylic acid.

reconstituted soil (peat and compost, 4:1, v/v; mixed with sand, 3:1, v/v). Five seeds per pot were sown to avoid germination failure and after the seedlings establishment two healthy plants of nearly equal size in each pot were maintained. The pots were kept in the environmental growth chamber (Khera KI-261, Delhi) with day/night temperatures at 24 °C/18 °C (± 3 °C) and relative humidity of 68% ± 5%. Plants (2 per pot) were subjected to either 0 (control) or 100 mmol L^{-1} NaCl. Plants were saturated on alternate days with 150 mL of 100 mmol L^{-1} NaCl solution. In all there were thirty two pots for the experiment. In the four cultivars, GB content and plant dry mass were recorded at 30 days after sowing (DAS). On the basis of plant dry mass tolerance index of the cultivars was calculated and the salt-tolerant and salt-sensitive types were selected. In another experiment, the selected salt-tolerant cultivar 711 (tolerance index: 71.8%) and salt-sensitive cultivar WH542 (tolerance index: 40.0%) were subjected to 0 (control) or 100 mmol L^{-1} NaCl and grown for 30 DAS under the same procedure and plant growth conditions. The number of pots used in this experiment was sixteen. The treatments in these experiments were arranged in a factorial randomized block design and four replicates for each treatment were maintained ($n = 4$). In these salt-tolerant and salt-sensitive cultivars, variation in ethylene evolution, N-assimilation (N content, NR activity), oxidative stress (H_2O_2 content, chlorophyll fluorescence (F_v/F_m)), GSH and oxidized glutathione (GSSG) content and redox state (GSH/GSSG) were examined.

Glycinebetaine estimation

GB was measured using the method of Grieve and

Grattan (1983) by estimating betaine-periodite complex. Dried and finely ground leaf (0.5 g) was mechanically shaken with 20 mL of deionized water for 24 h at 25 °C. The samples were then filtered and the filtrates were diluted 1:1 with 1 mol L^{-1} H_2SO_4. Aliquots (0.5 mL) were measured into centrifuge tubes and cooled in ice water for 1 h. Cold KI-I_2 reagent (0.2 mL) was added and then reactants were gently stirred. The tubes were stored at 4 °C for 16 h and then centrifuged at 10 000 × *g* for 15 min at 0 °C. The supernatant was carefully aspirated and the absorbance was measured after 2 h at 365 nm by using a spectrophotometer. Reference standards of GB (50–200 μg mL^{-1}) were prepared in 1 mol L^{-1} H_2SO_4.

Plant dry mass and tolerance index

Each plant was washed repeatedly with running tap water to wipe off the adhering foreign particles. After blotting, the plants were dried in a hot air oven at 80 °C until constant weight and dry weight was determined. Tolerance index was calculated as the ratio of dry mass of NaCl treated plants to dry mass of control plants and expressed as a percentage.

Measurement of ethylene

Ethylene evolution was measured by cutting 0.5 g leaf material into small pieces that were placed in 30 mL tubes containing moist paper to minimize evaporation from the tissue and were stopper with secure rubber caps and placed in light for 2 h under the same condition used for plant growth. Earlier experiment showed that 2 h incubation time was adequate for ethylene detection without the interference of wound-induced ethylene, which began after 2 h of leaf incubation (data not shown). A 1 mL gas sample from the tubes was withdrawn with a hypodermic syringe and assayed on a gas chromatograph (Nucon 5700, New Delhi, India) equipped with a 1.8 m Porapack N (80–100 mesh) column, a flame ionization detector and data station. N was used as the carrier gas. The flow rates of N, hydrogen and oxygen were 30, 30 and 300 mL min^{-1}, respectively. The detector was at 150 °C. Ethylene identification was based on the retention time and quantified by comparison with the peaks from standard ethylene concentration.

