1. INTRODUCTION
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Soil salinity is one of the most important problems of crop production in arid and semi-arid zones. In India, an estimated total of 7.2 million hectares land is salt affected; out of this 4.5 million hectares is saline but non-alkaline and 2.7 million hectares is alkaline. Exploitation of genetic variability for salt tolerance in plant improvement programs has seldom been considered. However, marked differences with regard to salt tolerance exist among genera, species and varieties of various field crops. Data on diversity in salt tolerance at the intraspecific level will be of greater significance for crop improvement programs. The plant response to salt and such other abiotic stresses is complex and involves a large number of events.

Salinity is an environmental stress that limits growth and development in plants (Greenway and Munns. 1980; Boyer, 1982; Bohnert et al., 1995; Tsugane et al., 1999; Hasegawa et al., 2000; Bohnert et al., 2001; Borsani et al., 2001; Ghoulam et al., 2002; Girija et al., 2002). The effects of saline soils on plant growth have been a focus of research for nearly 100 years because salt stress is a major stress, limiting crop productivity (Fougere et al., 1991). High soil salinity poses a problem to many agriculturally unuseable areas in the world
and it can be expected that this problem becomes more severe due to the need of keeping even marginal land under cultivation and as a consequence of irrigation in arid areas (Bohnert et al., 1999). The sensitivity of crop plants toward salinity is one of the major factors causing agricultural losses in particularly arid regions (Greenway and Munns, 1980; Boyer, 1982). Worldwide, about 33% of the irrigated land is affected by salinity, and more land is not being irrigated because of salinity (Marschner, 1993).

Salt tolerance of plants is a complex phenomenon that involves morphological and developmental changes as well as physiological and biochemical processes. These aspects have been covered periodically in several reviews (Greenway and Munns, 1980; Hanson and Hitz, 1982; Rajasekaran et al., 2000). Most studies have been descriptive and have not clearly elucidated mechanisms by which salinity inhibits plant growth and metabolism (Munns, 1993; Cheeseman, 1998). In recent years, some progress has been made in the study of molecular processes involved in the physiological and metabolic adaptations of plants subjected to desiccation, salt stress or cold.

The salt stress is the result of two factors: First, a decline in water uptake caused by the increase in external osmotic pressure (Osmotic stress) and secondly, physiological disturbances in plant cells caused by an ionic imbalance in the cytoplasm of the plant cells (ionic stress) (Flowers et al., 1977; Hayashi
et al., 2000; Almansouri et al., 2001; Houle et al., 2001). Survival and growth of plants in saline environments is the result of adaptative processes such as ion transport and compartmentation, osmotic solute synthesis and accumulation that lead to osmotic adjustment, and protein turnover for cellular repair (Munns and Termaat, 1986; Poljakoff-Mayber, 1998; Heidari et al., 2000; Hong et al., 2000).

The physiological events usually associated with salinity stress are decline in photosynthesis, turgor changes, metabolite accumulation, increases in radical oxygen species, disturbances of carbon and nitrogen allocation as well as changes in ion homeostasis (Bohnert and Jensen, 1996; Tsugane et al., 1999; Heidari et al., 2000; Hamilton and Heckathorn, 2001; Hamilton et al., 2001; Liu and Staden, 2001). Physiological, molecular and biochemical analyses have uncovered multiple mechanisms which seem to act synergistically and additively to enable plants to cope with high salinity. Some genetic studies revealed stress tolerance to be governed by multiple genes (Bohnert et al., 2001; Kawasaki et al., 2001). Accumulation of compatible, low molecular weight osmolytes, such as sugar alcohols, special amino acids, and glycine betaine, has been suggested as a major mechanism that may underlie the adaptation or tolerance of plants to osmotic stresses (Yancey et al., 1982; Alhakimi and Hamada, 2001; Hartzendorf et al., 2001).
Salt accumulation in spinach (*Spinacia oleracea* L.) leaves inhibited photosynthesis by decreasing stomatal and mesophyll conductances to CO₂ diffusion and then impaired ribulose-1,5-bisphosphate Carboxylase/Oxygenase (Bongi and Loreto, 1989; Delfine et al., 1998; Querghi et al., 2000). Higher photosynthetic rates would require an increase of rubisco activity, whereas lower photosynthetic rates would indicate a reduction of rubisco characteristics in salt-stressed leaves (Delfine et al., 1999). The first effect as salinity increases is change in stomatal conductance as terrestrial plants encounter water stress (Morgan, 1984; Schulze, 1986). Initially, reduced stomatal aperture leads to lower intracellular CO₂ concentrations and a decrease in carboxylation capacity (Delfine et al., 1998; Sanchez-Rodriguez et al., 1999; Querghi et al., 2000). It is not yet established whether water or a different regulator, such as ABA, is the signal leading to the partial or complete closure of stomata under salt stress conditions (Bostock and Quatrano, 1992; Bray, 1993). Salt accumulation caused a 25% reduction of the intercellular spaces in the mesophyll of spinach leaves. This could have caused a more tortuous path for CO₂ directed toward the chloroplast and was suggested to be responsible for the observed photosynthesis reduction associated with low mesophyll conductance in salt-stressed leaves (Delfine et al., 1998). Mesophyll CO₂ conductance may
be affected by low osmotic potential of the liquid phase in salt stressed leaves (Delfine et al., 1999).

