Chapter: 5

DISCUSSION
Plants are frequently exposed to stress factors, which are defined as external conditions that adversely affect growth, development, or productivity. Response to stress may occur within few seconds (e.g., a change in the phosphorylation status of protein), within few minutes and hours (e.g., a change in gene expression) or several days (e.g., alterations in cellular ultrastructure). Salt stress affects many aspects of plant metabolism and, as a result, growth and yields are reduced (Pasternak et al., 1995). Salinity stress is known to elicit complex effects on plant metabolism resulting from ion toxicity, water deficit, and nutrient imbalances (Pasternak, 1987; Werner and Finkelstein, 1995).

In the present investigation seedlings of *Zea mays* cultured in Hoagland’s nutrient solution supplemented with different concentrations of NaCl showed markedly reduced shoot length, reduced RWC, decreased moisture content and a
correspondingly higher % dry matter content. Thus, seedlings which were growing in nutrient solution supplemented with 250 mM NaCl showed an almost 2 fold reduction in shoot length compared to the untreated controls. These results are in agreement with similar observations made on lentil (Ashraf and Waheed, 1990), *Hordeum* spp. (Mano and Takeda, 1998), *Zea mays* (Çiček and Cakirlar, 2003), *Phaseolus* spp. (Bayuelo-Jimenez et al., 2002; Stoeva and Kaymakanova, 2008) who have also demonstrated the inhibitory effects of salt stress on shoot length, moisture content and RWC. Kuhad *et al.* (1987), Greenway and Munns (1980) and Shanon and Nobel (1995) have ascribed the reduction in shoot length to delayed seed germination as a consequence of salt stress in pearl millet and tomato. In the present investigation the seedlings were of same age at the time of treatment. Hence the reduction in shoot length cannot be ascribed to delayed seed germination. The increased % dry matter content of seedlings cultured in nutrient solution supplemented with different concentrations of sodium chloride could be ascribed to the increased loss of moisture from these seedlings as a consequence of more negative water potential of the ambient nutrient solution. Water status of a plant is known to be highly sensitive to salinity and therefore is dominant in determining the plant responses (Stepien and Klobus, 2006). The loss of cellular water in salt stressed tissues, as observed in the present investigation, would lead to a significant decrease in the water potential of the plants and consequently their ability to maintain turgor. Even though both the varieties of maize viz. Gujarat Makki and RCM1-1, showed the inhibitory effects of NaCl on growth and moisture retention capacity the reduction in moisture content in the variety RCM1-1 was much less marked compared to that in
Gujarat Makki. Results of the present investigation clearly demonstrate the ameliorative effects of \( \text{CaCl}_2 \) and ABA on the salt stress induced suppression of growth in the seedlings. Similar observations have been made by Akinci and Simsek (2004) in cucumber. ABA treatment has been reported to enhance draught tolerance, as evidence by higher RWC and lower electrolyte leakage, in tall fescue (Jiang and Huang, 2002). Similar observations have also been reported for maize by Jiang and Zhang (2003).

While the seedlings of *Zea mays* var. Gujarat Makki cultured in nutrient solution supplemented with 250 mM NaCl showed almost 40% reduction in the total chlorophyll content, those of the variety RCM1-1 registered only a marginal decrease in the tissue content of total chlorophyll. The differential responses of the two varieties to sodium chloride, as reflected in differences in changes in the tissue content of total chlorophyll, indicate the differences in the tolerance levels of the two varieties to salt stress. Reduction in the tissue content of total chlorophyll in plants under salt stress have been reported in sorghum (Netondo *et al.*, 2004), rice (Baek *et al.*, 2006) and sunflower (Turhan *et al.*, 2008). Notendo *et al.* (2004) have reported almost 58% decrease in the content of chlorophyll a and 68% decrease in the content of chlorophyll b in seedlings of cultured under 250 mM NaCl. In the present investigation seedlings of *Zea mays* var. Gujarat Makki cultured in nutrient solution containing 250 mM NaCl showed almost 40% reduction in the total chlorophyll content. Reddy and Vora (1986) have ascribed the reduction in chlorophyll to either the inhibitory effects of salinity on chlorophyll synthesis or the acceleration of
chlorophyll degradation as a response of plants to salinity. Result of the present investigation showed that Ca\(^{2+}\) and ABA prevented the salt stress induced reduction in the total chlorophyll content of maize seedlings. Agarwal et al. (2004) have made similar observations about the effect of ABA on chlorophyll content of salt stressed leaves.