NR activity and N content

NR activity in leaf was measured by preparing enzyme extract using the method of Kuo *et al.* (1982). Leaves (1.0 g) were frozen in liquid N_2, ground to a powder with a mortar and pestle, and then stored at −80 °C. The powder was thawed for 10 min at 4 °C and homogenized in a blender in 250 mmol L^{-1} Tris-HCl buffer, pH 8.5, containing 10 mmol L^{-1} cysteine, 1 mmol L^{-1} ethylenediamine tetraacetic acid (EDTA), 20 μmol L^{-1} flavin adenine dinucleotide (oxidized) (FAD), 1 mmol L^{-1} dithiothreitol (DTT), and 10% (v/v) glycerol. The homogenate was centrifuged at 10 000 × *g* for 30 min at 4 °C (REMI CPR24, New Delhi, India). Activity of NR was assayed spectrophotometrically as the rate of nitrite production at 28 °C adopting the procedure of Nakagawa *et al.* (1984). The assay mixture contained KNO_3 (10 mmol L^{-1}), N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulphonic acid) (HEPES) (0.065 mol L^{-1}; pH 7.0), nicotinamide adenine dinucleotide (reduced) (NADH) (0.5 mmol L^{-1}) in phosphate buffer (0.04 mmol L^{-1}; pH 7.2) and enzyme in a final volume of 1.5 mL. The reaction was started by adding NADH. After 15 min the reaction was terminated by adding 1 mL of 1 mol L^{-1} HCl solution containing 1% sulfanilamide followed by adding 1 mL of 0.02% aqueous N-1-napthylethylene-di-amine-dihydrochloride (NED). The absorbance was read at 540 nm after 10 min. Leaf N content was determined in acid-peroxide digested material using the method of Lindner (1944).

Water potential and osmotic potential

Leaf water potential was measured on the second leaf from the top (fully expanded young leaf) of the plant by using the water potential system (Psypro, WESCOR, USA). The leaf used for water potential measurement was frozen in liquid N_2 in sealed polythene bags which were thawed and cell sap was extracted with the help of a disposable syringe. The extracted sap was used for the determination of osmotic potential using a vapour pressure osmometer (5520, WESCOR, USA).

Content of H_2O_2

The content of H_2O_2 was determined following the method of Okuda *et al.* (1991). Fresh leaf tissues (50 mg) were ground in ice-cold 200 mmol L^{-1} perchloric acid. After centrifugation at 1 200 × *g* for 10 min, perchloric acid of the supernatant was neutralized with 4 mol L^{-1} KOH. The insoluble potassium perchlorate was eliminated by centrifugation at 500 × *g* for 3 min. The reaction was started by the addition of peroxidase and the increase in absorbance was recorded at 590 nm for 3 min.

Efficiency of PSII

Efficiency of PSII was estimated as chlorophyll fluorescence of fully expanded second leaf from top u-

sing chlorophyll fluorometer (OS-30p, Hudson, USA). Plants were dark-adapted for 30 min, minimal fluorescence (F_0) was measured during the weak measuring pulses and a saturating pulse was used to obtain maximal fluorescence (F_m) and efficiency of PSII was calculated.

GSH and GSSG contents and redox state

GSH was assayed by an enzymic recycling procedure (Griffith, 1980) in which it was sequentially oxidized by 5,5′-dithiobis-2-nitrobenzoic acid and reduced by nicotinamide adenine dinucleotide phosphate (reduced) (NADPH) in the presence of glutathione reductase. For specific assay of GSSG, the GSH was masked by derivatization with 2-vinylpyridine. Fresh leaf tissue (0.5 g) was ground in liquid N_2 using a pestle and mortar. The ground tissue was suspended in 0.5 mL 5% sulphosalicylic acid and centrifuged at 12 000 × g for 10 min. A 300 µL aliquot of supernatant was removed and neutralized by addition of 18 µL 7.5 mol L^{-1} triethanolamine. One 150 µL sample was then used to determine concentrations of GSH plus GSSG. Another sample was pretreated with 3 µL 2-vinylpyridine for 60 min at 20 °C to mask the GSH by derivatization, to allow the subsequent determination of GSSG alone. In each case 50 µL aliquots of the two samples were mixed with 700 µL 0.3 mmol L^{-1} NADPH, 100 µL 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and 150 µL buffer containing 125 mmol L^{-1} sodium phosphate, 6.3 mmol L^{-1} EDTA (pH 6.5). A 10 µL aliquot of glutathione reductase (GR) (5 U mL^{-1}) was then added and the change in absorbance at 412 nm monitored at 30 °C. Redox state was presented as the ratio of GSH to GSSG.