Photochemical efficiency was reduced in salt stressed leaves due to a decrease in the fraction of open photosystem II centers (Delfine et al., 1999; Misra et al., 1999; Lu and Zhang, 1999). A reduction in chlorophyll content and an increase in the chlorophyll a/b ratio were observed in salt-stressed leaves. Low chlorophyll affects light absorbance but is unlikely to change light partitioning between photosystems (Delfine et al., 1999). A high chlorophyll a/b ratio also indicates that the ratio between PS II/PS I content changes in stressed leaves (Anderson, 1986). The quantum yield of PSII was significantly lower in salt stressed leaves. While analyzing the components of PS II yield and the efficiency of energy dissipation in leaves exposed to salinity stress, it was observed that both qP (photochemical quenching) and, Φexc (excitation energy capture by open PS II centers) were severely affected (Delfine et al., 1999). However, a drought induced reduction in pigments contents was previously reported in several species, including Pea (Moran et al., 1991) and Nerium oleander (Demmig-Adams et al., 1988).

Osmotic stress, caused either due to the loss of water or increase in soil salinity, reduces growth and productivity of plants (Verma, 1999; Hasegawa et al., 2000). Accumulation of one or several metabolites has been observed in
many species during water and salinity stress (Ford, 1984; Rhodes and Hanson, 1993; Hanson et al., 1994). The accumulation of presumably osmoprotective compounds is a common response to salt stress in many organisms (Flowers et al., 1977; Binzel et al., 1988; Yancey et al., 1982) and in suspension cells of glycophytes and halophytes (Wyn Jones et al., 1983). Accumulation of these compounds during salt stress indicates osmoregulation as cellular trait of plants (Treichel, 1975; Csonka, 1989). The synthesis and accumulation of osmolytes in higher plants (proline, glycine betaine, polyols and fructon) under salinity stress is best known (Mc Cue and Hanson, 1990; Ishitani et al., 1995; Kishor et al., 1995; Pilon-smits et al., 1995; Holmstrom et al., 1996; Hong et al., 2000). Generally the term “osmoprotectant” might be used for substances that had some benefit to the whole plant to maintain meristem activity, as long as the precise nature and function of their action is not known (Bohnert and Jensen, 1996). The correlation between metabolite accumulation and increased tolerance to drought and high salinity is strong (Mc Cue and Hanson, 1990; Bray, 1993; Bohnert et al., 1995). As a rule, these metabolites are connected to the main flow of carbon and nitrogen in plant cells by short pathways, i.e. few genes and enzymes are involved in metabolite synthesis (Hasegawa et al., 2000). It is believed that high concentrations of metabolites lead to water
retention and/or the ability to take up water when the osmotic pressure of the external medium is high (Bohnert et al., 1999).

The accumulation of metabolites under salinity stress may be "Compatible osmolytes" (Williams and Gounaris, 1992; Talibart et al., 1994). The osmolytes, or the so-called compatible solutes (Brown, 1976), are neutral under physiological pH, have a low molecular mass, a high solubility in water, and are nontoxic to the organisms even when accumulated at a high concentration. It has also been argued that oxidative reactions leading to these metabolites might decrease over-reduction of the photosystem (Serrano and Gaxiola, 1994; Papageorgion and Murata, 1995). A specific function in radical scavenging has been suggested for some metabolites (Smirnoff and Cumbes, 1989). Accumulating osmolytes could support the well-documented antioxidant function of carotenoids (Telfer et al., 1994). If sufficient amounts of compatible solutes were present, they could react with the hydroxyl-radicals, thereby protecting lipids, DNA, proteins and macromolecular structures from degradative reactions leading to cell destruction (Bohnert et al., 1999; Tsugane et al., 1999). The reaction of compatible solutes with hydroxyl radicals, most likely, involves hydrogen abstraction forming H₂O₂, leaving behind a much-less reactive carbon radical in the solute molecule and it reacts with oxygen and is
dissipated as a peroxide by the ascorbate or any other peroxidase systems (Bohnert et al., 1999).

Excess NaCl in the growth medium induces structural changes in bean roots, as well as leakage of ions correlated with alterations of the cell membranes (Cachorro et al., 1995). It was also reported that NaCl treatment leads to changes in the lipid composition of bean roots (Cachorro et al., 1993; Surjus and Durand, 1996) and affects the proton extrusion activity, which appears to be partially dependent on a H⁺-ATPase associated with the plasmalemma. Salt stress leads to slower of root-and leaf-derived cells, while cells established from hypocotyls tissue are growth-arrested under stress (Thomas et al., 1992).

Studies on the effects of water stress on plant life and performance are many. In the cortex the intercellular-to-cellular-area ratio was significantly decreased in the NaCl-stressed roots, reflecting a reduction in the apoplast in response to the increased NaCl concentration in the growth medium (Hilal et al., 1998). Saline stress retards primary xylem differentiation. Roots of plants treated with NaCl were shorter and had fewer secondary roots than the controls (Hilal et al., 1998).

