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Differential Response of Scavenging of Reactive Oxygen Species in Green Gram Genotype Grown under Salinity Stress

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Abstract: Salinity stress affects the metabolism of plant cells leading to severe crop damage and loss of yield. Oxidative stress is one of the major causes of salinity that may be responsible for the tissue damage. The present endeavor is based on the immediate responses on enzymatic and non-enzymatic to salinity-induced oxidative stress in two high yielding green gram (*Phaseolus aureus*) cvs. salt tolerant (T-44) and salt sensitive (SML-32). Under salinity stress, the salt tolerant cv. T-44 showed the higher activity of ROS scavenging enzyme, catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), Glutathione Reductase (GR), glutathione-S-transferase (GST) and enhanced levels of ascorbate (ASC) and glutathione (GSH), than the sensitive cv. SML-32. While, superoxide dismutase (SOD), monodehydroascrobate reductase (MDAR) and dehydroascorbate reductase (DHAR) were lower in cv. T-44 than cv. SML-32, it indicated lesser extent of membrane damage (lipid peroxidation) and lower levels of H_2O_2. The high levels of CAT activity indicate efficient scavenging of H_2O_2, which is produced more by non-enzymatic means than via SOD in cv. T-44. These findings suggest that planned action of enzymatic as well as non-enzymatic ROS scavenging machineries are essential to overcome the salinity-induced oxidative stress in green gram.

Key words: Antioxidants, antioxidant enzymes, *Phaseolus aureus*, salinity, salt stress

Introduction

Soil salinity is one of the major abiotic stresses affecting germination, plant growth and productivity (Misra and Dwivedi, 2004). Under normal conditions of growth and development, plants are inevitably exposed to different types of stress, which may cause increased production of Reactive Oxygen Species (ROS) (Hippeli *et al.*, 1999). These include superoxide radicals (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH), which cause tissue injury (Foyer *et al.*, 1994). It is now widely accepted that ROS are responsible for various stress-induced damage to macromolecules and ultimately to cellular structure (Fridovich, 1986; Davies, 1987; Imlay and Linn, 1988). ROS are highly reactive in the absence of any protective mechanism. They can seriously disrupt normal metabolism through oxidative damage to membrane lipids, proteins, pigments and nucleic acids

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(Fridovich, 1986; Davies, 1987; Imlay and Linn, 1988). Plants have evolved various protective mechanisms to eliminate or reduce ROS (Foyer *et al.*, 1994), one of the protection mechanisms is antioxidant system, composed of both non-enzymatic and enzymatic antioxidants (Foyer *et al.*, 1994). Consequently the role of antioxidant enzymes viz., SOD, APX, GR, DHAR and CAT and metabolites like ASC, GSH, -tocopherol, flavonoids, carotenoids responsible for the scavenging of ROS becomes very important (Bowler *et al.*, 1992; Moran *et al.*, 1994; Menconi *et al.*, 1995).

These ROS are detoxified by the sequential and simultaneous action of a number of enzymes including GR, GST, CAT, APX, GPX, DHAR, MDAR, SOD and metabolites like, ascorbic acid, glutathione, -tocopherol, flavonoids, carotenoids, etc. Superoxide dismutase (SOD; EC 1.15.1.1) is located in various cell compartments and it is a major scavenger of superoxide ($O_2^{.}$) and its enzymatic action results in the formation of H_2O_2 and O_2 (Smirnoff, 1993). The hydrogen peroxide produced is then scavenged by catalase (CAT; EC 1.11.1.6.) and a variety of peroxidases (POD; EC 1.11.1.7). Catalase, which is located in peroxisomes, glyoxysomes and mitochondria, apparently absent in the chloroplast, dismutates mostly photorespiratory/respiratory H_2O_2 into water and molecular O_2 (Asada, 1992; Willekens *et al.*, 1997) whereas POD decomposes H_2O_2 by oxidation of co-substrate such as phenolic compounds and/or antioxidants. ASC and GSH are the important components of the ASC-GSH cycle responsible for the removal of H_2O_2 in different cellular compartments (Jimenez *et al.*, 1997). ASC and GSH directly interact with and detoxify oxygen free radicals (superoxide and hydroxyl) and thus contribute significantly to non-enzymatic ROS scavenging (Polle, 2001). Some work has been reported on the generation of ROS and plant antioxidant in relation to salinity stress (Kalir and Poljakaff-Mayber, 1981; Hernandez *et al.*, 1993; Gueta-Dahan *et al.*, 1997). Salt stress induces conditions of oxidative stress (Sing and Choudhuri, 1990; Hernandez *et al.*, 1999; Zhu, 2000). Changes in the activity of antioxidant enzymes in response to salinity (Hernandez *et al.*, 1994; Fadzilla *et al.*, 1997; Sharata and Tal, 1998; Meneguzzo *et al.*, 1999) were different in tolerant and sensitive cultivars (Gossett *et al.*, 1994; Olmos *et al.*, 1994; Meloni *et al.*, 2003; Vaidyanathan *et al.*, 2003).

