4. DISCUSSION
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Salt is one of the most serious environmental factors limiting the productivity of crop plants (Borsani et al., 2001; Ghoulam et al., 2002; Girija et al., 2002). Several physiological and biochemical processes are affected by salinity, particularly photosynthesis and nitrate assimilation, which largely influenced plant growth. High concentration of NaCl arrest plant development and leads to plant death (Van der Moezel., 1988; Bohnert et al., 1999; Tsugane et al., 1999; Kawasaki et al., 2001). Several interacting events are triggered in plants by salt stress, including the inhibition of enzyme activities in metabolic pathways, decreased uptake of nutrients in roots, decreased carbon use efficiency, and the denaturation of protein and membrane structure (Tsugane et al., 1999). The stresses most commonly associated with water deficits are drought, salinity and low temperature (Bohnert et al., 1994; Houle et al., 2001).

In the present study, the rate of photosynthesis (Figure 1) in *C. junghuhniana* (CSIRO 19491, 19489) showed decreased rates (7%, 11% respectively) with 150mM salinity concentration compared to their respective control plants while *C. equisetifolia* (CSIRO 18355, 18271) showed inhibited photosynthesis with 50mM (both 12%), 100mM (24%, 25% respectively) and 150mM (45%, 48%
respectively) when compared to respective control plants. *C. cunninghamiana* (Local) showed decrease with 100mM (10%) and with 150mM (15%) compared to control plants. Salt accumulation in leaves might first inhibit photosynthesis by decreasing stomatal and mesophyll conductances to CO$_2$ diffusion and is known to impair 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 in rubisco activity, where as lower photosynthetic rates would indicate a reduction of rubisco characteristics in salt-stressed leaves (Delfinr et al., 1999). The effects of salinity has been reported to change the stomatal conductance in several terrestrial plants (Morgan, 1984; Schulze, 1986). Prolonged closure of stomata, however, leads to lower intracellular CO$_2$ concentrations and a decrease in carboxylation capacity (Delfine et al., 1998; Sanchez-Rodriguez et al., 1999; Querghi et al., 2000). In the present study, the rates of photosynthesis were differentially affected in three Casuarina species under the influence of salinity. However, our data on photosynthetic characteristics under salinity stress clearly demonstrate that *C. junghuhmiana* is much superior in terms of photosynthesis rates compared to *C cunninghamiana* and *C equisetifolia*. 
The effect of salinity stress on the photochemical activities (Photosystem System II activity) in isolated chloroplasts of tree species is very little understood. The results in this investigation shows the marked effect of salinity stress on the photoreduction activity of DCPIP by isolated chloroplasts (Figure 2) DCPIP photoreduction varied among the three Casuarina species with different concentrations salinity. The reduction in photochemical efficiency in salt stressed leaves was presumed to be due to a decrease in the fraction of open photosystem II centers (Delfine, 1999; Rokka et al., 2000). In *C. junghuhniana* DCPIP photoreduction decreased by 6% in CSIRO 19491 and by 8% in CSIRO 19489 with 150mM salinity concentrations compared to respective control plants while in *C. equisetifolia*, the photoreduction of DCPIP decreased by 22% in CSIRO 18355 and by 24% in CSIRO 18271 when compared to respective control plants. Decreased photo system II activity was observed in *C. cunninghamiana* with 150mM salinity by 13% compared to control plants. Quantum yield of PSII was reported to be significantly low in salt stressed leaves and the efficiency of energy dissipation in leaves exposed to salinity stress was affected (Delfine et al., 1999). During the salinity stress, it was also recently reported that dephosphorylation of the PSII reaction center proteins D1 and D2 occurs as well as of the chlorophyll a binding protein CP43 (Rokka et al., 2000). It is thus believed that the reduction in photosystem II
activity in salt stressed plants might be a crucial factor in determining the photosynthetic productivity in Casuarina plants.

