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Brinjal (*Solanum melongena* Linn.) Varieties Accumulate both Na^+ and K^+ under Low NaCl Stress, but Exclude Na^+ and Accumulate K^+ under High Salt Levels

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ABSTRACT

The effect of NaCl stress (0 – 200 mM) was investigated on the accumulation of mineral nutrient and antioxidant enzyme activities in two brinjal varieties 'MEBH 10' (salt sensitive) and 'MHBJ 112' (salt tolerant) that differ in their salt sensitivity. NaCl stress resulted in an increase in sodium (2.55- and 3.10-fold), potassium (1.95- and 2.65-fold) and calcium (1.55- and 1.48-fold more) content in the seedlings of 'MEBH 10' and 'MHBJ 112' up to 100 mM NaCl level over control, respectively. The magnitude of accumulation of Na^+ and K^+ ions was more in salt tolerant variety 'MHBJ 112' as compared to salt sensitive variety 'MEBH 10'. Both the lines maintained significantly lower Na^+/K^+ but not Na^+/Ca^{2+} ratios. Under high salt stress, brinjal varieties excluded Na^+ and accumulated K^+. Catalase (CAT) activity increased with increasing NaCl level in both varieties. About 225% more CAT activity in 'MEBH 10' and 249% more in 'MHBJ 112' was recorded at 200 mM NaCl as compared with the respective control. The activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) increased up to 100 mM NaCl but decreased at higher concentrations (150 – 200 mM) of NaCl. About 124% and 291% increase in SOD activity in 'MEBH 10' and 'MHBJ 112', respectively were recorded at 100 mM NaCl. Similarly, a 124% increase in APX activity in 'MEBH 10', 118% in 'MHBJ 112' and 175% and 168% increase in GPX activity was recorded in 'MEBH 10' and 'MHBJ 112', respectively at 100 mM NaCl. Thus the mechanism of high salt tolerance in brinjal appears to be reduced Na^+, increased K^+ and by maintaining higher activity of antioxidant enzymes.

Keywords: antioxidative enzymes, salt stress, sodium exclusion, *Solanum melongena*
Abbreviations: APX, ascorbate peroxidase; **CAT**, catalase; **DMRT**, Duncan's multiple range test; **EDTA**, ethylenediaminetetraacetic acid; **GPX**, guaiacol peroxidase; **H_2O_2**, hydrogen peroxide; **NBT**, nitroblue tetrazolium chloride; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase

INTRODUCTION

Soil salinity is one of the most serious abiotic stresses which influences crop productivity and induces water deficit even in well-watered soils by decreasing the osmotic potential of soil solutes (Seckin *et al*. 2009). Soil salinity affects plants through osmotic effects, ion-specific effects, and oxidative stress (Pitman and Lauchli 2002). High salt levels can influence the balance of other ions within cells, leading to ion deficiencies (Marschner 1995). NaCl stress while increases the concentrations of Na^+ and Cl^-, decreases in the concentrations of K^+ and Ca^+ (Mansour *et al*. 2005). Excess Na^+ concentration exert ion cytotoxicity with varying levels according to the salt tolerance capacity of plants (Diédhiou and Golldack 2006). Na^+ and Cl^- limit the absorption of other ions and nutrients which result in nutrient imbalance, leading to inhibition of plant growth. Na^+ replaces K^+ due to physicochemical similarities, which may lead to ion cytotoxicity in many biochemical reactions (Kumar *et al*. 2008). The conformational changes and loss of functions of proteins may also be evidenced which consequently lead to ion cytotoxicity as Na^+ and Cl^- ions penetrate the hydration shells and interfere with the non-covalent interactions between their amino acids (Chinnusamy *et al*. 2005). During salinity, plant adaptations are of three distinct types: osmotic stress tolerance, Na^+ or Cl^- exclusion, and the tolerance of tissue to accumulated Na^+ or Cl^- (Munns and Tester 2008).