Thus, an understanding of the enzymatic and non-enzymatic ROS scavenging machineries is critical for identifying key components involved in oxidative stress tolerance. Besides, scarce attention has been pointed on such antioxidant responses in the third most important pulse crop, green gram (*Phaseolus aureus*) of India subjected to short term salt stress. Therefore, the present study elucidates such responses in two genotypes of green gram, T-44 (salt-tolerant) and SML-32 (salt sensitive) upon exposure to different concentrations of NaCl.

Materials and Methods

Plant Material, Growth, Stress Conditions

Seeds of green gram (*Phaseolus aureus*, Family Leguminosae) cultivar, T-44; salt tolerant and SML-32; salt sensitive cultivars were well screened in the lab and surface sterilized with 1% sodium hypochlorite and germinated as described by Misra and Dwivedi (2004). Three concentrations of NaCl, viz., 50, 100 and 200 mM, were used for tolerant cultivar T-44 while three concentrations of NaCl, viz., 1, 10 and 50 mM were used for sensitive cultivar SML-32 (Misra and Dwivedi, 2004). Starting with 4 h soaked seeds (zero hour of seed germination) the germinated seeds were taken out at 24 h intervals up to 5 days, root and shoot (along with cotyledons) were separated from the seeds. The experiments were repeated thrice with three replicates of each treatment.

Enzyme Extraction and Enzyme Assays

A crude enzyme extract was prepared by homogenizing 500 mg of tissue (root and shoot along with cotyledons) in 0.1 M Tris HCl buffer, pH 7.5, 0.5 mM EDTA, at 4°C. The homogenate was centrifuged at 30,000-x g for 30 min. The supernatant was used as the crude enzyme preparation.

Glutathione reductase (EC 1.6.4.2) activity was assayed according to method of Smith *et al.* (1988) by following the increase in absorbance at 415 nm due to glutathione reduction of 5,5'-dithiobis-2-nitro benzoic acid (DTNB). The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.5), 1 mM EDTA, 0.70 mM DTNB in 0.01 M sodium phosphate buffer, 0.1 mM NADPH, 1mM GSSG. Reaction was started by the addition of enzyme extract.

Glutathione-S-transferase (EC 2.5.1.18) activity was measured by Mannervik and Guthenberg (1981) by following the decrease in the absorbance at 340 nm due to glutathione (GSH) oxidation. The final assay volume of 1 ml contained 0.1 mM sodium phosphate buffer, pH 6.5, 1 mM GSH, 1 mM 1-chloro-2, 4-dinitrobenzene (CDNB) in ethanol. Reaction was started by the addition of enzyme extract.

Ascorbate peroxidase activity (EC 1.11.1.11) was determined according to Asada and Badger (1984) measuring the decrease in 290 nm due to ascorbate oxidation (E = 2.8 /mM/cm). The enzyme activity was calculated in terms of μmol of ascorbate oxidized per minute at 28±3°C.

Dehydroascorbate reductase (EC 1.8.5.1) activity was assayed by monitoring the increase in absorbance at 265 nm due to ascorbate formation (E = 14/mM/cm) (Nakano and Asada, 1981). The enzyme activity was defined in terms of μmol of ascorbate formed per minute at 28±3°C.

Monodehydroascorbate reductase (EC 1.1.5.4) activity was assayed by following the oxidation of NADPH (E = 6.2/mM/cm) at 340 nm (Hossaion *et al.*, 1984).

Catalase (EC 1.11.1.6) activity was determined by Upadhyaya *et al.* (1985). The assay mixture contained 20 mM sodium phosphate buffer, pH 7.5, 0.025 % H_2O_2 and enzyme extract. The decomposition of H_2O_2 was measured at 240 nm.

Superoxide dismutase (EC 1.15.1.1) activity was measured by the photochemical method as described by Giannopoliti and Ries (1977) with slight modification. The reaction mixture consisted of 20 mM sodium phosphate buffer pH 7.5, 0.1 mM EDTA and 10 mM methionine, 0.1 mM P-nitro blue tetrazolium chloride (NBT) in ethanol, 0.005 mM riboflavin and enzyme extract. Blanks were kept in the dark and others were illuminated for 30 min. Total SOD activity was defined as the amount enzyme required to cause 50 % inhibition of the rate of NBT reduction at 560 nm.