Exposure to salinity stress caused a decrease in pigment content in all the three Casuarina species in response to varying salinity concentrations (50, 100, 150mM). In the present study, total chlorophyll (Figure 3) content decreased in C. equisetifolia (CSIRO 18271) by 8%, 14%, 19% with 50mM, 100mM and 150mM salinity treated plants respectively compared to control plants while in C. junghuhniana (CSIRO 19491), total chlorophyll content decreased by 6% with 150mM compared to control plants (Figure 3). C. cunninghamiana (Local) showed decreased chlorophyll content with 100mM salinity by 6% and with 150mM by 14% compared to control plants. Similar trend of salinity stress affected pigment composition in terms of chlorophyll-a (Figure 4) and chlorophyll-b (Figure 5) was noticed in the three Casuarina species. It is thus evident from our data that the chloroplast integrity has been damaged under stressful conditions in Casuarina species (Kaiser et al., 1981). The present study on the pigment composition in response to salinity stress clearly showed that the C. junghuhniana maintained high pigment content than C. cunninghamiana and C. equisetifolia even when subjected to salt stress.

Our data also demonstrate that salt stress reduced the Casuarina leaf protein content with different concentrations (Figure 6). Decrease in the rate of
protein biosynthesis during stressed conditions has been studied (Singla and Grover, 1994). Protein content was significantly decreased in *C. equisetifolia* (CSIRO 18355, 18271) with 50mM salinity (15%, 13% respectively), 100mM (25%, 26% respectively) and 150mM (47%, 49% respectively) compared to respective control plants, while *C. junghuhniana* (CSIRO 19491, 19489) showed reduction with 150mM salinity (12%, 15% respectively) compared to their respective control plants. Further, *C. cunninghamiana* showed the reduction in foliar protein with 100mM salinity (12%) and 22% reduction with 150mM salinity treatment, compared to control plants. Hsiao (1973) reported that the water stress affects the polysomes and protein synthesis and we presume that salinity also affects protein synthesis in Casuarina. The results in this study clearly indicates that the protein synthetic machinery in the Casuarina species are affected by the salinity stress, but the reduction in protein content varied with different salinity concentrations among the three Casuarina species.

The quantitative estimation of of nucleic acids (DNA and RNA) in different Casuarina species in response to different salinity concentrations clearly showed that salt stress has reduced the nucleic acid contents (*Figures 7 and 8*). DNA content in *C. junghuhniana* decreased by 10% in CSIRO 19491 and by 13% in CSIRO 19489 with 150mM salinity compared to respective control
plants while in *C. equisetifolia* DNA content significantly decreased by 35% in CSIRO 18355 and by 40% in CSIRO 18271 compared to respective control plants. *C. cunninghamiana* showed decrease by 19% at 150mM salinity compared to control plants. The contents of RNA were also similarly affected in different Casuarina species (Figure 8). It was reported that the environmental stress induce the reactive oxygen species (ROS) which attack nucleic acids causing lipid peroxidation, protein denaturation and DNA mutation (Bowler et al., 1992). The soluble protein content and nucleic acid levels in our study indicate that *C. junghuhniana* was superior in retaining most of its protein and nucleic acids than *C. cunninghamiana* and *C. equisetifolia* subjected to varying salinity concentrations.

The relationship between salinity stress and carbohydrate accumulation patterns in green leaves is very interesting. In the present study, the general trend was that carbohydrate content was reduced in all the Casuarina species. But the decrease in carbohydrate content varied among the three Casuarina species. The sink systems of the plant compete for the limited carbon supplies under salinity which affect the overall plant growth and yield (Munns and Termaat 1986, Daie, 1996). As a consequence, the different growth responses to salinity can be presumed and interpreted as resulting from changes in the allocation and partitioning of photoassimilates (Poljakolf-Mayber and Lerner,
The regulation of carbon allocation and partitioning would 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). Hence we investigated the total sugars as well as the contents of sucrose and starch as influenced by salinity stress in Casuarina. Total sugar content (Figure 9) decreased in *C. junghuhnia* (CSIRO 19491, 19489) with 150mM salinity (18%, 20% respectively) compared to the respective control plants while *C. equisetifolia* showed decrease of total sugar content with all the salinity treatments: 50mM salinity (21%, 25% respectively), 100mM salinity (41%, 46% respectively) and 150mM salinity (59%, 65% respectively) compared to respective control plants. However, *C. cunninghamiana* showed decrease in total sugar content with 100mM salinity (14%), 32% reduction with 150mM salinity when compared to control plants.