One of the biochemical changes that occurs when plants are subjected to salt stress is the production of reactive oxygen species (ROS) such as the superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\bullet$). ROS can have a detrimental effect on normal metabolism through oxidative damage to lipids, proteins, and nucleic acids (Mittler 2002). Most plants react to environmental stresses with an effective ROS scavenging system involving antioxidant molecules like carotenoids, ascorbate, glutathione, and tocopherols as well as antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) (Greenway and Munns 1980; O'Neill 1983). Recent studies have demonstrated that the activities of these antioxidant enzymes and the levels of antioxidant molecules increase and are corelatable to various environmental stresses (Hernández *et al*. 2000; Sekmen *et al*. 2007). Such a correlation was observed between NaCl induced salt stress tolerance and antioxidative responses in different plant systems such as rice (Vaidyanathan *et al*. 2003; Benavente *et al*. 2004), *Sorghum* species (Jogeswar *et al*. 2006) and *Sesuvium portulacastrum* (Lokhande *et al*. 2010, 2011).

Brinjal (*Solanum melongena* Linn.), is an important vegetable crop plant cultivated throughout India. It is popular amongst small-scale farmers and low income consumers due to which it is often described as poor man's vegetable. The literature that exists on eggplant tolerance to soil salinity is contradictory; some classified as a moderately

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sensitive (Heuer *et al*. 1986; Savvas and Lenz 1996), whereas others (Unlukara *et al*. 2010) reported that it is sensitive to water stress caused by salinity. Chinnusamy *et al*. (2005) described the threshold level of brinjal for salinity as 1.1 dS m^{-1}. Akinci *et al*. (2004) also reported that the eggplant is affected negatively by increasing salt at the germination and seedling stages. Meager information is available on the response of NaCl induced salinity stress on antioxidant enzyme activities in brinjal. Recently, we assessed the differential response of brinjal varieties to NaCl stress in terms of physiological and biochemical parameters including seed germination and growth, chlorophyll contents, lipid peroxidation, proline, glycine betaine and total soluble sugars (Ahire and Nikam 2011). In the present investigation, efforts were made to study the changes in mineral nutrients and antioxidant enzyme activities in salt tolerant and sensitive brinjal varieties.

MATERIALS AND METHODS

Plant material, salinity treatment and culture conditions

Seeds of salt sensitive 'MEBH 10' and tolerant variety 'MHBJ 112' that were previously tested in our laboratory (Ahire and Nikam 2011) were purchased from the Market Yard, Pune (Maharashtra, India) and used for the experimentation. Seeds of both varieties were surface sterilized with 0.1% (w/v) mercuric chloride for 2 min and then washed five times with sterile distilled water. Twenty five seeds of each variety were sown in a sterile Petri dish (10 cm diameter, Axygen, India) containing two layers of germination paper (1 mm thick, Modern Paper Ltd., India). Initially, the germination paper was moistened with 10 ml of distilled water considered as control and different concentrations of NaCl solutions (i.e. 50, 100, 150 and 200 mM). Every day, 2 ml of NaCl solutions (treatment) and distilled water (control) was applied to respective Petri dishes and the observations were recorded on the 14^{th} day after sowing. The Petri dishes were maintained at room temperature in the dark.

Determination of mineral nutrients

The seedlings were washed with distilled water to remove surface contaminants and soaked on tissue paper followed by drying at 60°C for 48 h in an oven. The dried seedlings were ground to powder and 200 mg powder from each treatment in triplicate was submerged in 10 ml of 35% (v/v) HNO_3 (Qualigens, Mumbai, India) overnight at room temperature followed by acid digestion at 100°C still the acid was evaporated and finally the residue was dissolved in 30 ml of distilled water. Then the samples were filtered using Whatman filter paper No. 1 (Whatman International Ltd., Maidstone, England). Minerals such as sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) were measured by atomic absorption spectrophotometer (AA-7000, Labindia Analytical Instruments Pvt. Ltd., Mumbai, India). The standard solutions of minerals (Na^+, K^+ and Ca^{2+}) were purchased from Qualigens and were used for quantification of mineral content in the samples.