Guaiacol peroxidase (EC 1.11.1.7) activity was measured according to Gasper *et al.* (1975) by following the increase in absorbance at 420 nm due to oxidation of guaiacol (E = 26.6/mM/cm). The enzyme activity was measured in terms of mol of guaiacol oxidized at 28±1°C.

All the enzyme activities were calculated in terms of katal.

Determination of Soluble Proteins

Soluble protein was measured by the Bio-Red micro assay modification of the Bradford (1976) procedure using bovine serum albumin as standard.

Determination of Non-enzymatic Contents

Hydrogen peroxide content was estimated by determine the absorbance of titanium-hydroperoxide complex (Mukherjee and Choudhari, 1983). Tissue samples (500 mg) were homogenized in 12 mL of cold acetone. The homogenate was filtered through Watman number 1 filter paper. To whole of the extract 5 mL of titanium reagent was added followed by 6 mL of concentrated ammonium solution to

precipitate titanium-hydroperoxide complex. After centrifugation for 5 min at 12,000xg, the supernatant was discarded and precipitate was dissolved in 1 M sulfuric acid. It was re-centrifuged to remove undissolved material and absorbance was recorded at 415 nm against blank. Concentration of H_2O_2 was determined using standard curve plotted with known concentration of H_2O_2.

The level of lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) content (Health and Packer, 1968). Tissue samples (500 mg) were homogenized in 10 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000xg for 5 min to 2 mL of aliquot of the supernatant 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95°C for 40 min and then immediately cooled in ice bath. After centrifugation at 12,000xg for 15 min to remove suspended turbidity the absorbance of supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The TBARS content was calculated using its absorption coefficient of 155 m $mol^{1}cm^{1}$.

Ascorbate (ASC) was determined according to the some modification of the procedure Law *et al.* (1983). Tissue samples (500 mg) were homogenized with 1.0 mL of 5% metaphosphoric acid and centrifuged at 20,000xg for 20 min at 28°C. The supernatant was treated with dithiothreitol. The supernatant (0.3 mL) was added to 0.6 mL of 100 mM phosphate buffer (pH 7.5) containing 5 mM EDTA and 0.1 mL of 0.5% (w/v) *n*-ethylmaleimide. After adding 0.4 mL of 10% TCA, 0.4 mL of 44% (v/v) orthophosphoric acid, 0.4 mL of 4% (w/v) *o*'-dipyridyl in 75% (v/v) ethanol and 0.2 mL of 3% (w/v) $FeCl_3$ the mixture was incubated at 450°C for 30 min the color developed was measured at 525 nm. Total ASC was calculated by using a standard curve of ascorbate (pure).

Total glutathione (GSH) was estimated by the modified Griffith (1980) method. Tissue samples (500 mg) were homogenized in 1.8 mL of 5% (w/v) sulphosalicylic acid. The homogenate was centrifuged at 12,000xg for 15 min at 28°C. The supernatant (1.0 mL) was neutralized with 1.5 mL of 0.5 M phosphate buffer (pH 7.5) containing 10 mM EDTA, 0.2 mL of 6 mM 5,5'-dithiobis-(2-nitrobenzoic acid), 0.1 mL of 2 mM NADPH and 0.1 U of Baker's yeast glutathione reductase (GR). The change in the absorbance at 412 nm was measured at 28±2°C.

Statistical Analysis

Each treatment was analyzed with at least three replicates and standard deviation (SD) was calculated. Statistical analysis was performed using the Student's t-test; $p<0.05$ was considered statistically significant.

Results

Effect of Salinity Stress on HO Content

Figure 1A depicted the change in H_2O_2 content during plant growth (from day 1 to day 5) in absence (nonsaline control) and in the presence of different concentrations of salinity stress in T-44 (salt tolerant cultivar) and in SML-32 (salt sensitive cultivar). Cultivar T-44 has steady state levels of H_2O_2 irrespective of the level of NaCl in the medium. While SML-32 showed significant increase (approximately 2.0 fold) in the H_2O_2 content on day 5th of the plant growth, in the presence of 50 mM NaCl. A change in H_2O_2 is a good indicator of the status of ROS scavenging capacity of plants under oxidative stress.