This study provides an evidence that the synthesis and accumulation of structural and non structural carbohydrates in all Casuarina species is dependent upon salinity concentration. In addition to playing a central role in metabolism, soluble sugars such as glucose and sucrose may help regulate many developmental and physiological processes in plants (Koch, 1996; SmeEkens, 1998; Scheen et al., 1999; Yu, 1999). For example, sugar levels have been postulated to play an important role in determining the time at which some plant
species flower (Gibson, 2000). Our data showed that reducing sugar (Figure 10) content decreased in *C. junghuhniana* (CSIRO 19491) by 17% in 150mM salinity-treated plants while in *C. equisetifolia* (CSIRO 18271) reducing sugars showed significant decrease by 27%, 52%, 69% with 50mM, 100mM and 150mM salinity-treated plants respectively, compared to control plants. In *C. cunninghamiana* the decrease was low with 100mM salinity (12%) and with 150mM it was by 29% compared to control plants. Non-reducing sugar content (Figure 11) in various Casuarina species in response to salinity stress also showed similar pattern.

It was hypothesized that due to limitation in the supply of structural and non structural carbohydrates in Casuarina leaves, plant growth will be significantly affected due to limited supply of energy and carbon skeletons during various stages of growth. Sugars also are thought to help control key metabolic processes such as photosynthesis (Krapp et al., 1993; Koch, 1996). The starch content in all the Casuarina species increased with increase in salinity concentration (Figure 12). In *C. junghuhniana* (CSIRO 19491,19489) starch content increased with 150mM salinity (in both cases 16%) compared to their respective control plants, while *C. equisetifolia* showed increase in starch with 50mM salinity (both 13%), 100mM salinity (both 19%) and with 150mM salinity (25%, 26% respectively) compared to their respective control plants. *C.
*cunninghamiana* showed increase in starch with 100mM salinity (13%) and 150mM salinity (22%) compared to control plants. The data indicate that starch accumulation as well as its degradation and mobilization is an important physiological criterion in determining the rates of carbon fixation. Starch accumulation is also an important criterion to understand carbohydrate partitioning efficiency. It is presumed that unless the accumulated starch is degraded and mobilized into various regions of plants, further carbon assimilation would not be effective and feed back inhibition of photosynthesis becomes operational. The present study on foliar sucrose (Figure 13) content showed that in *C. junghuhniana*, sucrose content decreased by 30% in CSIRO 19491 and by 35% in CSIRO 19489 at 150mM salinity when compared to respective control plants while among *C. equisetifolia*, sucrose content significantly decreased by 64% in CSIRO 18355 and 69% in CSIRO 18271 compared to respective control plants. In *C. cunninghamiana* sucrose content decreased by 46% with 150mM salinity compared to control plants. Carbohydrate studies on three casuarina species with response to salinity stress clearly indicates that *C. junghuhniana* had an effective carbohydrate partitioning mechanism which might contribute for efficient photosynthesis, as already observed for this species.
Proline content of salt affected Casuarina species showed increased levels with increased salinity (50, 100, 150mM) (Figure 14). Accumulation of proline is a widespread plant response to environmental stress (Yancey et al., 1982; Delauney and Verma, 1993; Kavi Kishor, et al., 1995; Roosens et al., 1999; Hayashi et al., 2001). The foliar accumulation of proline in tree species in response to environmental stress is little known. The present study demonstrated that *C. junghuhniana* (CSIRO 19491, 19489) showed significant increase of proline content with all salinity treatments: 50mM salinity (30%, 31% increase respectively), 100mM salinity (44%, 46% increase respectively) and 150mM salinity (55%, 52% increase respectively), compared to the respective control plants while *C. equisetifolia* (CSIRO 18355, 18271) showed increase with 150mM salinity (46%, 44% increase respectively) compared to their respective control plants. Increase in proline was observed in *C. cunninghamiana* with 100mM salinity (35%) and 150mM salinity (42%) compared to control plants. Proline interacts with enzymes to preserve protein structure and activity within the cell and the *in vitro* studies have shown that high concentrations of proline reduce enzyme denaturation attributable to heat and high NaCl (Rajendrakumar et al., 1994). Proline may protect proteins and membranes from damage by inactivating hydroxyl radicals or highly reactive chemical species that accumulate when stress inhibits electron-transfer
processes (Smirnoff and Cumbes, 1989; Saradhi et al., 1995). Other known attributes of proline are its interaction with membrane systems (Rudolph et al., 1986), regulating cytosolic activity (Venekamp, 1989), balancing the ratio of NADH/NAD$^+$ (Alia and Saradhi, 1991) and also acting as a energy source (Kohl et al., 1998). It has also been reported that the osmotic stress caused either due to the loss of water or increase in soil salinity, reduce growth and productivity of plants (Verma, 1995).