Statistical analysis

Data were analyzed statistically using the Statistical Package for the Social Sciences (SPSS, 10.0 for Windows). Data are presented as mean ± standard error ($n = 4$). Analysis of variance was performed and F-value was calculated at $P < 0.05$. For significant data least significant difference (LSD) was determined. Each mean and standard error in the figures represents four replicate measurements. Bars marked by the same letter are not significantly different.

RESULTS

Comparison of cultivars for GB, plant dry mass and tolerance index under salt stress

Salt-treated wheat plants exhibited greater accumulation of GB than control plants. Maximum accumulation of 45.4% was observed in wheat cultivar 711, whereas cultivar WH542 exhibited minimum accumulation of 14.3% in comparison to control. The other two cultivars, PBW343 and 3765 had GB content values in between these two extremes. Cultivar PBW343 and 3765 accumulated 33.3% and 22.2% GB under salt stress compared to their respective control (Fig. 2).

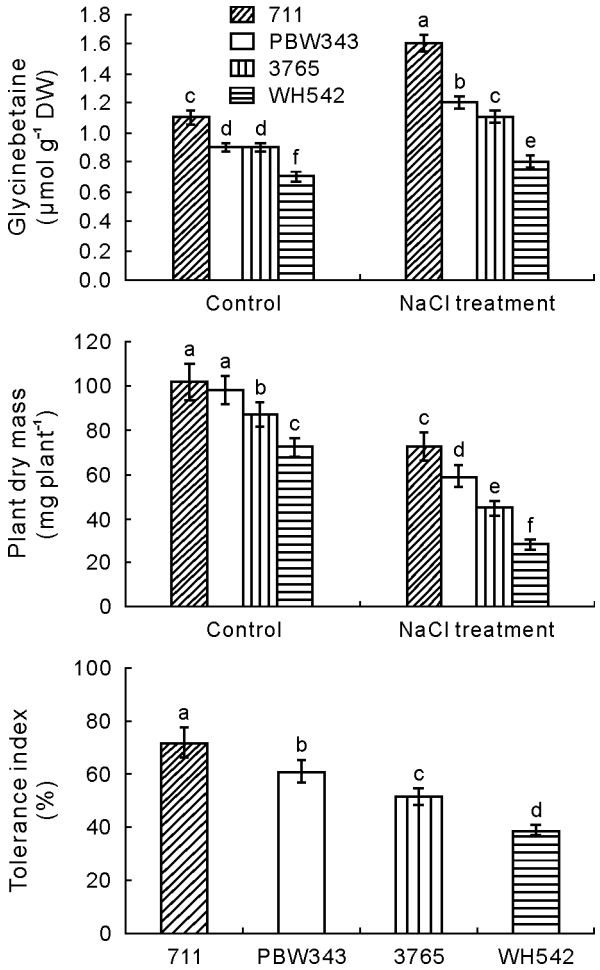

Fig. 2 Glycinebetaine content, plant dry mass and tolerance index of wheat (*Triticum aestivum* L.) cultivars treated with 100 mmol L^{-1} NaCl at 30 days after sowing. Error bars represent standard error of the mean ($n = 4$). Bars marked by the same letter are not significantly different at $P < 0.05$.

Treatment of 100 mmol L^{-1} NaCl significantly reduced plant dry mass in all the four cultivars. A reduction in plant dry mass of 28.2% in cultivar 711 and 61.0% in cultivar WH542 was observed under salt stress compared to control. Moreover, highest tolerance index of 71.8% was found in cultivar 711 followed by 61.1% in cultivar PBW343, 51.3% in cultivar 3756, and 38.9% in cultivar WH542. The cultivar 711 showed greatest tolerance index and cultivar WH542 least tolerance index among all the cultivars (Fig. 2).