There are multiple genes that seem to act in concert with increase NaCl tolerance and certain proteins involved in salinity stress protection have been recognized (Yeo et al., 1990; Yeo, 1992; Bohnert and Jensen, 1996; Hare et al.,
1996; Borsani et al., 2001; Kawasaki et al., 2001). Therefore, linking the expression of a gene to a higher degree of tolerance within a genotype provides an important argument for a role in adaptation (Hasegawa et al., 2000; Borsani et al., 2001; Kawasaki et al., 2001). Sets of proteins, or in vitro translation products, present at different levels in tolerant versus sensitive genotypes have regularly been observed. For example, salt-induced proteins are reported in Lophophyrum, a salt-tolerant wheat relative (Gulick and Dvorak, 1987), and also in a salt tolerant barley cultivar (Ramagopal, 1987; Hurkman et al., 1989). ABA or cold induced proteins were shown in a freezing-tolerant alfalfa cultivar (Mohapatra et al., 1989). The expression level of a number of specific genes has been reported to be correlated with salt, desiccation, or cold tolerance of varieties or cell lines such as HAL1 overproduction in yeast (Gaxiola et al., 1992), the salt induction of osmotin in tobacco (LaRosa et al., 1989), the early salt induced LEA gene from Lophopyrum (Gulick and Dvorak, 1992; Galvez et al., 1993), the chilling induction of a Gly-rich protein from alfalfa (Mohapatra et al., 1989), and a group 2 LEA protein of wheat (Houde et al., 1992).

Environmental stresses, such as salinity (Hermondez et al., 1995; Tsugane et al., 1999), high light intensity (Cakmak and Marschner, 1992), herbicide exposure (Aono et al., 1995), air pollutants (Ranieri et al., 1996), drought (Zhang and Kirkham, 1994), water logging (Monk and Fagertedt and Crawford,
1987) and heavy metal toxicity (Weeks and Clijster, 1996) cause oxidative stress in higher plants including several species. Salt stress give rise to oxidative stress, as suggested by the increase in activities of antioxidant enzymes in response to high salinity, and by the correlation of salt tolerance with antioxidant enzyme levels (Gossett et al., 1994; Olmes et al., 1994; Sehmer et al., 1995; Tsugane et al., 1999). Increasing evidence shows that much of the injury to plants due to various environmental stresses is associated with oxidative damage through direct or indirect formation of reactive oxygen species (ROS) (Scandalios, 1993; Acevedo et al., 2001; Hamilton and Heckathorn, 2001; Nishihara et al., 2001; Hegedus et al., 2001). The ROS including superoxide (\(\cdot O_2^-\)), hydrogen peroxide (\(H_2O_2\)), hydroxyl radicals (\(\cdot OH\)) and singlet oxygen (\(\cdot O_2\)), are inevitable byproducts of cell metabolism. Under normal conditions, the production and destruction of these radicals is well regulated in the cell metabolism. In chloroplasts, superoxide radical is produced by photoreduction of \(O_2\) at photosystem I (PSI) and photosystem II (PSII). Singlet \(O_2\) is formed by energy transfer to \(O_2\) from triplet excited state chlorophyll (Niyogi, 1999). \(H_2O_2\) can originate, in turn, from the spontaneous or enzyme-catalyzed dismutation of superoxide radical. Other subcellular compartments of leaves, such as peroxisomes and mitochondria are also potential generators of superoxide radical and \(H_2O_2\) (Del Rio, 1991). These
ROS attack lipids, proteins and nucleic acids, causing lipid peroxidation, protein denaturation and DNA mutation (Bowler et al., 1992). To prevent such damage, plant cells have an antioxidative system consisting of: 1. low molecular weight antioxidants, such as ascorbate, α-tocopherol, glutathione and carotenoids, and 2. protective enzymes that operate in the following way: Superoxide radicals are scavenged by superoxide dismutase (SOD), while the product of this reaction, H$_2$O$_2$ can be detoxified by the catalase and in the ascorbate-glutathione cycle, which includes ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase (Scandalios, 1993; Palatnik et al., 2002). However, under stress conditions, the formation of these radicals might be in excess, thus creating oxidative stress (Yu and Rengel, 1999; Nakano and Asada, 1981).