Antioxidant enzyme assays

1. Extraction

The treated and control fresh samples (500 mg) were homogenized in 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA (Hi Media, Mumbai, India) and 1% (w/v) polyvinylpyrrolidone (PVP 40000; Hi Media) with chilled mortar and pestle. The homogenate was filtered with single layered cheese cloth and centrifuged at 10,000 rpm for 20 min at 4°C. An appropriate aliquot/dilution of the supernatant was used as a crude enzyme(s) for determination of antioxidant enzyme activities. Soluble protein content in the enzyme extract was determined according to Lowry *et al*. (1951) using bovine serum albumin (BSA; Merck, Mumbai, India) as a standard.

2. SOD assay

Total superoxide dismutase (SOD) enzyme (EC 1.15.1.1) activity was assayed according to Becana *et al*. (1986) by inhibition of the photochemical reduction of nitroblue tetrazolium chloride (NBT; Hi Media). The reaction mixture (1 ml) containing 50 mM phosphate buffer (pH 7.0) and 0.1 mM EDTA to which an oxygen-generating system containing 14.3 mM methionine, 82.5 µM NBT, and 2.2 µM riboflavin (all from Hi Media), prepared freshly *in situ*, was added. The reaction was initiated by adding 25 µl of crude enzyme. The entire system was kept 30 cm below the light source (six 15 W fluorescent tube light; Philips, Kolkata, India) for 30 min. The reaction was stopped by switching off the tube light. For light blank, all the reactants without enzyme extract was incubated in light as for the samples, whereas all the reactants along with 25 µL enzyme extract were incubated in dark for dark blank. The reduction in NBT was measured by monitoring the change in absorbance at 560 nm (UV 1800, Shimadzu, Japan). The readings of light blank were used in calculation of enzyme units. One unit of SOD enzyme was defined as the amount that produces 50% inhibition of NBT reduction under the assay conditions and expressed as units of SOD activity mg^{-1} protein.

3. CAT assay

Catalase (CAT) enzyme (EC 1.11.1.6) activity was measured by following the decomposition of hydrogen peroxide (H_2O_2) as described by Cakmak and Marschner (1992) with minor modifications. The activity was measured in a reaction mixture (1 ml) containing 50 mM phosphate buffer (pH 7.0) and 300 mM H_2O_2 (Qualigens). The reaction was initiated by adding 50 µl enzyme extract and the activity was determined as a result of H_2O_2 decomposition by monitoring the decrease in absorbance at 240 nm (ε = 36 mM^{-1} cm^{-1}) for 2 min at an interval of 15 s. The slope of readings between the time interval considered as ΔA and the enzyme activity was expressed as µ Kat of CAT activity mg^{-1} protein.

4. APX assay

Ascorbate peroxidase (APX) enzyme (EC 1.11.1.11) activity was determined according to Nakano and Asada (1981). The reaction mixture (1 ml) contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate and 0.1 mM H_2O_2. The reaction was started by adding 50 µl of crude enzyme. Ascorbate oxidation was monitored for 1 min by measuring the decrease in absorbance at 290 nm at every 15 s (ε = 2.8 mM^{-1} cm^{-1}). The enzyme activity was expressed as µKat of APX activity mg^{-1} protein.

5. GPX assay

Guaiacol peroxidase (GPX) enzyme (EC 1.11.1.7) activity was assayed according to Hemeda and Klein (1990). The reaction mixture (1 ml) contained 50 mM phosphate buffer (pH 7.0), guaiacol (Hi Media), 200 mM H_2O_2 and 10 µl enzyme extract. The reaction was started by adding 200 mM H_2O_2. The increase in absorbance due to oxidation of guaiacol (ε = 26.6 mM^{-1} cm^{-1}) was monitored at 470 nm. Enzyme activity was expressed as µKat mg^{-1} protein.