Effect of salt stress on ethylene evolution

The two cultivars 711 and WH542 showed differential sensitivity to salt stress and ethylene evolution. Ethylene evolution was significantly greater in both the cultivars under salt stress than control plants. Ethylene evolution in cultivar 711 increased to about 6 times, while there was an increase of 5 times ethylene in cultivar WH542 in comparison to their controls (Fig. 3).

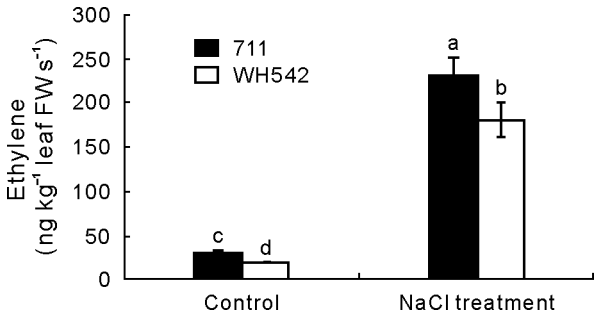

Fig. 3 Ethylene content of wheat (*Triticum aestivum* L.) cultivars treated with 100 mmol L^{-1} NaCl at 30 days after sowing. Error bars represent standard error of the mean ($n = 4$). Bars marked by the same letter are not significantly different at $P < 0.05$.

Effect of salt stress on N assimilation

N content and NR activity decreased under salt stress in both the cultivars, but the reduction in cultivar 711 was lesser than cultivar WH542. N content in cultivar 711 and cultivar WH542 was reduced by 11.1% and 44.6%, while NR activity was reduced by 10.9% and 52.4% with 100 mmol L^{-1} NaCl compared to the respective control (Fig. 4).

Effect of salt stress on water relations

Salt stress significantly decreased the water potential and osmotic potential in both the cultivars, but cultivar WH542 showed greater reduction than cultivar 711. Water potential and osmotic potential were reduced by 54.3% and 42.2% in WH542 and by 24.4% and 13.9% in cultivar 711, respectively, compared to control (Fig. 5).

Effect of salt stress on oxidative stress, GSH and GSSG contents and redox state

Salt stress induced oxidative stress in both the cultivars in terms of H_2O_2 content and chlorophyll fluorescence. Under salt stress both the cultivars showed an increase in H_2O_2 content, but the increase in cultivar 711 was lesser than cultivar WH542. In contrast, there was a decrease in chlorophyll fluorescence in both

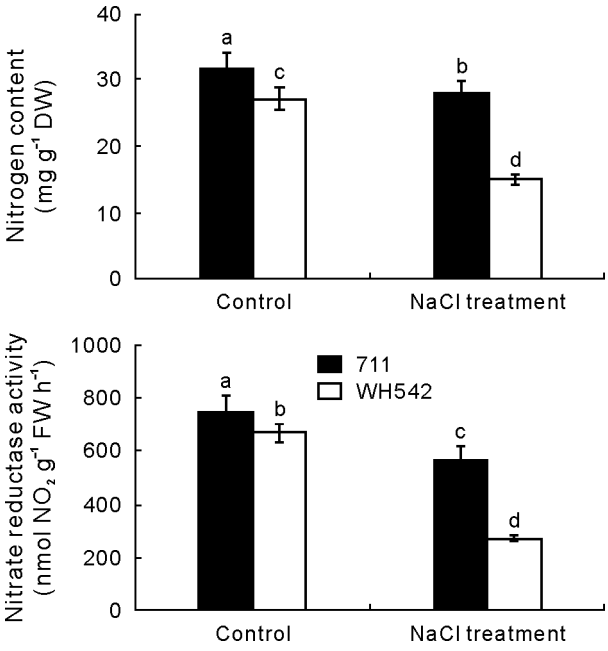

Fig. 4 Content of nitrogen and the activity of nitrate reductase of wheat (*Triticum aestivum* L.) cultivars treated with 100 mmol L^{-1} NaCl at 30 days after sowing. Error bars represent standard error of the mean ($n = 4$). Bars marked by the same letter are not significantly different at $P < 0.05$.