Among all organisms, the cellular concentration of dioxygen is highest in plants (Scandalios, 1993). Several Calvin-cycle enzymes within chloroplasts are extremely sensitive to H$_2$O$_2$ and high levels of H$_2$O$_2$ (the product of superoxide dismutation) directly inhibit CO$_2$ fixation (Kaiser, 1979). Superoxide and H$_2$O$_2$ can react in a “Haber–Weiss” reaction to generate the hydroxyl radical (OH), which is the most potent oxidant (Scandalios, 1993). The hydroxyl radical indiscriminately and rapidly attacks virtually all macromolecules, leading to serious damage in cellular components and mutations, and often leading to
irrepairable metabolic dysfunction and cell death (Scandalios, 1993; Carvalho and Amancio, 2002). Thus, O₂ although essential for the existence and survival of aerobic life, presents living organisms with a variety of physiological challenges collectively termed “Oxidative stress” (Scandalios, 1993). Under normal conditions, plants possess scavenging systems that keep active oxygen species below damaging levels (Larson, 1998). A drought induced limitation of photosynthesis is the exposure of plants to excess energy, which if not safely dissipated, may be harmful to PSII because of overreduction of reaction centers (Demmig and Adams, 1992) and increased production of reactive oxygen species in the chloroplasts (Smirnoff, 1993). Enzymatic antioxidant defenses include ascorbate peroxidase and GSH reductase, which are believed to scavenge H₂O₂ (Foyer et al., 1994; Lima et al., 2002) the catalase (CATs) and peroxidases that remove H₂O₂ very efficiently (Scandalios, 1993) and superoxide dismutases (SODs) that scavenge the superoxide anion. The CATs and SODs are the most efficient antioxidant enzymes. Their combined action converts the potentially dangerous superoxide radical (O₂⁻) and hydrogen peroxide (H₂O₂) to water (H₂O) and molecular oxygen (O₂), thus averting cellular damage. The combined action of SOD and CAT abate the formation of the most toxic and highly reactive oxidant, the hydroxyl radical (OH⁻), which can react indiscriminately with all macromolecules. Hence, increased SOD
alone may not render the plant to combat oxidative stress. Rather, an increase in H$_2$O$_2$-scavenging capacity may also be required to enable removal of H$_2$O$_2$ produced by enhanced SOD activity (Foyer et al., 1994; Eremin, 2001).

The biological role and significance of SODs as protective enzymes against O$_2$ toxicity are borne out in numerous studies with prokaryotes and lower and higher eukaryotes, including higher plants (Fridovich, 1986, Hassan and Scandalios, 1990; 1992; Tsang et al., 1991; Bowler et al., 1992; Racchi et al., 2001). Environmental conditions as drought, chilling, anoxia, and pathogenic injury have been correlated with SOD activity (Monk et al., 1989; Umayal et al., 2000). Photosynthetic electron transport chain contains, at the acceptor side of PSI, a number of autooxidizable enzymes that reduce oxygen to superoxide (Asada and Takahashi, 1987; Asada, 1994). The superoxide may mediate cyclic electron flow around PSI (Asada, 1994) or it may diffuse it to the stromal membrane surface, where it is dismutated to oxygen and H$_2$O$_2$ in nonenzymic and enzymic reactions. Superoxide can inactivate some metal containing enzymes such as the Fd-linked nitrate reductase, catalase and peroxidase (Asada and Takahashi, 1987). But the potent danger is that H$_2$O$_2$ can react with reduced metal ions, especially Fe, resulting in the formation of the hydroxyl radical. The hydroxyl radical initiates self-propagating reactions leading to peroxidation of membrane lipids, base mutation, breakage of DNA strands, and destruction of
proteins (Asada and Takahashi, 1987; Halliwell, 1987; Bowler et al., 1992). SODs are classified, according to their metal cofactor, as FeSOD, Cu/ZnSOD. Chloroplasts generally contain Cu/ZnSOD and, in a number of plant species, FeSOD (Van Camp et al., 1994).

Catalase is an efficient antioxidant enzyme that detoxify the H$_2$O$_2$ (Brisson et al., 1998; Krishnamurthy et al., 2000; Racchi et al., 2001; Palatnik et al., 2002). The relation of catalase activity to net photosynthesis was supported by studies with a tobacco mutant selected by screening for superior growth at elevated, near lethal O$_2$ levels (Zelitch, 1992), in which a correlation was obtained between elevated catalase and decreased photorespiration. In addition to the catalase reaction (2H$_2$O$_2$ → O$_2$ + 2H$_2$O), catalase can use H$_2$O$_2$ to oxidize organic substrates such as ethanol to acetaldehyde (H$_2$O$_2$ + CH$_3$CH$_2$OH → CH$_3$CHO + 2H$_2$O). The latter represents the peroxidatic activity of catalase. Reduced catalase levels increased photorespiration and elevated catalase levels decreased photorespiration (Brisson et al., 1998).

The enzyme Glutathione reductase (GR) (NAD(P)H: oxidized GSH oxidoreductase) catalyzes the reduction of GSSG to GSH and maintains a high cellular GSH/GSSG ratio (Halliwell and Foyer, 1978; Madamanchi et al., 1992; Polle, 2001). Reduced GSH is the major nonprotein sulfhydryl compound in all living organisms, including plants and it has been implicated by numerous
metabolic processes (Rennenberg, 1982), of which its action as an antioxidant is particularly important (Halliwell, 1984). Several studies have documented the accumulation of GSH in plants exposed to oxidative stress conditions (Burke et al., 1983; De Kok and Oosterhuls, 1983; Tanaka et al., 1985; Mahan and Burke, 1987). GSH content and metabolism in plants are affected by developmental and environmental conditions (Smith et al., 1990). GR is involved in the maintenance of a high cellular GSH/GSSG ratio and scavenging the hydrogen peroxide through the ascorbate-GSH cycle (Halliwell and Foyer, 1978). Under normal conditions, GSH and GR are involved in the detoxification of H$_2$O$_2$ in the light by the Mehler reaction in chloroplasts. Increased GR levels have been correlated with tolerance to low temperature stress (De Kok and Oosterhuls, 1983; Guy and Carter, 1984) and drought (Gamble et al., 1984). GR has been purified from chloroplasts (Connel and Mullet, 1986), leaf homogenates (Halliwell and Foyer, 1978; Wingsle, 1989), and roots (Bielawski and Joy, 1986).