Statistical analysis

Each Petri dish was considered as a replicate and all treatments were repeated three times; data are expressed as mean ± standard error (SE). Data were analyzed by analysis of variance (ANOVA) to detect significant differences between means. Means differing significantly were compared using Duncan's multiple range test (DMRT) at the 5% probability level using software SPSS version 9.0.

RESULTS

Two brinjal varieties differing in their salinity tolerance levels were exposed in the present study to find out their mechanisms of tolerance. In the present investigation, Na^+ content increased in both the salt sensitive and tolerant varieties as salinity increased from 0 to 100 mM. The magni-

Table 1 Effect of different concentrations of NaCl stress on Na^+, K^+ and Ca^{2+} ion concentration in seedlings of brinjal varieties.

NaCl (mM)	Na^+ (mmol g^{-1} DW)		K^+ (mmol g^{-1} DW)		Ca^{2+} (mmol g^{-1} DW)	
	MEBH 10	**MHBJ 112**	**MEBH 10**	**MHBJ 112**	**MEBH 10**	**MHBJ 112**
Control	0.89 ± 0.01 e	0.98 ± 0.02 e	03.74 ± 0.02 e	05.62 ± 0.03 e	1.60 ± 0.01 d	1.75 ± 0.02 d
50	2.06 ± 0.01 b	2.55 ± 0.01 b	07.31 ± 0.03 b	09.87 ± 0.04 b	1.95 ± 0.02 b	2.43 ± 0.02 b
100	2.30 ± 0.02 a	3.03 ± 0.02 a	10.97 ± 0.05 a	14.80 ± 0.04 a	2.53 ± 0.01 a	2.58 ± 0.01 a
150	1.12 ± 0.01 c	1.38 ± 0.02 c	06.44 ± 0.01 c	08.36 ± 0.02 c	1.73 ± 0.01 c	1.80 ± 0.02 c
200	0.98 ± 0.02 d	1.24 ± 0.01 d	05.64 ± 0.02 d	07.05 ± 0.03 d	0.55 ± 0.02 e	0.58 ± 0.02 e

Each value represents mean ± SE of three replications. Means followed by same letters within columns are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test. DMRT was applied to each variety separately.

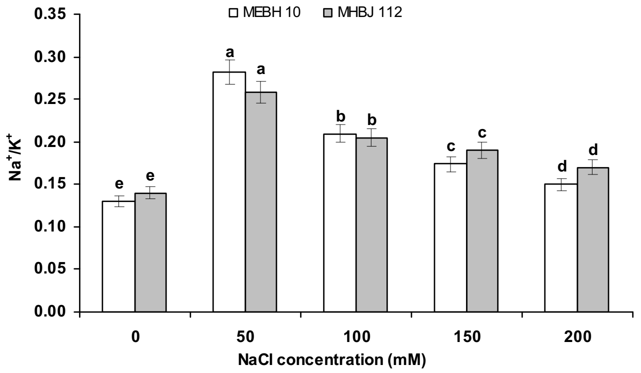

Fig. 1 Effect of different concentrations of NaCl stress on Na^+/K^+ ratio in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

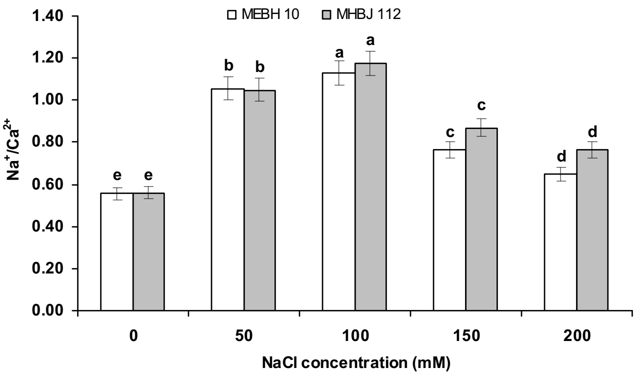

Fig. 2 Effect of different concentrations of NaCl stress on Na^+/Ca^{2+} ratio in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