The free amino acid content in response to salinity treatment showed increased accumulation of free amino acids with increasing salinity concentrations (50,100,150mM) (Figure 15). Amino acids constitute the major form of transported organic nitrogen in plants (Kishor, 1995). Amino acids are also involved in the osmotic adjustment and sometimes potential substrates for mitochondrial reactions (Kishor et al., 1995). Data on free amino acid content (Figure 15) showed significant increase in *C. junghuhniana* by 60% in CSIRO 19491 and by 57% in CSIRO 19489 at 150mM salinity treatments compared to respective control plants while in *C. equisetifolia* free amino acid content increased by 34% in CSIRO 18355 and by 30% in CSIRO 18271 at 150 mM salinity when compared to respective control plants. Free amino acid content in *C. cunninghamiana*, significantly increased by 43% with 150mM salinity compared to control plants. The response of proline and free
amino acids to salinity stress on three Casuarina species clearly showed that C. junghuhniana accumulated more proline and free amino acids than C. cunninghamiana and C. equisetifolia. Thus C. junghuhniana proved to be much superior in accumulating the osmoprotectants during stressful environment.

The antioxidant property of the ascorbic acid was important to evaluate drought tolerance among Casuarina species in response to different salinity concentrations (Figure 16). Ascorbic acid is an important antioxidant defense substance in plant cells (Rautenkranz et al., 1994). 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). Ascorbic acid was reported to serve as an antioxidant metabolite (Foyer et al., 1991). Oxygen free radicals may be scavenged by ascorbic acid or may lead to the production of H$_2$O$_2$, 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). In the present study, our data showed that ascorbic acid content
significantly increased in *C. junghuhniana* (CSIRO 19491) by 34%, 45%, 51% with 50mM, 100mM and 150mM salinity treated plants respectively while in *C. equisetifolia* (CSIRO 18271) ascorbic acid content showed an increase by 27% with 150mM salinity treated plants compared to control plants. In *C. cunninghamiana*, ascorbic acid content was increased with 100mM salinity by 27% and 150mM salinity by 33% compared to control plants. It was also reported that ascorbic acid plays a key role in detoxification of O$_2^-$ radical (Foyer et al., 1991) and it can react directly by reducing superoxide, hydrogen peroxide and hydroxyl radical, or quenching singlet oxygen (Walker et al., 1993). We believe that ascorbic acid plays a crucial role in scavenging reactive oxygen species which are produced during the salt stress Casuarina.