GSH plays several roles in plant defense system and in addition to its function as an antioxidant, intimately involved with the redox balance of the cell (Kunert and Foyer, 1993). GSH is also an important cofactor, both for enzyme activities and for enzyme synthesis, as well as being control to the metabolism of reduced sulfer (Schmidt and Kunert, 1986). The increased levels of GSH and ascorbate
may confer additional antioxidant protection to the chloroplast. Adaptation to drought may depend on different mechanisms, including the capacity to maintain high levels of antioxidants and to regenerate them through the induction of GR activity (Quartacci et al., 1994; Loggini et al., 1999). The catabolism of cytosolic H$_2$O$_2$ is likely to depend on the maintenance of reduced ascorbic acid and GSH, or the conversion of GR which contribute significantly to the ability to prevent the stress (Madarnanchi et al., 1992). The glutathione system is efficient provided that GSSG is rapidly reduced to GSH by GR (Bartling et al., 1993; Navari-Izzo and Izzo, 1994).

Peroxidases located in the cytosol also contribute to the scavenging of cytosolic H$_2$O$_2$ (Takahama, 1991; Mandal, 2000). Hydrogen peroxide is especially toxic in the chloroplasts because even at low concentrations it inhibits the Calvin cycle enzymes possessing exposed sulfhydryl groups, such as G3PDH and FBPase, thus reducing the photosynthetic carbon dioxide assimilation (Takeda et al., 1995). Plant peroxidases are among the enzymes where reaction intermediates were first identified, and their molecular and enzymatic properties have been characterized (Nakano and Asada, 1987). Guaiacol peroxidases are assumed to play a role in a broad spectrum of biological activities, such as the biosynthesis of lignin, biosynthesis of ethylene, degradation of indole-3-acetic acid, wound healing and defense against
pathogens (Chen et al., 1992). Many isozymes of guaiacol peroxidase have been found in plant tissues. Ascorbate peroxidase also is present in two forms: chloroplast and cytosol isozymes (Chen and Asada, 1989). Peroxidase acts in the scavenging of hydrogen peroxide (H$_2$O$_2$) and the scavenging of H$_2$O$_2$ by ascorbic peroxidase in chloroplasts has been shown to be indispensable to photosynthesis (Asada and Takahashi 1987). Ascorbate peroxidase is the key enzyme in the scavenging of hydrogen peroxide in chloroplasts with ascorbate as the electron donor (Nakano and Asada, 1980; Asada and Badger 1984; Hossain et al., 1984). Increase in the concentration of H$_2$O$_2$ in the chloroplasts, inactivates the PSII reaction center (Van Camp, 1996). Overexpression of cytosolic Apx increased tolerance to methyl viologen (herbicide) (Pitcher et al., 1994), supporting the conclusion of Sen Gupta et al., (1993) that increased Apx activity was important in determining stress tolerance. Apx would remove hydrogen peroxide in a reaction catalyzed by the peroxidase and, thus, it protects the enzyme (Chen and Asada, 1992). Ascorbate peroxidase (Moran et al., 1994) and other antioxidant enzymes (Zhang and Kirham, 1994) may play a role in maintaining low levels of hydrogen peroxide in the cells. H$_2$O$_2$, even at low concentrations, inhibits chloroplast sulfhydryl-containing enzymes by readily oxidizing their sulfhydryl groups. Therefore, it is important for plant cells to keep the levels of hydrogen peroxide low or to scavenge it efficiently.
Ascorbic acid is an important antioxidant defense metabolite in plant cells (Rautenkranz et al., 1994; Alhakimi et al., 2001). It protects plant cells against damage by oxygen free radicals, which may be produced as a result of disturbances of electron transfer processes or via autooxidation. Ascorbic acid as an antioxidant is oxidized to DHA (oxidized ascorbic acid). Oxygen free radicals may be scavenged by AA (reduced ascorbic acid) or may lead to the production of $H_2O_2$, which can be detoxified by ascorbic acid in the presence of ascorbate peroxidase (Castillo and Greppin, 1986). An additional involvement of ascorbic acid as an antioxidant was proposed for the reduction of oxidized tocopherol (Wefer and Sies, 1988). Tocopherol is associated with membrane lipids and is oxidized as it protects lipids against peroxidation (Suarna and Southwell-Keely, 1991). Ascorbic acid has been known to be synthesized in the cytosol (Loewus, 1980) and then translocated into the chloroplasts, vacuole, and apoplast. Translocation of ascorbic acid into chloroplasts has been studied in spinach and was suggested to occur by facilitated diffusion (Anderson et al., 1983; Beck et al., 1983). Regeneration of reduced ascorbic acid from oxidized ascorbic acid occurs in the cytosol (enzymatic) and chloroplasts (nonenzymatic), with glutathione as a reductant. The same compartments contain glutathione reductase for the regeneration of reduced glutathione (Halliwell, 1984; Rautenkranz et al., 1994).
Accumulation of proline (pro) is a widespread plant response to environmental stresses (Yancy et al., 1982; Delauney and Verma, 1993; Kavi Kishor et al., 1995; Roosens et al., 1999; Hayashi et al., 2001; Girija et al., 2002). Because of the high concentrations often observed, proline has a clear role as an osmoticum. Because of its zwitterionic, highly hydrophilic characteristics, proline acts as a “compatible solute”, i.e. it can accumulate to high concentrations in the cell cytoplasm without interfering with cellular structure or metabolism (Verma, 1999). Other functions of proline accumulation have also been proposed, including radical detoxification (Smirnoff and Cumbes, 1989) and regulation of cellular redox status by proline metabolism (Hare and Cress, 1997). NaCl treatment leads to proline accumulating from 5% to 40 to 50% of total amino acids depending on the length of stress and plant age (Treichel, 1975; Thomas et al., 1992). A genetic modification that increased the basal level of proline in tobacco, reduced the plants sensitivity to NaCl (Kavi Kishor et al., 1995). Proline accumulation is proposed to be part of the process of osmotic adjustment that contributes to the cellular adaptation of many plant species to drought, salinity, and other stresses (Morris et al., 1969; Stewart and Larher, 1980; Hanson and Hitz, 1982; Hong et al., 2000).