Seedlings of both varieties of maize cultured in Hoagland’s nutrient solution supplemented with different concentrations of sodium chloride showed markedly higher electrolyte leakage and higher cytosolic Na\(^+\) than the untreated controls. The magnitude of increase in electrolyte leakage and cytosolic Na\(^+\) increased with increasing concentration of NaCl. Similar observations about increased electrolyte leakage as a response to salt stress have been made for fox-tail millet (Sreenivasulu et al., 1999), mulberry (Sudhakar et al., 2001), lentil (Bandeoglu et al., 2004) and rice (Baek et al., 2006). The increased electrolyte leakage could be due to loss of membrane permeability as a consequence of free radical induced membrane lipid peroxidation. Baek et al. (2006) have suggested that changes in solute leakage could be used as indicators for determining membrane integrity and permeability during salt stress. Orcutt and Nilsen (2000) have suggested that cell membrane function may be also compromised as a result of Na\(^+\) replacing Ca\(^{2+}\), resulting in increased cell leakiness.

Our results on the effects of increased NaCl concentration in the ambient medium clearly identify enhanced uptake of Na\(^+\) as one of the early responses of the seedlings to salt stress. Increased ion accumulation in plant cells has been reported as an apparent consequence of salt stress (Hayward and Wadleigh, 1949; Grillot,
1954; Gorham et al., 1985; Shabala et al., 1998; Schachtman and Munns, 1992; Hasegawa et al., 2000; Neel et al., 2002; Tester and Davenport, 2003). Increase in cellular levels of Na\(^+\) as a response to salt stress has also been reported for other genotypes of maize (Alberico and Cramer, 1993; Erdei and Taleisnik, 1993; Azevedo-Neto and Tabosa, 2000). Alberico and Cramer (1993) have suggested that salt tolerance in maize was not related to shoot Na\(^+\) content but to the capacity of the cells to compartmentalize the ions in the vacuole thereby maintaining low Na\(^+\) content in the cytoplasm. In the present investigation, seedlings of both varieties of maize cultured in Hoagland’s nutrient solution containing different concentrations of sodium chloride showed markedly higher levels of cytosolic sodium than the untreated controls. However, the level of cytosolic sodium was significantly lower in var. RCM1-1 than the variety Gujarat Makki. RCM1-1 also showed lesser reduction in the content of total chlorophyll. While Azevedo-Neto et al. (2004) have indicated that leaf Na\(^+\) content or leaf soluble organic solute content had no relation with salt tolerance in maize, our results indicate that leaf cytosolic Na\(^+\) levels can be used as physiological markers for salt stress in maize.