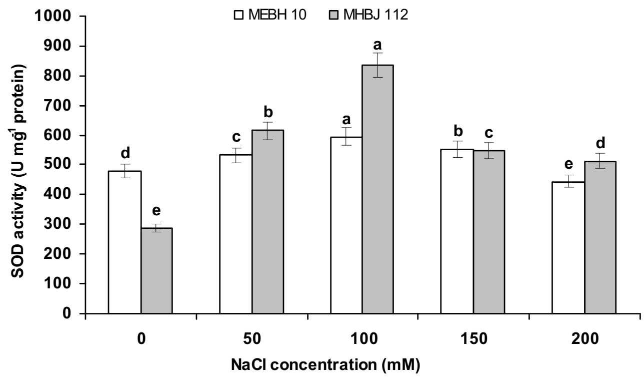

Fig. 3 Effect of different concentrations of NaCl stress on SOD activity in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

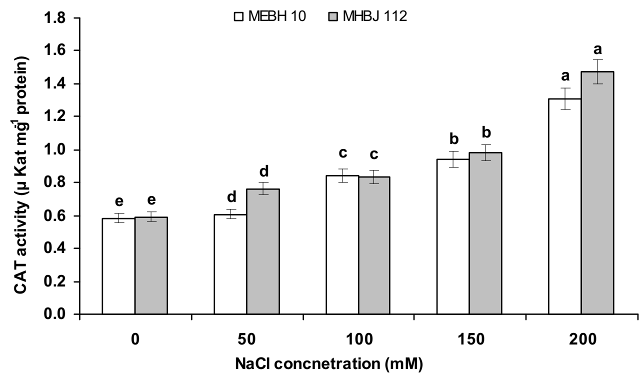

Fig. 4 Effect of different concentrations of NaCl stress on CAT activity in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

tude of increase in Na^+ content was higher in salt-tolerant 'MHBJ 112' than in salt-sensitive 'MEBH 10'. The increase in Na^+ content was observed up to 100 mM NaCl. But the behavior of these two lines differed at higher NaCl levels. Higher NaCl concentration resulted in decreased Na^+ content in seedlings of both varieties. On the other hand, higher accumulation of K^+ was noticed in a tolerant variety than in the susceptible one. Also, the quantity of Ca^{2+} was marginally higher in the tolerant than in the susceptible line (**Table 1**). Under low (50 and 100 mM) or higher (150 and 200 mM) NaCl stress the ratios of Na^+/K^+ was lower irrespective of the variety (**Fig. 1**). The Na^+/Ca^{2+} ratios were lower without stress, but under stress conditions, the ratios were higher regardless of the brinjal line (**Fig. 2**).

Activity of SOD increased with an increase in NaCl level up to 100 mM especially in the tolerant variety. At higher NaCl concentration, SOD activity decreased in both the varieties (**Fig. 3**). The SOD activity in control as well as in stressed seedlings of 'MHBJ 112' was significantly higher than those of 'MEBH 10' (**Fig. 3**). In contrast, increasing concentrations of NaCl increased the activity of CAT, more so in the tolerant line (**Fig. 4**). Specific activity of APX also increased like SOD until 100 mM NaCl level, but declined later. However, the activity was higher in the tolerant variety, 'MHBJ 112' (**Fig. 5**). More or less, an identical activity was also noticed in GPX in both cultivars and at all NaCl concentrations tested (**Fig. 6**).