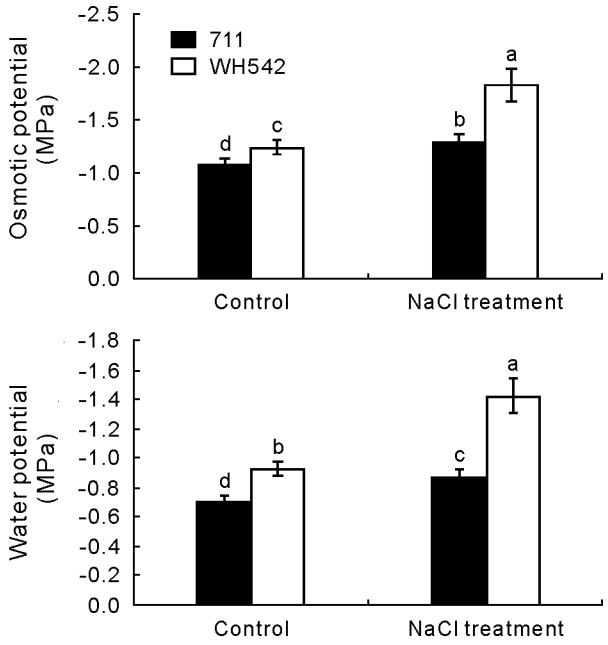

Fig. 5 Osmotic potential and water potential of wheat (*Triticum aestivum* L.) cultivars treated with 100 mmol L^{-1} NaCl at 30 days after sowing. Error bars represent standard error of the mean ($n = 4$). Bars marked by the same letter are not significantly different at $P < 0.05$.

the cultivars, and the reduction in chlorophyll fluorescence was 56.2% in cultivar WH542 and 15.0% in cultivar 711 in comparison to the respective control (Fig. 6).

Plants under salt stress exhibited maximum incre-

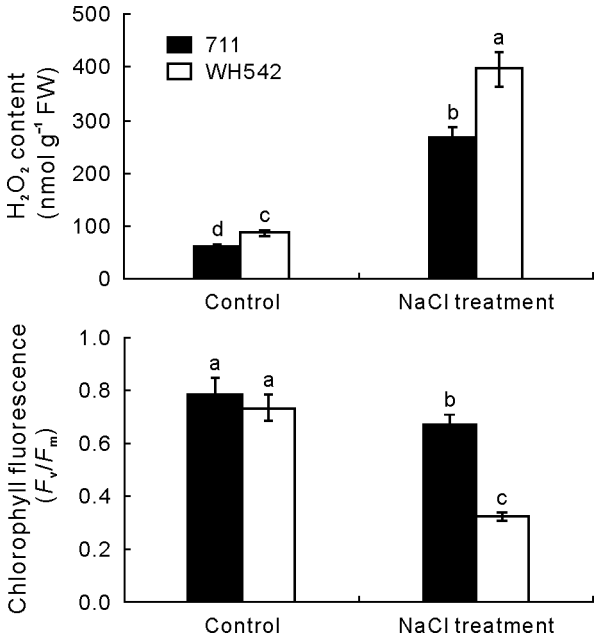

Fig. 6 H_2O_2 content and chlorophyll fluorescence (F_v/F_m) of wheat (*Triticum aestivum* L.) cultivars treated with 100 mmol L^{-1} NaCl at 30 days after sowing. Error bars represent standard error of the mean ($n = 4$). Bars marked by the same letter are not significantly different at $P < 0.05$.