The primary role of proline in osmoprotection may not be solely as an osmoregulatory osmolyte, but it also helps the cell to overcome oxidative stress
(Smirnoff and Cumbes, 1989). Other known attributes of proline, such as protecting enzymes from denaturation (Rajendrakumar, 1994), interacting with membrane systems (Rudolph et al., 1986), regulating cytosolic activity (Venekamp, 1989), scavenging free radicals (Smirnoff and Cumbes, 1989), balancing the ratio of NADH/NAD\(^+\) (Alia and Saradhi, 1991), and finally acting as a energy source (Kohl et al., 1998) may be more important for the overall health of the plant under abiotic/osmotic stress. High levels of endogenous proline help reduce free radicals generated during oxidative stress induced by the osmotic stress (Verma, 1999). During periods of drought or NaCl stress, plants increase their pools of free proline in excess of the demands of protein synthesis. Proline may interact with enzymes to preserve protein structure and activity within the cell. In vitro studies have shown that high concentrations of proline reduce enzyme denaturation attributable to heat and high NaCl (Pollard and Wyn Jones, 1979; Rajendrakumar et al., 1994). Proline may protect proteins and membranes from damage by inactivating hydroxyl radicals or other highly reactive chemical species that accumulate when stress inhibits electron-transfer processes (Smirnoff and Cumbes, 1989; Saradhi et al., 1995).

Primary metabolites such as soluble sugars including glucose and sucrose help regulate many developmental and physiological processes in plants (Koch,
1996; Smeekens, 1998; Scheen et al., 1999; Yu, 1999; Gibson, 2000). For example, sugar levels have been postulated to play an important role in determining the time at which some plant species flower (Gibson, 2000). Sugars are also thought to help control key metabolic processes such as photosynthesis (Krapp et al., 1993) and starch synthesis and breakdown (Koch, 1996). Strong evidence for the importance of sugars in controlling plant processes is also provided by reports that sugars help regulate the expression of a significant number of plant genes (Koch, 1996). The composition of different physiological processes and sink organs for the limited carbon supplies under salinity significantly affects overall plant growth and crop yield (Munns and Termaat 1986, Daie, 1996). As a consequence, the different growth responses to salinity were interpreted due to changes in the allocation and partitioning of photoassimilates (Poljakoff-Mayber and Lerner, 1994). Under saline conditions, carbohydrate availability does not seem to be the limiting factor for growth although the regulation of carbon allocation and partitioning may have an important influence in the maintenance of growth rate and yield (Sacher and Staples 1985, Munns and Termaat 1986, Munns 1993, Everard et al., 1994, Balibrea et al., 1999).

Sucrose is the main form of translocated sugar in most plants (Bruneau et al., 1991). In sucrose-transporting plants, the sucrose status of a tissue play a
crucial role in the regulation of metabolism, so that the hydrolysis of sucrose in the sink organs may determine the ability to import photoassimilates and the sucrolytic activities could be used as biochemical indicators of sink strength. On the other hand, sucrose export from mature leaves was related to sucrose synthesis (Geiger and Fondy 1991) and, therefore, to some extent the sucrose phosphate synthase activity could be used as an indicator of source strength since it catalyzes the regulatory step in sucrose synthesis during photosynthesis (Stitt et al., 1987). Sucrose synthesis is catalyzed by sucrose phosphate synthase (SPS) and sucrose-6-phosphate and its degradation is catalyzed by sucrose synthase or invertase (Lafta and Lorenzen 1995; Moore, 1995; Burleigh and Harrison, 1999). Sucrose is the principal and the preferred form of sugar transported to various sink tissues in plants (Cheng et al., 1996). A major control point for the portioning of photosynthate between sucrose and starch in the leaves is SPS (Huber, 1983; Huber et al., 1984; Kalt-Torres et al., 1987; Huber and Huber, 1992; Chen et al., 2001). SPS activity in mature leaves was reduced in plants subjected to water stress (Vassay and Sharkey, 1989; Vassey et al., 1991; Castrillo, 1992). SPS is localized in the leaf cytosol and is believed to contribute to the control of the flux of carbon fixation into sucrose (Cheng et al., 1996). SPS enzyme/protein has been well analyzed in terms of its invitro regulation and its kinetic properties (Huber and Huber, 1992; Stitt et al., 1998).
SPS is a predominant enzyme of the photosynthetic tissues but it is also detected in certain nonphotosynthetic tissues (Klein et al., 1993). SPS is believed to play a critical role in sucrose biosynthesis by coupling the Calvin cycle and starch turnover reactions in chloroplasts (Cheng et al., 1996).