DISCUSSION

Salt stress is associated with complex traits, which include osmotic stress, specific ion effect, ion imbalances and nutrient deficiency, especially potassium. Therefore, salt stress affects various physiological and biochemical mechanisms related to plant growth and development (Pitman and Lauchli 2002). Elevated NaCl causes an increase in Na^+ concentration and a decrease in K^+ and Ca^{2+} concentrations as reported earlier by Chartzoulakis and Loupassaki (1997) and Munns *et al*. (2002). Besides, accumulation of Na^+ ions changes ion balance ratio such as Na^+/Ca^{2+} and Na^+/K^+ in plants under saline conditions. In the present study, lower concentration of NaCl (up to 100 mM) increased Na^+ accumulation in both the brinjal varieties though the accumulation was higher in the tolerant line. This indicates that Na^+ accumulation may help the plants in balancing osmotic

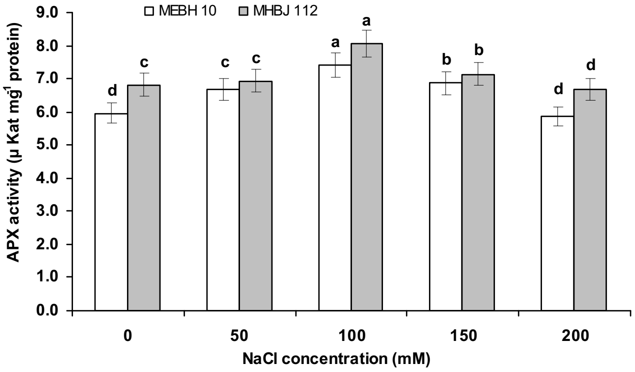

Fig. 5 Effect of different concentrations of NaCl stress on APX activity in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

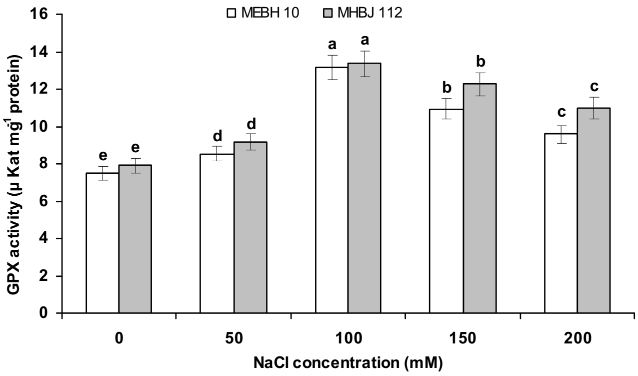

Fig. 6 Effect of different concentrations of NaCl stress on GPX activity in brinjal varieties. Each value represents mean of three replications and vertical bars indicate SE. Data are statistically significant at $P < 0.05$. DMRT was applied to each variety separately.