The effect of salinity on foliar nitrogen contents was analyzed to evaluate nitrogen metabolism among the three Casuarina species. The general observation was that foliar nitrogen content decreased with increasing salinity. Our results suggest that the photosynthetic capacity of leaves was closely related to foliar nitrogen content (*Figure 17*). Data on foliar nitrogen content (*Figure 17*) clearly demonstrated that in *C. junghuhniana* (CSIRO 19491, 19489) foliar nitrogen decreased with 150mM (23%, 30% respectively) compared to respective control plants while *C. equisetifolia* (CSIRO 18355, 18271) showed significant decrease in nitrogen content with all the salinity
concentrations: 50mM (28%, 22%), 100mM (36%, 35%) and 150mM (44%, 52%) compared to their respective control plants. In *C. cunninghamiana* foliar nitrogen content decreased with 100mM salinity (26%) and 150mM salinity (37%) compared to control plants. It has been reported that leaf N is involved in major alterations in carbon metabolism, including alterations in starch and sucrose contents in leaves (Hofstra et al., 1985; Fiehther and Schulze, 1992) and the synthesis of organic acids to provide carbon skeletons for amino acid synthesis and to act as counteranions and prevent alkalinization (Foyer and Ferrario, 1994). This study provide an evidence that the salt stress reduces the nitrate assimilation capacity in all the Casuarina species, but the percentage of reduction varied among the provenances. The study on foliar nitrogen content clearly showed that *C. junghuhniana* species has efficient nitrogen assimilation system compared to other species.

Our further investigations were on elemental analyses in the leaves of different Casuarina species with response to different salinity concentrations. 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⁺, are disturbed (Binzel et al., 1988; Niu et al., 1995; Hasegawa et al., 2000; Ghoulam et al., 2002). Excessive salinity has been considered the most important edaphic factor limiting the distribution of plants in certain natural
habitats, and constituting an increasingly severe agricultural problem in wide areas of the world. The effects of salinity on the growth of plant species which restrict salt uptake (excluders) differ from those showed by species with relatively high rates of uptake and translocation of salt ions (mainly Cl and/or Na) to the shoots (includers) (Marschner, 1986). The present study demonstrated that Na⁺ concentration (Figure 18) increased in leaves of *C. junghuhniana* (CSIRO 19491, 19489) with 150mM salinity (30%, 36% respectively) compared to respective control plants while in *C. equisetifolia* (CSIRO 19491, 19489) Na⁺ content increased with all the salinity concentrations: 50mM salinity (both 12%), 100mM salinity (both 21%) and 150mM salinity (59%, 64% respectively) compared to the respective control plants. In *C. cunninghamiana* (local) increase of Na⁺ was observed with 100mM salinity by 16% and with 150mM salinity by 43% compared to the control plants (Figure 18). In glycophytes, which comprise most of crop species, an inverse relationship has been reported between salt uptake and salt tolerance; that is, exclusion is the predominant strategy (Greenway and Munns, 1980).