In green leaves surplus carbohydrates generated during photosynthesis are stored in the form of starch in chloroplasts (Vally and Sharma, 1995). The level of chloroplastic starch undergoes a daily oscillation, where its level becomes higher during the day when photosynthesis favors its accumulation, and its level declines during the night because of its mobilization (Vally and Sharma, 1995). Although the metabolic pathway of transitory starch mobilization in leaves has not been fully elucidated, studies of storage organs such as seeds and tubers have revealed that starch degradation is mediated by a small group enzymes, α- and β-amylase (Beck and Ziegler, 1989). It is assumed that the above starch-degrading enzymes may also participate in the mobilization of transitory starch in chloroplasts (Vally and Sharma, 1995). As the $\delta$sink starch is exclusively localized in chloroplasts, it is reasonable to expect that starch-degrading enzymes too are localized in chloroplasts. However, β-amylase is found only in plants and certain species of bacteria (Nakamura et al., 1991). Seeds of cereals and soybean contain a large amount of β-amylase and this enzyme is
also present in vegetative tissues of plants, for example, in leaves, roots, and cotyledons (Steup, 1988).

The photosynthetic capacity of leaves is closely related to their nitrogen content. As leaf nitrogen content increases, the photosynthetic rate at any partial pressure of \( \text{CO}_2 \) is enhanced (Makino et al., 1994). Nitrate is the major source of nitrogen in most plants (Marschner, 1995; Walker et al., 2001). The addition of nitrate leads to increased rates of nitrate uptake (Siddiqi et al., 1990; Laine et al., 1995) and increased activities of nitrate reductase (NR) (Laine et al., 1995) and nitrite reductase (NiR) (Wray, 1993). Nitrate assimilation is the major process by which the majority of higher plant species obtain reduced nitrogen. In this process, nitrate, once taken up from the soil is reduced to nitrite by nitrate reductase (NR). Nitrite is translocated to the chloroplasts, where it is further reduced to ammonium by nitrite reductase. NR reduces nitrate in the cytosol (Rufty et al., 1986; Vaughn and Campbell, 1988; Fedorova et al., 1994) by using NADH, which is generated by carbon catabolism (Beevers and Hageman, 1980). As much as 25% of the energy generated by photosynthesis can be consumed in driving nitrate assimilation (Guerrero et al., 1981). When plants are exposed to nitrate, levels of NR and NiR increases drastically (Rajasekar and Delmulier, 1987; Solomonson and Barber, 1990).
When plant cells are exposed to salinity, mediated by high NaCl concentrations, kinetic steady status of ion transport for Na\(^+\) and Cl\(^-\) and other ions, such as K\(^+\) and Ca\(^{2+}\), are disturbed (Binzel et al., 1988; Niu et al., 1995; Ghoulam et al., 2002). It is generally accepted that plant cells must maintain a high ratio of K\(^+\) and Na\(^+\) ions in their cytoplasm if they are to grow successfully in saline environments (Greenway and Munns, 1980). Salinity affects physiology of plant through changes of the ionic status in the cells (Sultana et al., 2001; Kashem et al., 2000; Hasegawa et al., 2000). Thus, it is vital for the plant to re-establish cellular ion homeostasis for metabolic functioning and growth and to adapt to the saline environment (Niu et al., 1995). When plants are exposed to NaCl, ions reduce the apoplastic water potential and accumulate excessively in the cytosol (Binzel et al., 1988). Plant cells adjust to the water relations imbalance through osmotic adjustment by synthesizing compatible organic solutes and accumulating ions (Niu et al., 1995; Hamilton et al., 2001).