The content of cytosolic Na\(^+\), H\(_2\)O\(_2\) levels and electrolyte leakage in shoots tissue of both varieties of maize viz. Gujarat Makki and RCM1-1 showed a marked decline in the presence of 10 mM CaCl\(_2\) in the nutrient solution. Although several studies have reported no effect of Ca\(^{2+}\) variation in the millimolar range on shoot Na\(^+\) uptake (Aslam et al., 2003; Baba and Fujiyama, 2003; Yeo and Flowers, 1985), a reduction in shoot Na\(^+\) has been observed in others (Song and Fujiyama, 1996; Anil et al., 2005). Our results on the effects of external calcium on the levels of cytosolic
sodium in salt stressed plants are in agreement with similar observations on rice 
(Shah et al., 2003), wheat (Hussain et al., 2004), rice (Anil et al., 2005) and cotton 
(Cramer, 1986, 1997). Shah et al. (2003) have attributed the protective effects of 
Ca\(^{2+}\) on growth and Na\(^+\) exclusion in salt stressed rice seedlings. They have 
concluded that besides its role in overcoming the Na\(^+\) toxicity supplemental Ca\(^{2+}\) 
mitigated the osmotic components of salt stress by enhancing proline accumulation 
and also helped the salt stressed cells to maintain not ion homeostasis. Hussain et al. 
(2004) have attributed the inhibitory effect of external calcium on Na\(^+\) uptake to a 
regulation of xylem loading transporters in the plants. Cramer et al. (1987) and Liu 
and Zhu (1998) have suggested that externally supplied Ca\(^{2+}\) reduced the toxic 
effects of NaCl, presumably by facilitating higher K\(^+\)/Na\(^+\) selectivity. The tight 
Na\(^+\)/Ca\(^{2+}\) interaction has been ascribed to similar crystal ionic radii of the two ions 
(Allen et al., 1994; Cramer, 2002). While Ca\(^{2+}\) has crystal ionic radius of 0.099 nm, 
sodium ions have a crystal ionic radius of 0.097 nm. Zid and Grignon (1985), 
Grignon and Senetenac (1991) and Munns (2005) have attributed the Na\(^+\)/Ca\(^{2+}\) 
interactions to competition between Na\(^+\) and Ca\(^{2+}\) for negatively charged binding 
sites that have a high specificity for Ca\(^{2+}\).

Seedlings incubated in Hoagland’s nutrient solution lacking any additional 
supplements did not show any significant change in tissue level of free cytosolic K\(^+\) 
during the 144 hours of incubation. Similarly seedlings maintained over Hoagland’s 
nutrient solution containing either 10 mM CaCl\(_2\) or 100 µM ABA also did not show 
any significant change in tissue level of free cytosolic K\(^+\) during the 144 hours of 
incubation. On the other hand seedlings incubated in nutrient solution supplemented
with 200 mM NaCl showed a marked decrease in the cytosolic K⁺ levels during the 144 hours incubation period. The increase in cytosolic K⁺ in salt stressed seedlings affected the Na⁺/K⁺ ratio of the seedlings. There was no marked change in the Na⁺/K⁺ ratio in the shoot tissues of seedlings cultured in native Hoagland nutrient solution during the 144 hours of incubation. However, seedlings incubated in nutrient solution containing 200 mM sodium chloride showed a consistent increase in Na⁺/K⁺ ratio of the shoot tissues with progressive time. These seedlings registered a 6 fold increase of Na⁺/K⁺ ratio of the shoot tissues during the 144 hours of incubation. On the other hand seedlings incubated in nutrient solution containing 200 mM NaCl and 10 mM CaCl₂ or 200 mM NaCl and 100 μM ABA showed a markedly lower ratio of Na⁺ to K⁺ than the corresponding control seedlings which were incubated in nutrient solution supplemented with only 200 mM NaCl. However, seedlings pretreated with either 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl, and 10 mM CaCl₂ or 200 mM NaCl, and 100 μM ABA showed a significantly higher ratio of Na⁺ to K⁺ than the corresponding controls which were not pretreated with either verapamil or EGTA. These seedlings registered nearly six fold increase in the level of ratio of Na⁺ to K⁺ during the 144 hours of incubation.

The reduction in K⁺ content in the shoot tissues of maize seedlings under salt stress has also been reported by Hajibagheri et al. (1987), Alberico and Crammer (1993), Azevedo-Neto and Tabosa (2000) and Azevedo-Neto et al. (2004). Munns (2002) has suggested that salt induced growth inhibition in salt sensitive genotypes could be mainly due to metabolic changes resulting from ion imbalance or ion
toxicity during salt stress. Our results on the effect of pretreatments with calcium channel blocker verapamil or calcium chelator EGTA on Na+/K+ ratio in seedlings of maize under salt stress indicate involvement of Ca^{2+} ions in exclusion of Na^{+} from the cells during stress.