Lipid Peroxidation as an Indicator of Oxidative Damage

TBARS content increased with plant growth and salinity levels in both the cultivars (Fig. 1B). SML-32 showed more TBARS content under nonsaline control and salinity treatments than T-44 at
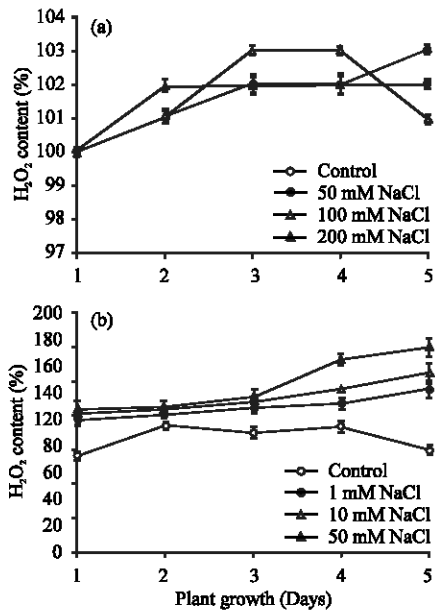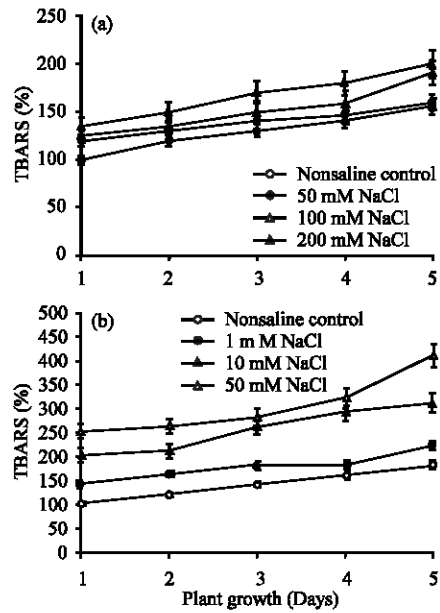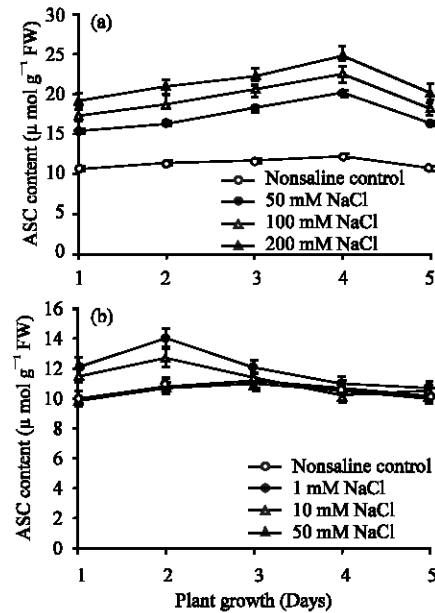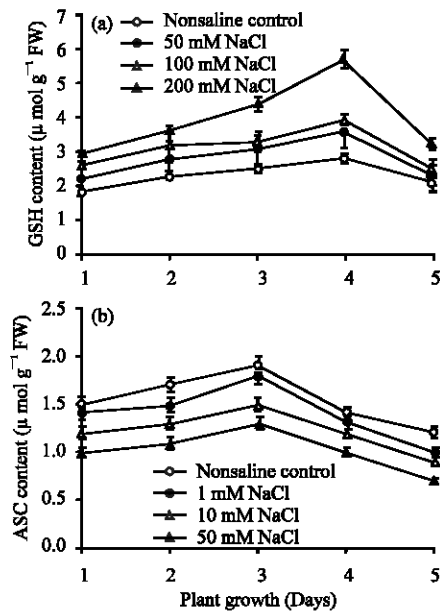
Fig. 1A