Na\(^+\) acts as a competitor of K\(^+\) uptake (Watab et al., 1991; Schroeder et al., 1994). Vacuolar compartmentation of Cl\(^-\) is an essential adaptation for NaCl tolerance. It is widely assumed at present that the death of plant cells exposed to saline conditions is caused by a high ratio of Na\(^+\) and K\(^+\) ions in the cytoplasm, which is due to drastic increases in the influx of Na\(^+\) ions into the cells and in the efflux of K\(^+\) ions from the cells (Katsuhara and Tazawa 1988; Nakamura et
al., 1992; Niu et al., 1995). Under saline conditions, the large electrochemical Na\(^+\) gradient results in passive Na\(^+\) uptake into root cells (Smith and Walker, 1989; Allen et al., 1995; Tyeman and Skerrett, 1999; Buschmann et al., 2000). Long term Na\(^+\) influx by Na\(^+\)-permeable channels/transporters can elevate the cytoplasmic Na\(^+\) concentration to toxic levels and trigger a variety of detrimental cellular events (Volkmar et al., 1999). Plants have various mechanism for controlling the cytoplasmic concentration of Na\(^+\) ions, such as sequestration of the Na\(^+\) ions into the vacuole via the action of a Na\(^+\)/H\(^+\) antiporter at the tonoplast (Blunwaid and Poole, 1987; Garbarino and DuPont, 1988) and pumping out Na\(^+\) ions across the plasmalemma through Na\(^+\)/H\(^+\) exchange mechanisms (Watabd et al., 1986; Buschmann et al., 2000). The essential role of Ca\(^{2+}\) ions has been extensively documented with respect to various cellular functions that are associated with the growth and development of plants (Bangerth, 1979; Roux and Slocum, 1982; Hepler and Wayne, 1985). In particular, regulation of membrane functions is thought to be one of the most important roles of Ca\(^{2+}\) ions in plant cells (Tazawa et al., 1987). Ca\(^{2+}\) is known to have ameliorative effects on plants under high-salinity conditions (LaHaye and Epstein, 1969) and also was known to reduce low-affinity Na\(^+\) uptake (Rengel, 1992). Studies of salt-affected soils have shown that increases in exchangeable Na, accompanied by decreases in exchangeable Ca\(^{2+}\), may result
in ion imbalances that adversely affect plant growth (Grieve and Maas, 1987; Nakamura et al., 1992). High external Na⁺ reduces the activity of Ca²⁺ ions in the root medium and decreases the amount of Ca²⁺ that is available for uptake by the plant (Cramer and Lauchli, 1986; Cramer et al., 1986). A decrease in Ca²⁺ and Mg²⁺ contents in leaves under salt stress would imply a higher salt tolerance (Guerrier, 1984; Francois, 1988) Salinity also changes the anion concentrations in plants.

Casuarinas are a distinctive group of Angiosperms belonging to the family Casuarinaceae. This family consists of 11 tree species found in South East Asia, Malaysia, Melanasia, Polynesia, New Calendona and Australia. Among Casuarinas, Casuarina equisetifolia is traditionally grown along the coastal areas throughout India. Casuarina species grown for wide range of services and products, of which, wind breaks, production of poles and pulp wood are considered the most important. The demand for Casuarina wood for paper and pulp production is increasing. Although Casuarina has been planted extensively by farmers, government and quasi government agencies in India, Little attention has been paid to the genetic improvement of this species.

Casuarinas are large evergreen tree, with a straight stem and numerous long, slender drooping, jointed, 6-8 angle leafless dwarf branchlets arising from rough, woody branches. The dwarf branchlets are partly deciduous, green and
perform the functions of leaves. Bark is brown, rough, fibrous and exfoliating in longitudinal strips. Wood is hard, liable to cracks and split. The Casuarina in appearance resembles a feathery conifer. The tree is short lived, seldom surviving over more than fifty years even under favourable situations, and in less favourable conditions rarely reaching an age of 25 years before becoming hollow and misshapen.

Casuarina is also an important tree species grown extensively in many parts of the world. The productivity of Casuarina is highly influenced by environmental factors such as sub optimal temperature, drought, frost, salinity and alkalinity. Under favourable conditions, it attains a height of 32 m or more. Casuarina thrives best in areas in proximity of the sea on loose sands, sometimes within few yards of high-tide level. Casuarinas have a wide range of environmental adaptability and occupy from arid deserts to high rainfall coastal sites in both tropical and temperate zones. Salt and water stress usually affect the growth and ion balances of Casuarina species (Van der Moezel et al., 1988; Sanchez-Rodriguez et al., 1999). Casuarina equisetifolia is the most extensively planted Casuarina with major areas in India and is planted for a variety of purposes (Doran and Hall, 1983). Casuarina junghuhniana and Casuarina cunninghamiana also grow in a wide variety of ecological situations in India (Boland, 1989). Several farmers favour planting Casuarina in the coastal areas.
because of its commercial viability. The present study was undertaken to evaluate salinity tolerance among three Casuarina species usually used for cultivation in India.

OBJECTIVES

The productivity of crop plants is greatly affected by salt stress. The progressive salinization of soil, estimated at around 20% of irrigated land has made the genetic improvement of salt tolerance an urgent priority for the future of agriculture.

In spite of extensive research on plant stress, only a few salt tolerant cultivars have been developed. The development of salt tolerant plants has been hampered by the difficulty in the genetic dissection of this multigenic trial and by the lack of knowledge about the physiological processes that limit growth under salt-stress conditions.

Although much information is available on the agronomic aspects of Casuarina, very little is known about the effects of salinity on physiological and biochemical aspects of tree species, especially in Casuarina. Our main objective of the present study was to evaluate the salinity stress-induced changes on certain physiological and biochemical characteristics which include photosynthesis, pigment composition, photochemical activities, osmolytes,
nucleic acids, carbohydrates, nitrogen metabolism and elemental analysis. Very few data are available on antioxidative enzyme systems such as superoxide dismutase, catalase, glutathione reductase, ascorbate peroxidase, peroxidase and stress induced accumulation of proteins in Casuarina species. Hence we initiated this study to understand salinity stress-induced antioxidative metabolism also in three Casuarina species.