H\textsubscript{2}O\textsubscript{2} levels, MDA concentration and electrolyte leakage are routinely estimated parameters to assess the extent of oxidative stress in plants. Increases in the level of H\textsubscript{2}O\textsubscript{2} and MDA upon salt stress has been reported in rice (Dionisio-Sese and Tobita, 1998; Lee et al., 2001), mulberry (Sudhakar et al., 2001), wheat (Sairam and Srivastava, 2002) and lentil (Bandeoglu, 2004). The increase was shown to be related to the magnitude of stress and correlated with membrane lipid damage. The reactive oxygen species produced as a result of stress can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc, causing lipid peroxidation, protein denaturing and DNA mutation (Breusegem et al., 2001; Scandalios, 1993; Quiles and Lopez, 2004). ROS are also known to cause oxidative damage to chlorophyll (Alscher et al., 1997; Pastori and Foyer, 2002).

In the present investigation seedlings cultured in nutrient solution supplemented with 200 mM NaCl registered a more than 3 fold increase in the level of H\textsubscript{2}O\textsubscript{2} during the 144 hours of incubation. However seedlings cultured in nutrient solution supplemented with 10 mM CaCl\textsubscript{2} and 200 mM NaCl registered only 2 fold increase in the level of H\textsubscript{2}O\textsubscript{2} during the 144 hours of incubation. The increase in tissue levels of H\textsubscript{2}O\textsubscript{2} during the 144 hours of incubation was, however, significantly higher in seedlings pretreated with the calcium channel blocker verapamil. These
results indicate the ameliorative role of calcium ions in preventing oxidative damage to tissues of salt stressed plants. Similar observation on the role of calcium ions in preventing oxidative damage in salt stressed plants have been made by Cachorro et al. (1993), Gong et al. (1997) and Hawighorst (2007). Neill et al. (2002b) and Pastori and Foyer (2002) have explained that H$_2$O$_2$ was produced under salt stress either by the dismutation of superoxide and transported from the apoplast to the cytosol or was generated in chloroplasts, mitochondria and peroxisomes from where it would move into cytosol. H$_2$O$_2$ has also been shown to be involved in the cellular signaling process as secondary messengers to induce a number of genes and proteins involved in stress defenses including SOD, CAT, APX, GR and POX (Lamb and Dixon, 1997; Karpinski et al., 1999; Morita et al., 1999; Desikan et al., 2001; Neill et al., 2002a; Vranová et al., 2002). Our results clearly show a marked increase in the MDA content of leaf tissues in seedlings cultured in nutrient solution supplemented with 200 mM NaCl. The MDA content, however, significantly lower in seedlings cultured in nutrient solution supplemented with 200 mM and 10 mM CaCl$_2$. On the other hand seedlings pretreated with either verapamil or EGTA, before transfer to nutrient solution containing 200 mM NaCl and 10 mM CaCl$_2$, had markedly higher levels of MDA. Analysis of changes observed in the level of protein carbonylation and the MDA content with salt stress, as observed in the present investigation, indicate that membrane lipid peroxidation rather than protein carbonylation was the major cause of enhanced electrolyte leakage during salt stress. MDA content is often used as an indicator of lipid peroxidation resulting from oxidative stress (Smirnoff, 1995), and its accumulation is considered a manifestation of the detriment of ROS in plants.
Increase in the MDA content in seedlings pretreated with calcium channel blocker verapamil or calcium chelator EGTA clearly indicates the protective role of Ca\(^{2+}\) in preventing membrane lipid peroxidation during salt stress in maize. Gilroy et al. (1991), McAinsh et al. (1990, 1992), Chen et al. (2001) and Yang and Poovaiah (2002) have suggested that ABA could trigger Ca\(^{2+}\) influx thereby enhancing the concentration of cytosolic Ca\(^{2+}\) in the cells. The enhanced cytCa\(^{2+}\) could through a downstream signaling cascade induce other physiological responses in the salt stressed plants cells. Behl and Jeshke (1981) have suggested that EGTA or verapamil disrupts the deployment of Ca\(^{2+}\) as second messenger thereby blocking the action of ABA in preventing salt stress induced damage to plant cells.