Fig. 1B

Fig. 1C

Fig. 1D

Fig. 1: Effect of salinity levels on (A) H_2O_2, (B) Lipid peroxidation (TBARS content), (C) Ascorbate, (D) Glutathione, in shoots of green gram genotypes, upon stress with varying concentration of NaCl during plant growth, (a) T-44 (salt tolerant) and (b) SML-32 (salt sensitive). Each value represents mean of three independent observations and SD determined. Data are statistically significant at $p<0.05$
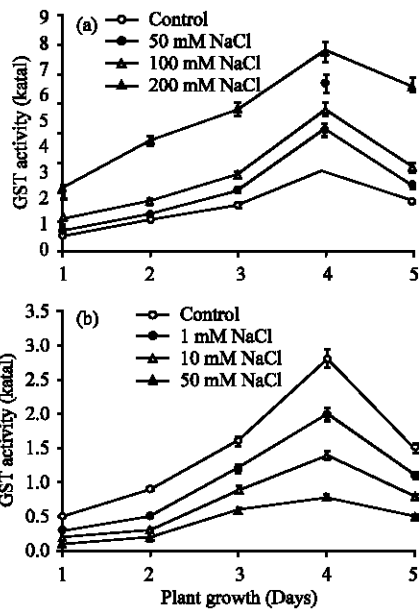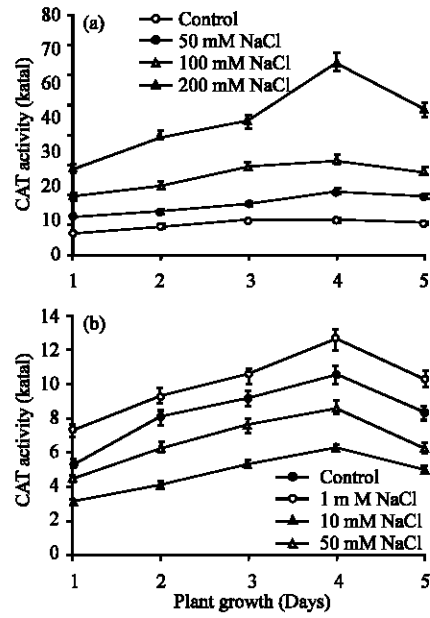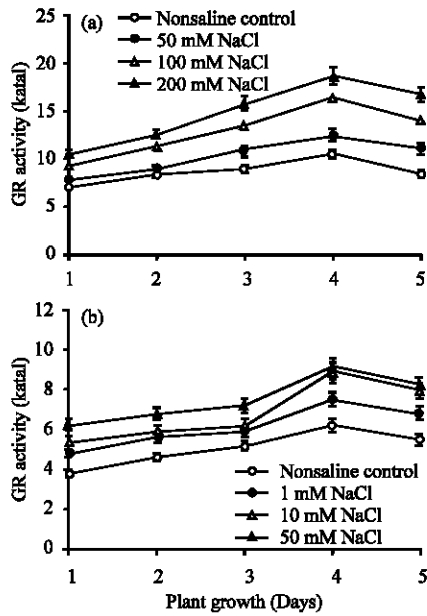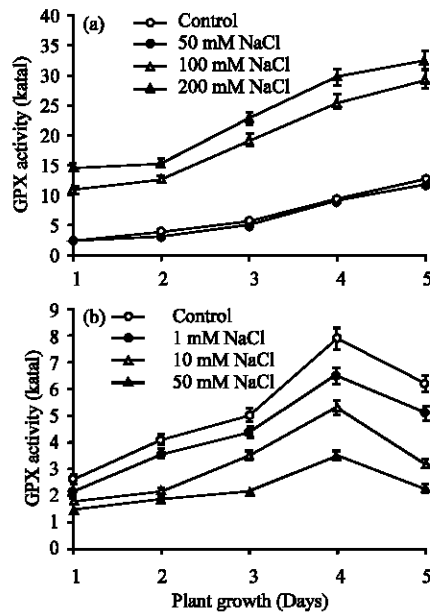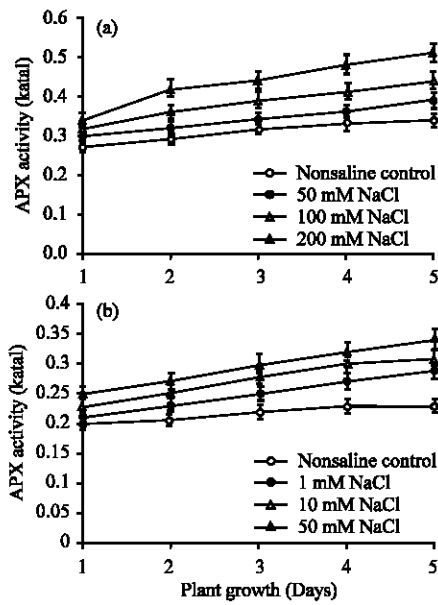
Fig. 2A