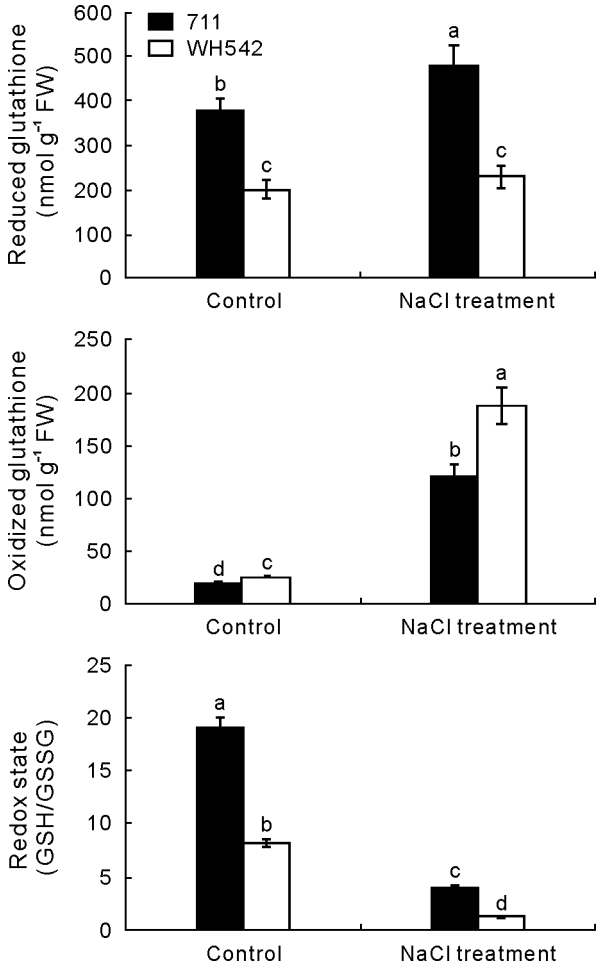

Fig. 7 GSH and GSSG contents and redox state (GSH/GSSG) of wheat (*Triticum aestivum* L.) cultivars treated with 100 mmol L^{-1} NaCl at 30 days after sowing. Error bars represent standard error of the mean ($n = 4$). Bars marked by the same letter are not significantly different at $P < 0.05$.

ase in GSH content in cultivar 711 and maximum increase in GSSG content in WH542. The increase in GSH content was 26.3% in cultivar 711 and 15.1% in cultivar WH542, whereas GSSG content increased by 6 times in cultivar 711 and 7.6 times in cultivar WH542 in comparison to control (Fig. 7). The redox state (GSH/GSSG) decreased under salt stress in both the cultivars, but a lesser decrease of 78.9% in cultivar 711 and greater decrease of 85.0% in cultivar WH542 was observed in comparison to control (Fig. 7).

DISCUSSION

Salinity is one of the important environmental factors that limits the distribution and productivity of major crops (Nazar *et al.*, 2011a). Agricultural productivity in arid and semiarid regions of the world is very low due to accumulation of salts in soils (Munns, 2002). The survival of plants under salt stress depends on their ability of salt tolerance, which differs not only among different genera and species, but also within the different organs of the same species (Ismail, 2003). The present study has shown that GB content increased in all the cultivars in the presence of salt stress, but cultivar 711 exhibited the highest value. Dry mass and salt tolerance index were also greatest in this cultivar, whereas cultivar WH542 had the lowest GB content, dry mass and tolerance index. Thus, cultivar 711 appeared to be a salt-tolerant type and cultivar WH542 as a salt-sensitive type. As an adaptation to stress conditions many plants accumulate some high soluble compounds (osmoprotectants) to raise osmotic potential in the cytoplasm and stabilize proteins and membranes. One such osmoprotectant is GB which appears to be a critical determinant of salt tolerance (Zhang *et al.*, 2009). GB has been shown to involve in reducing H_2O_2 content and increasing the antioxidant defense mechanism and salt tolerance (Demiral and Türkan, 2004; Banu *et al.*, 2009). It is reiterated that synthesis of GB requires methyl group donated by SAM, a precursor of ethylene (Chen and Murata, 2002), and it is likely that ethylene is also involved in GB-mediated tolerance. There is evidence that the increase of betaine results from the increased supply of the precursor glycine and SAM upon salt stress (Waditee *et al.*, 2005). The individual importance of GB and ethylene in salt tolerance has been shown (Grumet and Hanson, 1986; Lutts and Bouharmont, 1996; Liang *et*