Compared to the untreated controls salt stressed seedlings of both varieties of maize showed increased levels of AsA and GSH. While the tissue level of GSH showed a progressive decrease with time that of AsA increased progressively with time till 144 hours of incubation. The tissue concentration of AsA also showed a significant increase with progressing time in seedlings cultured in nutrient solution supplemented with 100 \(\mu\)M ABA. While the seedlings incubated in nutrient solution supplemented with 200 mM NaCl and either 10 mM CaCl\(_2\) or 100 \(\mu\)M ABA showed marginally higher tissue level of GSH, those pretreated with verapamil showed markedly lower tissue levels of GSH than the corresponding controls. On the other hand salt stressed seedlings had significantly higher tissue concentration of AsA than the untreated controls. The increased accumulation of AsA in ABA treated seedlings is indicative of the role of ABA depending signaling cascade in regulation of
Ascorbate- glutathione cycle. El-baky et al. (2003) have reported significant elevation in the level of GSH in three onion cultivars under salt stress. On the contrary results of the present investigation do not show any significant increase in GSH content during salt stress. Our results clearly show increase in the AsA content in leaf tissues of the seedlings in response to salt stress. Zushi and Matsuzoe (2007) have observed similar changes in AsA content in salt stressed tomato cv. House momotaro. They have also demonstrated the cultivar specific response of the plants in respect of accumulation various components of the antioxidative system. It was shown that while the cultivar House momotaro showed increase in AsA levels in response to salt stress the cultivar mini carol showed decreased levels of AsA under salt stress. El-baky et al. (2003) have suggested that the proportional contribution of various components of the antioxidative system in plants under salt stress was always species dependent and hence any change in any of the various components of the antioxidative system would depend on the tolerance level of the species/cultivar. Using a transgenic tobacco system, Lee et al. (2007) have demonstrated that simultaneous expression of multiple antioxidant enzymes was more effective than single or double enzyme expression systems for developing transgenic plants with enhanced tolerance to stresses. It was shown that mature leaves of transgenic plants expressing three antioxidant genes viz. Cu/ZnSOD, APX and DHAR had nearly 2 fold higher DHAR activity, higher ratio of reduced ascorbate (AsA) to DHA and oxidized glutathione (GSSG) to reduced Glutathione (GSH). The higher levels of AsA and lower levels of GSH in the variety RCM1-1, as observed in the present indication, is indicative of greater salt tolerance capability in this variety. This also
indicates the involvement of Ascorbate-Glutathione pathway in alleviating salt stress induced oxidative damage in the seedlings.

Results from the present study clearly revealed marked increases in the activities of APX, POX and SOD in the shoot tissues of maize seedlings under salt stress. While the salt stressed seedlings maintained higher activities of APX and POX throughout 144 hours of incubation, the activity of GR showed marked increase during the initial 48 hours of stress after which it showed marked decrease with progressing time. Similar pattern of change in activity was recorded for CAT. Simultaneously there was a sharp increase in the tissue content of MDA and protein carbonylation between 48 and 144 hours of stress. The sharp increase in MDA levels and protein carbonyls between 48 to 144 hours of stress indicates that salt stress induced oxidative damage to lipids and proteins might after 48 hours of incubation in nutrient solution supplemented with 200mM NaCl.