Fig. 2B

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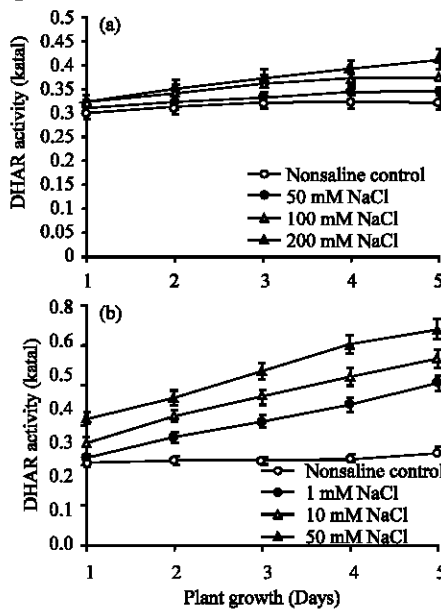
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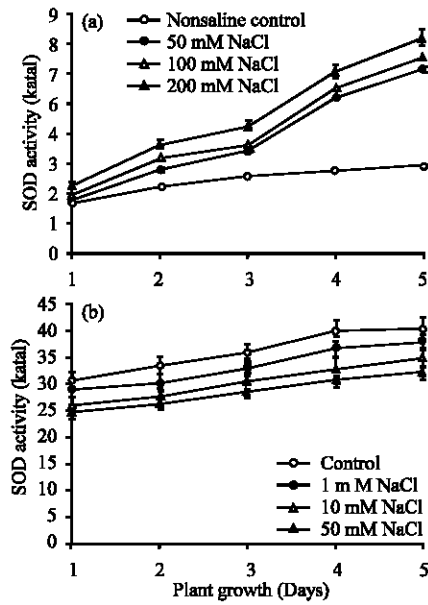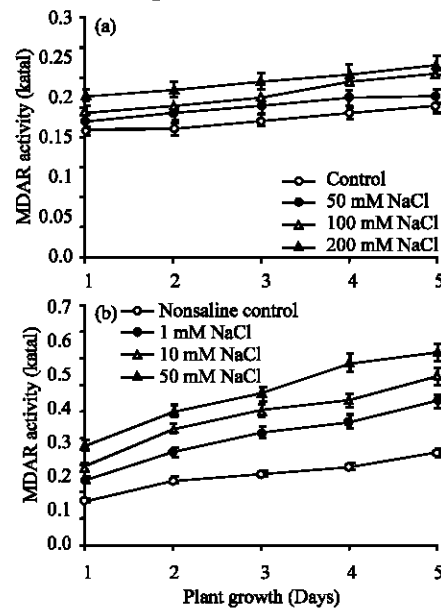
Fig. 2H

Fig. 2: Effect of salinity levels on (A) GST, (B) CAT, (C) GR, (D) GPX, (E) APX, (F) SOD, (G) DHAR and (H) MDAR activity in shoots of green gram genotypes, upon stress with varying concentration of NaCl during plant growth, (a) T-44 (salt tolerant) and (b) SML-32 (salt sensitive). The GST activity was calculated in terms of katal. Each value represents mean of three independent observations and SD determined. Data are statistically significant at p<0.05

all stages. TBARS level on day 5th of the plant growth in SML-32 was approximately 2.3 fold than non-saline control, while in T-44 it was only 1.3 fold. This indicates that T-44 is able to tolerate salinity-induced oxidative damage better than SML-32.

Influence of Salinity Stress on ASC and GSH Contents

We investigated the modulations of the levels of ASC and GSH in response to different concentrations of salinity-induced oxidative stress in shoots of cvs. T-44 and SML-32 (Fig. 1C and D). GSH and ASC are the common antioxidants used by plants to reduce ROS levels *in vivo*. In shoots of cv. T-44, the level of ASC and GSH were significantly increased (p<0.5) in presence of higher concentration of salinity while in cv. SML-32, the level of ASC was firstly increased in presence of 1 mM NaCl and decreased in presence of higher concentration of salinity (10 and 50 mM NaCl). The GSH level was decreased in the presence of higher concentration of NaCl (Fig. 1C). T-44 showed approximately 2.2 fold increase ASC and GSH levels on day 4th of the plant growth in the presence of 200 mM NaCl.

Modulation of ROS Scavenging Enzymes under Salinity Stress

In cv. T-44 (Fig. 2Aa) GST activity increased about 2.5 fold (p<0.05) in presence of 200 mM NaCl with the increasing concentration of salinity on day 4th of plant growth over the respective non-saline control. In contrast, in cv. SML-32, GST activity decreased with increasing salinity. Approximately 3.5 fold decrease in GST activity was observed on day 4 (peak activity) than the non-saline control. The GST activity in roots of both the cvs. was also investigated and found to be similar to that of shoots of the respective cultivars regardless of salinity (data not shown).

The H_2O_2 content significantly varied between cvs. T-44 and SML-32. Therefore, the activities of major H_2O_2 scavenging enzymes, GPX and CAT were determined in both the cvs. under salinity stress (Fig. 2B and D). CAT activity was significantly higher in T-44 in non-saline control as well as in the presence of different concentrations of salt as compared to SML-32. In T-44, CAT activity increased approximately 5.4 fold in presence of 200 mM NaCl on day 4th of plant growth, while in SML-32, it decreased about 2.0 fold in presence of 50 mM NaCl. Thus, the enzymatic detoxification of H_2O_2 seems to be highly efficient in cv. T-44.