potential at lower salt stress levels. Increase in Na^+ content in leaf tissues of brinjal was reported by Chartzoulakis and Loupassaki (1997) and Unlukara *et al*. (2010) with increasing salinity in the medium. Such an increase in Na^+ content was also noticed in other plants such as rice (Kumar *et al*. 2008). Salinity induced increase in Na^+ and depletion of K^+ have been reported previously in a number of crops including brinjal (Yasar *et al*. 2006; Ulunkara *et al*. 2010), rice (Nguyen *et al*. 2005; Kumar *et al*. 2008), mungbean (Zayed and Zeid 1998) and wheat (Sairam *et al*. 2002). The accumulation of Na^+ and concomitant decrease in K^+ levels appears to be one of the general characteristics of salt susceptible plant species (Lokhande *et al*. 2011). Yasar *et al*. (2006) reported earlier a decrease in K^+ concentration in the sensitive callus than the tolerant line of brinjal. Contrary to the above, our results portrayed a different scenario here. K^+ accumulated in both the varieties, but the accumulation was higher in the tolerant variety than in the susceptible (**Table 1**). When the salt concentration in the medium increased, both Na^+ and K^+ concentrations increased in the seedlings till 100 mM NaCl stress. At higher NaCl stress (150 and 200 mM), Na^+ content decreased surprisingly while the accumulation of K^+ is still higher. Na^+/K^+ ratio was less in both 'MHBJ 112' and 'MEBH 10' varieties. Higher K^+/Na^+ ratios have been reported in some salt tolerant plants (Liu *et al*. 2012). This may be ideal for ion homeostasis under salt stress conditions. Thus, it appears that brinjal is able to exclude Na^+ if the cellular concentrations are higher, and accumulate K^+ to counter the ion imbalance. Accumulation of Ca^{2+} ion also followed an identical pattern in both the varieties. Ulunkara *et al*. (2010) reported an increase in Ca^{2+} content with increasing NaCl concentration in brinjal. But Yasar *et al*. (2006) reported more decrease in Ca^{2+} concentration in salt sensitive than the tolerant callus line of brinjal. Such a distinct accumulation pattern was not observed in the present study. Low Na^+/K^+ or high K^+/Na^+ and lower Na^+/Ca^{2+} ion ratios were reported to be associated with the relatively salt tolerant lines in many species (Dvorak *et al*. 1994; Pérez-Alfocea *et al*. 1996). A high K^+/Na^+ ratio in the cytosol is essential for normal cellular functions of plants. Na^+ competes with K^+ uptake and may block the K^+ specific transporters or binding sites under salinity. This results in more accumulation of toxic ions such as Na^+, but less K^+ concentration which is necessary for enzymatic reactions and osmotic adjustment (Zhu 2003). In previous studies, it was observed that tolerant lines regulated the osmotic potential more effectively by avoiding the uptake of Na^+ and Cl^- and a simultaneous absorption of more essential ions like K^+ (Shanon and Noble 1995; Sivritepe *et al*. 2003). The Na^+/K^+ ratio was low in control as well as in NaCl treated lines irrespective of the varieties (**Fig. 1**). A similar increase in Na^+/K^+ ratio was recorded in *Vigna radiata* (Sumithra *et al*. 2006). On the other hand, Na^+/Ca^{2+} ratio was low in control (untreated) seedlings of both varieties. But the Na^+/Ca^{2+} ratio remained higher in salt treated seedlings in both the lines (**Fig. 2**). As has been seen in other species, in this study also, Na^+/K^+ and Na^+/Ca^{2+} ratios appeared to determine salinity tolerance in brinjal. Similarly, reducing Na^+/Ca^{2+} ratio under saline condition were recorded in tomato (Al-Harbi 1995). Levitt (1980) reported that a high Na^+/Ca^+ ratio results in increased cell permeability. Our results are in agreement with that of Akinci *et al*. (2004) who also reported higher Na^+/Ca^{2+} ratio in brinjal during germination. Such a ratio was also reported in other species (Abdel-Rahman 1983; Hasegawa *et al*. 1986).