The first enzyme of the ascorbate-glutathione cycle, APX, catalyzes the reduction of \( \text{H}_2\text{O}_2 \) to water and has high specificity and affinity for ascorbate as reductant (Asada, 1999). In the present investigation, APX activity in salt stressed seedlings was 54% higher than the untreated controls. Higher activity of APX was also recorded in ABA treated seedlings. Seedlings cultured in nutrient solution supplemented with 200 mM NaCl along with either 10mM CaCl\(_2\) or 100\(\mu\)M ABA also showed higher activity of the enzyme. The differential effects of sodium chloride on the APX activity of the two varieties of maize, as observed in the present investigation, indicate the differences in the tolerance levels of the two varieties to salt stress. Guetha-Dahan et al. (1997) have suggested that APX was a key enzyme in
determining salt tolerance in *Citrus* as its constitutive activity was much higher in salt tolerant cultivar. APX activity has also been shown to be higher in tolerant cultivars of pea (Hernandez *et al.*, 1999), mulberry (Sudhakar *et al.*, 2001), tomato (Rodriguez-Rosales *et al.*, 1999) under salt stress. Compared to the untreated controls, seedlings cultured in nutrient solution supplemented with 200 mM NaCl and 10 mM CaCl₂ or 200 mM NaCl and 100 μM ABA also showed higher activities of POX and GR. These results indicate activation of the antioxidant enzyme systems in the seedlings as an immediate response to salt stress. The activation of antioxidant enzyme systems indicates could be a consequence of the seedlings sensing higher levels of ROS in the tissues. These results also indicate that Ca²⁺ or ABA supplementation might have a role to play in preventing generation/accumulation of ROS in the salt stressed plants. Similar observations have been reported in foxtail millet (Sreenivasulu *et al.*, 2000), citrus (Arbona *et al.*, 2003) and maize (Jiang and Zhang, 2003).

SDS-PAGE profiles for total soluble proteins extracted from leaf tissues of the seedlings did not reveal any marked changes in the protein profiles in seedlings under salt stress. While there was no marked variation in the number of bands there were variations in the intensities of some bands particularly the two having molecular mass of 50 kD and 90 kD. Kongngern *et al.* (2005) have also shown increase in the intensity of 90 kDa band in salt stressed rice seedlings. These observations reflect changes in the expression of genes during when the seedlings are subjected to salt stress.

Our results have revealed the presence of 3 activity zones for APX isozymes
in shoot tissues of maize seedlings. The intensity of activity zones was higher in seedlings cultured in nutrient solution supplemented with 200 mM NaCl but lower in seedlings cultured in nutrient solution supplemented with NaCl and 10 mM CaCl$_2$ or NaCl and 100 μM ABA. Results of the present investigation have also clearly revealed the presence of 5 isoenzymes of SOD in the leaf tissues of maize seedlings. With the help of appropriate inhibitors these isozymes have been identified as These isozymes were characterized as MnSOD, FeSOD (I and II) and Cu/ZnSOD (I and II).

**Which isozyme showed greater changes?**

Lee (2001) has reported enhanced intensities of APX isoymes IV, V, VI and VII in salt stressed rice with APX isoymes I, II and II showing no significant change in response to salt stress. Enhanced activities of three isoymes of APX in response to salt stress, as observed in the present investigation, is indicative of its role in alleviating the disruptive effect of H$_2$O$_2$ in the shoot tissues during salt stress.

Our results on changes in the profile and intensity of isoymes of SOD in leaf tissues under salt stress are in agreement with earlier findings in a number of plant species including wheat (Sairam and Srivastava, 2002), pea (Hernandez et al., 1999), tomato (Kurepa et al., 1997) and french bean (Kwiatowski and Kaniuga, 1984). Lee (2001) have reported enhancement of expression of Cu/Zn-SOD-1, II and Mn-SOD-II isoforms by salt stress in rice. On the other hand the salt sensitive varieties Hitomebore and IR28 of rice have been shown to exhibited decreased SOD activity, increased lipid peroxidation, increased electrolyte leakage.
and high Na⁺ accumulation under high salinization (Dionisio and Tobita, 1998). It has been suggested that low SOD activity could favour accumulation of oxygen radical species which would cause oxidative damage to membranes thereby enhancing electrolyte leakage.