Salt-induced alteration in the specific activities of various enzymes involved in ASC-GSH cycle, in cvs. T-44 and SML-32 (Fig. 2C and E-H) were also determined. The SOD activity in cv. SML-32 was about ten times more than that of cv. T-44 (Fig. 2F). However, SOD activity in cv. T-44 was increased approximately 2.5 fold in presence of 200 mM NaCl on day 5th of the plant growth while in cv. SML-32, the SOD activity was decreased concurrently up to 1.3 fold in presence of 50 mM (maximum salt stress) on day 5th of plant growth, but still the value was four times higher than that of cv. T-44 (Fig. 2F). The activities of other enzymes in the ASC-GSH cycle, viz., APX (Fig. 2E), DHAR (Fig. 2G), MDAR (Fig. 2H) and GR (Fig. 2C), increased with increasing levels of salinity stress in cvs. T-44 and SML-32.

Discussion

Salinity is one of the most important abiotic stresses, which directly affects crop productivity. Unlike drought, salinity stress is an intricate phenomenon, which included osmotic stress, specific ion effect, nutrient deficiency, etc., thereby affecting various physiological and biochemical mechanisms associated with plant growth and development (Misra and Dwivedi, 1995; Misra and Gupta, 2005;

Misra and Dwivedi, 2004) in addition to oxidative stress (Hasegawa *et al.*, 2000). Plants have developed various combating mechanisms to cope with the deleterious effect of salinity stress. In the present study, salt stress caused significant H_2O_2 elevation in the shoots of green gram cvs. The accumulation of H_2O_2 has been reported to function as an intercellular signal (Levine *et al.*, 1994) and it stimulates a number of genes and proteins involved in stress responses, such as catalase, peroxidase and alternative oxidase (Prasad *et al.*, 1994; Vanlerberghe and McIntosh, 1996). However, ROS can also be scavenged non-enzymatically by antioxidants like ASC and GSH (Noctor and Foyer, 1992). The present study was based on the interplay of enzymatic and non-enzymatic ROS scavenging mechanisms operational in green gram, cvs. SML-32 (salt sensitive) and T-44 (salt tolerant). This would shed light on the key components employed by the salt-tolerant cv. T-44 to combat salinity induced oxidative stress. This, in turn may lead to the identification of genes for plant transformation to achieve abiotic stress.

Present findings suggest that an increased H_2O_2 generation in the salt-stressed shoots of green gram may be induced by the induction of SOD and it may function in the signaling of oxidative enzymes associated with H_2O_2 scavenging system, particularly an ASC-GSH cycle. Therefore, changes in the activity of antioxidant enzymes in the response to salt stress, causing the generation of ROS, were investigated in details.

Superoxide radicals are toxic by-products of oxidative metabolism. Thus, the dismutation of superoxide radicals into H_2O_2 and O_2 by SOD is necessary to protect the plant tissues from damage. Generally, it has been found that the SOD activity (both constitutive and induced) of salt-tolerant cvs. is higher than the sensitive cvs. It has been shown that salt-tolerant cotton (Gossett *et al.*, 1994), barley (Acar *et al.*, 2001), tomato (Sharata and Tal, 1998) and wild beet (Bor *et al.*, 2003) exhibit higher constitutive and induced levels of SOD as compared to their salt sensitive cvs. while, just opposite trend was observed in salt tolerant rice cv. PK (Vaidyanathan *et al.*, 2003). However, the salt-tolerant green gram cv. T-44 seems to be an exception to this general rule. Dionisio-Sese and Tobita (1998) also reported that the constitutive level of SOD in the salt-tolerant cv. PK was lower than that of the other sensitive rice cvs. Present study also shows that the SOD activity (both constitutive and induced) of cv. T-44 is significantly lower than that of the sensitive cv. SML-32 throughout the plant growth from day 1 to 5. These results suggest a possibility that SOD may induce the overproduction of H_2O_2 to eliminate the toxicity of superoxide radicals in the shoots of green gram plants subjected to salt stress. The low level of SOD activity in the presence of salt might be showed the lesser extent of membrane damage.