Differences in the capacity for Na and Cl exclusion also exist among cultivars or species. For example, the higher salt tolerance in certain cultivars of wheat, barley and citrus was related to a more effective restriction of shoot transport of
both Na and Cl (Marschner, 1986); whereas in soybean cultivars and grapevine rootstocks it was primarily related to the restriction of Cl transport (Downton, 1977; Lauchli and Wieneke, 1979), and a retention of Na in root and restriction of its translocation to the shoot seem to play an important role in the salinity tolerance of wild relatives of pigeonpea (Subbarao et al., 1990). The present study, shows that C. junguhumiana leaves accumulate Cl⁻ (Figure 19) which significantly increased by 32% in CSIRO 19491 and by 35% in CSIRO 19489 at 150mM salinity treatments compared to the respective control plants. In C. equisetifolia Cl⁻ content was increased by 62% in CSIRO 18355 and by 57% in CSIRO 18271 with 150mM salinity when compared to respective control plants. In C. cunninghamiana Cl⁻ content increased by 49% at 150mM salinity compared to the control plants. Classifying species as excluders or includers is helpful in demonstrating the principles of adverse effects of (or adaptation to) salinity, but in reality very few glycophytes are strict excluders or includers; most are intermediate types (Marschner, 1986). From our study it is evident that the accumulation of Na⁺ and Cl⁻ in leaves showed varying pattern of accumulation among different Casuarina species with increasing salinity concentration. However, we believe that the Na⁺ and Cl⁻ ion taken from the soil are transport and efficiently to the shoot system in all Casuarina species, which could facilitate non-accumulation of Na⁺ and Cl⁻ in the roots.
The nutrients Na\(^+\), Ca\(^{2+}\) and K\(^+\) are not only involved in the regulation of photosynthesis but have other cellular regulatory function, which are directly or indirectly involved in growth and physiology of the plant. K\(^+\) plays a predominantly osmotic role in plants (Marshall and Porter, 1991). In the present study, the results showed that K\(^+\) (Figure 20) concentration decreased in *C. junghuhniana* (CSIRO 19491) by 31\% in 150mM salinity treated plants compared with control plants while in *C. equisetifolia* (CSIRO 18271) K\(^+\) content decreased by 24\%, 49\%, 57\% in 50mM, 100mM and 150mM salinity treated plants respectively compared with respective control plants and *C. cunninghamiana* showed decrease in K\(^+\) content with 100mM salinity by 19\% and 150mM salinity by 43\% compared to the control plants (Figure 20). High Na/K ratios are known to inhibit many enzyme activities (Flowers et al., 1977) and are characteristics of glycophytes in saline conditions. Salt tolerance of three Casuarina species was found to be correlated with maintenance of lower Na K ratios in needles (Aswathappa and Bachelard, 1986). 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 1986, 1988; Van der Moezel et al., 1988). Our results suggest that *C. junghuhniana* leaves had less efflux of K\(^-\)
compared to other species. It is well known that K⁺ plays crucial role in several physiological processes including photosynthesis and stomatal opening.

The essential role of Ca²⁺ ions has been extensively documented with respect to various cellular functions that are associated with the growth and development of plants (Hepler and Wayre, 1985). In the present study, the data on foliar Ca²⁺ concentration (Figure 21) clearly showed that in *C. equisetifolia* (CSIRO 18355, 18271), Ca²⁺ significantly decreased with all the salinity concentrations: 50mM salinity (both 8%), 100mM salinity (17%, 20% respectively), and 150mM salinity (49%, 58% respectively) when compared to respective control plants, while *C. cunninghamiana* showed decrease in Ca²⁺ with 100mM salinity (11%) and with 150mM salinity (33%) compared to the control plants while the decrease in Ca²⁺ content in *C. jutigl7uhnniana* (CSIRO 19491, 19489) was 12%, 16% respectively with 150mM salinity when compared to respective control plants (Figure 21). Regulation of membrane integrity and transport is thought to be one of the most important roles of Ca²⁺ ions in plant cells (Tazawa et al., 1987). Salt stress is known to inhibit the supply of Ca²⁺ to the shoot in several species (Lynch and Lauchli, 1985). Maintenance of higher levels of Ca²⁺ and Mg²⁺ contents in leaves under salt stress would certainly imply a higher salt tolerance (Guerrier, 1984; Francois, 1988). Magnesium concentration (Figure 22) decrease in *C. jutigl7uhnniana*
(CSIRO 19491, 19489) with 150mM salinity (16%, 18% respectively) compared to respective control plants, while *C. equisetifolia* (CSIRO 18355, 18271) showed significant decrease in Mg\(^{2+}\) content with 50mM salinity (11%, 12% respectively), 100mM salinity (23%, 26% respectively) and 150mM salinity (52%, 58% respectively) when compared to their respective control plants. *C. cunninghamiana* showed a decrease in magnesium content with 100mM salinity (11%) and 150mM salinity (32%) compared to their control plants. These results suggest that the leaves of *C. junghuhniana* maintained higher levels of Ca\(^{2+}\) and Mg\(^{2+}\) even under higher saline conditions.