Even though our results showed a marginal decrease in CAT activity in the tissues during salt stress, the activity of the enzyme was enhanced in the presence of CaCl₂ or ABA. Salt stressed seedlings of maize showed higher activity of CAT-I and CAT-II isozymes. CAT activity has also been found to increase under salt stress in soybean (Comba et al., 1998), tobacco (Bueno et al., 1998), cucumber (Lechno et al., 1997) and mulberry (Sudhakar et al., 2001). However, the activity of the enzyme did not show any marked change under salt stress in potato (Benavides et al., 2000) and rice (Lin and Kao, 2000). Khan et al. (2002) have even reported salt stress induced decrease in the activity of the enzyme in rice.

The last enzyme of ascorbate-glutathione cycle, GR, catalyzes the NADPH-dependent reduction of oxidized glutathione. It is the rate-limiting enzyme and is involved in the maintenance of reduced glutathione required for the regeneration of ascorbate (Sudhakar et al., 2001). GR activates in glutathione-ascorbate cycle and converts GSSG to reduced glutathione (GSH) (Asada, 2000; Vega et al., 2003). In addition, GR regulates GSH/GSSG ratio and supplies GSH for GPX and DHAR, which convert H₂O₂ to H₂O and reduce oxidized ascorbate, respectively. Although GR acquires the reduction power from NADPH, H⁺, it dissipates this power and, in turn, increases NADP⁺/NADPH, H⁺ ratio. Results of the present investigation showed
a marked increase in the activity of glutathione reductase within 24 hours of transfer to nutrient solution supplemented with 200 mM NaCl. Seedlings cultured in nutrient solution supplemented with 200 mM NaCl along with either 10mM CaCl$_2$ or 100μM ABA also showed higher activity of the enzyme. Increased activity of GR in response to salt stress has also observed in rice (Lin and Kao, 2000), soybean (Comba et al., 1997), mulberry (Sudhakar et al., 2001), tomato (Guetha-Dahan et al., 1998) and *Chrysanthemum morifolium* (Hossain et al., 2004). On the other hand, Bueno et al. (1998) have reported that salt stress increased the activity of all antioxidant enzymes except GR in tobacco plant. Sudhakar et al. (2001) mentioned that elevated levels of GR activity could increase the ratio of NADP$^+$/NADPH thereby ensuring the availability NADP$^+$ to accept electrons from photosynthetic electron transport chain, thus minimizing the reduction of oxygen and formation of superoxide radicals. Salt stress induced elevation of activities of SOD, CAT and APX have been reported in seedlings of *Catharanthus roseus* (Elkahoui et al., 2004), rice (Lee, 2001) and *Bruguiera gymnorrhiza* (Lin, 2001). In the present study, the activity of GR showed a marked decrease after 48 hours of treatment. The decrease in the activity of GR after 48 hours of treatment could lead to limitation of the glutathione-ascorbate cycle and NADP$^+$/NADPH,H$^+$ ratio leading to accumulation of ROS and a consequent damage to membranes.

Results from the present investigation showed that NaCl stress induced a significant increase in the production H$_2$O$_2$ and the activities of SOD, CAT, APX and GR, and the content of AsA. Jiang and Zhang (2001, 2003) have shown a
significant increase in $\text{H}_2\text{O}_2$ in seedlings of maize as a response to exogenous application of ABA. The increase in $\text{H}_2\text{O}_2$ levels was followed by increase activities of SOD, CAT, APX and GR. On the basis of their observations they have suggested the involvement of ABA dependent downstream signaling cascade in modulating the physiological responses of the seedlings to salt stress. Results of the present investigation have clearly revealed that pretreatment of the plants with verapamil or EGTA reduced the ameliorative effect of $\text{CaCl}_2$ and ABA in the seedlings during salt stress. Behl and Jeshke (1981) have suggested that EGTA or verapamil disrupt the deployment of $\text{Ca}^{2+}$ as second messenger, and then block the action of ABA. Results of the present investigation clearly indicate a link between salt stress and oxidative stress in maize seedlings. The results also indicate a crosstalk between $\text{Ca}^{2+}$ and ROS through ABA. This cross talk induces increases in the activities of antioxidant enzymes in the shoot tissues of maize seedlings.