al., 2009), but the physiological significance and relationship between GB and ethylene taken together in inducing salt-tolerance have not been studied. In the salt-tolerant cultivar 711, the GB and ethylene contents were higher than the salt-sensitive cultivar WH542. Under salt stress ethylene evolution increased through the increased availability of SAM. SAM provided methyl groups for the synthesis of GB during the formation of ethylene. Thus, the differential response of cultivars 711 and WH542 can be attributed to diverse capacity for ethylene and GB synthesis under salt stress.

Salt-tolerant cultivar with higher GB and ethylene had higher GSH content. GSH has been recognized to take part in the removal of excess reactive oxygen species (Noctor and Foyer, 1998; Tausz and Grill, 2004) generated under salt stress. Ethylene has been reported to control GSH synthesis under ozone stress (Yoshida *et al.*, 2009). They have shown that ethylene-insensitive types produced less GSH and were ozone-sensitive compared to ethylene-sensitive types. However, the relationship between ethylene and GSH in salt-tolerant and salt-sensitive types has not been studied. In the present study, the salt-tolerant cultivar had higher ethylene, GSH and maximal fluorescence with lesser H_2O_2 content than the salt-sensitive cultivar.

Plant dry mass reduction due to salt stress is also attributed to nutrient imbalance and reduction in water potential and osmotic potential. Under salt stress, accumulation of excess Na^+ and Cl^- causes negative impacts on the acquisition and homeostasis of essential nutrients and water balance (Fageria *et al.*, 2008) making it more difficult for water and nutrients to move through the root membrane (Volkmar *et al.*, 1998). In both the cultivars salinity decreased the leaf water potential and osmotic potential. The reduction in osmotic potential in salt stressed plants mainly occurs due to the accumulation of inorganic ions (Na^+, Cl^- and K^+) (Hasegawa *et al.*, 2000) limiting the availability of water to plants. The salt-tolerant cultivar had higher leaf water potential and osmotic potential than the salt-sensitive cultivar because of its ability to restrict Na^+ and Cl^- ions. The decrease in water potential impacts negatively on nutrient uptake resulting in reduced nutrients concentration in the leaf (Khorshidi *et al.*, 2009; Khan *et al.*, 2010). Botella *et al.* (1993) suggested that the metabolism of N compounds played a key role in the ability of plants to tolerate salinity. N uptake rates in plants have been found to decrease with high concentrations of NaCl salinity (Silveira *et al.*, 2001). In the present study, N content and NR activity decreased with salt. In the salt-tolerant cultivar lesser reduction in N content and NR activity was observed in comparison to the salt-sensitive cultivar. Salt-tolerant cultivar with comparatively high N content had higher ethylene evolution. N takes part in the synthesis of GB and GSH and also influences ethylene evolution under salt stress. Thus, under salt stress the cultivar 711 with higher GB content and ethylene evolution showed maximal tolerance to salt stress with higher N accumulation, GSH content and redox state in comparison to cultivar WH542. This showed that both GB and ethylene had a relationship in salt tolerance, however, the interaction between them needs to be researched further. The direct involvement of ethylene in GB-mediated salt stress tolerance needs to be tested in further experiments using ethylene action inhibitors.

CONCLUSIONS

Conclusively, it may be said that GB and ethylene are both involved in salt stress tolerance in wheat. The salt-tolerant cultivar 711 had greater GB and ethylene contents and exhibited higher tolerance to salt stress, whereas salt-sensitive cultivar WH542 with lesser GB and ethylene contents showed lesser tolerance to salt stress. The cultivar 711 also had higher N content, NR activity, GSH content, and redox state and lesser oxidative stress than cultivar WH542. Under salt stress increased availability of SAM resulted in more ethylene formation and release of methyl groups which were used in the synthesis of GB. However, the involvement of ethylene in GB-mediated tolerance still remains to be tested.

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