Salt stress leads to oxidative stress through an increase in reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$) and hydroxyl ($OH^{\bullet}$) radicals. ROS can alter normal cellular metabolism through oxidative damage to lipids, proteins and nucleic acids (Imlay 2003). To alleviate the oxidative damage initiated by ROS, plants have developed defensive antioxidative system, including low-molecular mass antioxidants as well as antioxidative enzymes such as SOD, CAT, APX, GPX and GR. In the present study, higher SOD activity was noticed in the salt tolerant line compared to the susceptible one (**Fig. 3**). An about 124 and 291% increase in SOD activity in 'MEBH 10' and 'MHBJ 112', respectively was recorded at 100 mM NaCl. SOD decreases the formation of superoxide radical and may cause severe damage to membranes, proteins and DNA (Neto *et al*. 2006). Similarly, Wei *et al*. (2009) reported the increase in SOD activity in grafted and non-grafted seedlings of brinjal upon exposure to excess calcium nitrate stress. Similar results have been reported by Neto *et al*. (2006), and they observed a more pronounced reduction in the activity of SOD in a salt-sensitive maize cultivar than in a salt-tolerant one. The excess H_2O_2 produced by SOD in response to salt stress is taken care by both CAT and APX. Catalases have been mainly associated with the removal of H_2O_2 in microbodies (Scandalios 1997) and catalyze either the direct decomposition of H_2O_2 or the oxidation by H_2O_2 of substrates. In the present study, CAT activity also increased, more so in the tolerant variety, with increasing concentrations of NaCl (**Fig. 4**). About 225% more CAT activity in variety 'MEBH 10' and 249% more in variety 'MHBJ 112' was recorded at 200 mM NaCl as compared with respective control. Similar results were documented by Kumar *et al*. (2009) in rice seedlings, where higher CAT activity was recorded in the seedlings of salt tolerant cultivar 'Panvel-3' than that of salt sensitive cultivar 'Karjat-3' and an intermediate activity was noticed in cultivar 'Kalarata', a moderately tolerant cultivar. In contrast to this, Jogeswar *et al*. (2006) reported a reduction in CAT activity with an increase in salt concentration in sorghum. Lokhande *et al*. (2011) also reported a decline in the activity of CAT with an increase in salt stress in the facultative halophyte *Sesuvium portulacastrum*. It is known that the enzymes of the ascorbate-glutathione cycle are involved in the removal of H_2O_2 (Noctor and Foyer 1998). APX requires a reductant (ascorbate) and has a higher affinity for H_2O_2 allowing for the scavenging of small amounts of H_2O_2 in more specific locations (Dat *et al*. 2000). In the present investigation, APX activity increased till 100 mM NaCl level in both the varieties. Further, higher APX activity was recorded in the salt tolerant variety 'MHBJ 112' at 100 mM NaCl (**Fig. 5**). Approximately, 124% increase in AXP activity in 'MEBH 10', 118% in 'MHBJ 112' was recorded in variety 'MEBH 10' and 'MHBJ 112' respectively at 100 mM NaCl (**Fig. 5**). Similar observations were recorded in grafted and non-grafted seedlings of brinjal when exposed to the excess calcium nitrate stress (Wei *et al*. 2009). Likewise, elevation in the APX activity was recorded by Vaidyanathan *et al*. (2003) in rice seedlings exposed to salt stress. The increased APX activity in the present investigation could be due to the activation of pre-existing APX or due to the synthesis of APX upon salt exposure as also has been opined by Parida *et al*. (2004). GPX is characterized by their broad specificity with respect to an electron donor, and both guaiacol and pyrogallol have been used as electron donors in assays of their activity. This type of peroxidase participates in a great number of physiological processes, such as the biosynthesis of lignin (Halbrock and Grisebach 1979), and plant development and organogenesis via the degradation of IAA (O'Neil and Scot 1987) or the biosynthesis of ethylene. SOD generates H_2O_2 which is eliminated by GPX (Rios-Gonzalez *et al*. 2002). In the present investigation, GPX activity increased by 2-fold with an increase in salt concentration in both the varieties up to 100 mM NaCl level over that of the untreated seedlings. The overall increase in GPX activity was marginally higher in the salt tolerant variety 'MHBJ 112' compared to 'MEBH 10' (**Fig. 6**). About a 175% and 168% increase in GPX activity was recorded in 'MEBH 10' and 'MHBJ 112' respectively at 100 mM NaCl (**Fig. 6**). Similar results were observed in maize and sunflower seedlings when exposed to salt treatment (Rios-Gonzalez *et al*. 2002). The present study indicates that enhancement in the activity of GPX (**Fig. 6**) may serve as an intrinsic defiance tool to resist NaCl-induced salt stress (Csiszár *et al*. 2004). Such an increase in GPX was also recorded by Vaidyanathan *et al*. (2003) in salt-tolerant as well as in salt-sensitive cultivars of rice when exposed to salinity.

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