Apart from having lower SOD activity, cv. T-44 also exhibited nearly steady state level of H_2O_2 under NaCl stress. This does not mean that cv. T-44 is experiencing low or no oxidative stress, as it showed higher activities of antioxidant enzymes like CAT, GPX, APX, MDAR, DHAR, GR and GST upon exposure to NaCl. This implies that cv. T-44 does not majorly depend on SOD activity for detoxification of O_2^*; it has an alternative, non-enzymatic routes for conversion of O_2^*-to H_2O_2 using antioxidants like GSH and ASC (Noctor and Foyer, 1992). In support of this view, upon exposure to salt, a significant enhancement in the total levels of ASC and GSH in cv. T-44 as compared to cv. SML-32. It has been suggested that the accumulation of H_2O_2 levels caused by various environmental stresses would result in the combined activity of CAT and APX in order to protect plant cells (Mizuno *et al.*, 1998). In the present study, however, salt stress caused higher activities of CAT and APX in salt-tolerant cv. T-44 aid in the rapid detoxification of H_2O_2 thus produced, so that a steady state level of this ROS is maintained even under high salt stress. Under salinity stress, cv. T-44 showed a lower level of lipid peroxidation (TBARS content) and H_2O_2 content than the salt sensitive cv. SML-32. H_2O_2 generated at the intercellular space of the plant subjected to salt stress to diffuse first

into the cytosol, where APX is localized. Cytosolic APX has a higher affinity for H_2O_2 than CAT does (Asada, 1992) and then diffuses into peroxisome, where CAT is typically found. CAT and APX are the major H_2O_2 detoxifying enzymes in plants. Under salinity stress, an increase in the activity of GPX was observed in both the cvs. The CAT activity was significantly higher in cv. T-44 than that of cv. SML-32. This partly accounts for the enhanced H_2O_2 level in the sensitive cv. SML-32 under salt stress. High levels of H_2O_2 can also accelerate processes like Haber-Weiss reaction, resulting in the formation of OH radicals that can cause lipid peroxidation (Loggini *et al.*, 1999). This is reflected in the greater extent of lipid peroxidation in cv. T-44 upon exposure to NaCl. In contrast, in salt sensitive cv. SML-32, a lower level of lipid peroxidation and a steady level of H_2O_2 content under stress. These results suggest that under salt stress sensitive cv. SML-32 suffers mainly from the toxic effects of H_2O_2 (due to lack of efficient H_2O_2 detoxification mechanism).

Plant possesses antioxidant systems in the form of enzymes such as GR, GST, CAT, POD and SOD and other metabolites (Hernandez *et al.*, 1994; Smirnoff, 1993). These antioxidant enzymes and metabolites are reported to increase under various environmental stresses (Hernandez *et al.*, 1993; Gueta-Dahan *et al.*, 1997; Yu and Rengel, 1999). Increased levels of antioxidant enzymes were found in tolerant cvs. than the susceptible ones (Hernandez *et al.*, 1994; Fadzill *et al.*, 1997; Sharata and Tal, 1998; Hernandez *et al.*, 2000; Sairam *et al.*, 2002; Sreenivasulu *et al.*, 2000; Sudhakar *et al.*, 2001), suggesting that higher antioxidant enzymes activity have a role in imparting tolerance to these cultivars against environmental stresses. In this background, the higher GR, GST, CAT, GPX, APX and SOD activity in T-44 under increasing salinity stress, signifies its relative tolerance to salinity stress, while SML-32 was inferior on that account.

An overview of the presented data has suggested that the antioxidant enzymes in salt tolerant cv. T-44 during plant growth under saline stress was found to characterize by high level of CAT, APX, GPX, GR, GST activity and ASC, GSH concomitant with a low level of SOD, MDAR, DHAR activity and H_2O_2 in the presence of higher concentration of NaCl stress. The salt sensitive cv. SML-32 was characterized by just opposite trend to that observed in salt tolerant cv. T-44. It has been suggested that salt tolerant cv. T-44 may have a better protection against ROS by increasing the activity of antioxidant enzymes under salt stress. In conclusion, salt stress may induce more severe oxidative stress in green gram cv. SML 32 than cv. T 44. The protective mechanism of ROS scavenger, which included both antioxidant enzymes and non-enzymes against salt stress in the green gram genotype, could be speculated as follows: (1) Higher levels of antioxidants (enzymes-CAT, APX, GPX, GR, GST and non-enzymes-ASC, GSH) could compensate for lower SOD activity to detoxify O_2^{*-} and thus prevent oxidative damage to plants under stress as exemplified by salt-tolerant cv. T-44. (2) The elucidation of the regulatory mechanism of genes involved in enzymatic and non-enzymatic ROS scavenging components (ASC and GSH) against various environmental stresses and relationship of the genes to these enzymes require further analysis.

Acknowledgements

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