RuBP Carboxylase, the key photosynthetic enzyme, was used to assess the photosynthetic capacity of the various Casuarina species with response to different salinity concentrations (50, 100, 150mM). RuBP Carboxylase, located in the chloroplast stroma, is the most abundant protein on earth and constitutes up to 50% of total chloroplast protein (Ellis, 1979). About 90% of the dry weight of plants is derived from CO\(_2\) assimilated by the RuBP carboxylase reaction during photosynthesis (Brisson et al., 1998). In the present study, salinity effects on RuBP carboxylase suggest that *C. junghuhniana* (CSIRO 19491, 19489) showed only slight decrease in the activity with 150mM salinity (6%, 7% respectively) compared to respective control plants, while *C. equisetifolia* (CSIRO 18355, 18271) showed significant decrease with 50mM salinity.
salinity (6%, 7% respectively), 100mM salinity (10%, 11% respectively) and 150mM salinity (17%, 23% respectively) compared to their respective control plants. Decrease in _C. cunninghamiana_ was also observed with 100mM salinity (6%) and 150mM salinity (9%) compared to their control plants. The expression of the photosynthesis genes like _rbcS_ was strongly inhibited by salt stress (Tsugane et al., 1999). The study on RuBP carboxylase clearly showed that _C. junghuhniana_ possess significantly higher activities even under higher saline conditions which were positively related to the photosynthetic rates.

Much of the injury to plants caused by the stress exposure is associated with oxidative damage at the cellular level (Anderson et al., 1995; Kaminaka et al., 1999). Under normal conditions, plants possess scavenging systems that keep active oxygen species below damaging levels (Larson, 1988). When the plant is stressed, the production of active oxygen can exceed the capacity of the scavenging systems, resulting in oxidative damage and thus, the ability of a plant to improve its active-oxygen-scavenging capacity may be a key element in stress tolerance (Anderson et al., 1995). SOD catalyzes the first step in the scavenging system of active oxygen by the disproportion of superoxide anion radicals to hydrogen peroxide and molecular oxygen (Kaminaka et al., 1999). Widespread losses of forests and crops due to saline soils is a common phenomenon occurs the world. Among all organisms the cellular concentration
of dioxygen is highest in plants (Scandalios, 1993). Hydrogen peroxide is especially toxic in the chloroplasts because even at low concentrations it inhibits the Calvin cycle enzymes thus reducing the photosynthetic carbon dioxide assimilation (Takeda et al., 1995). Increase in the concentration of H₂O₂ in the chloroplasts, results the inactivation of PSII reaction center (Van Camp et al, 1996). Hence one of our main objective was to investigative oxidative stress metabolism in different species of Casuarina to assess the salinity tolerance among the provenances.

Resistance to salinity occurs when a plant withstands the imposed stress, and this may arise from either tolerance or a mechanism that permits avoidance of the stress. The loss of the ability to scavenge free radicals during stress is generally attributed to a decrease in activity of antioxidative enzymes SOD, CAT, and Peroxidase (Pastori and Trippi, 1993). The present study demonstrated that SOD activity (Figure 24) significantly increased in C. junghuhnniana (CSIRO 19491, 19489) with all the salinity concentrations: 50mM salinity (12%, 11% respectively), 100mM salinity (both 19%) and 150mM salinity (25%, 23% respectively) compared to their respective control plants while among C. equisetifolia SOD activity increased with 150mM salinity by 12% in both CSIRO 18355, 18271 compared to the respective control plants. In C. cunninghamiana (Local) significant increase of SOD
observed with 100mM salinity by 11% and with 150mM salinity by 17% compared to the control plants (Figure 24). The biological role and significance of SODs as protective enzymes against O₂ toxicity in prokaryotes and lower and higher eukaryotes, including higher plants are of great interest (Bowler et al., 1992). Superoxide can inactivate some metal containing enzymes such as the Fd-linked nitrate reductase, catalase, and peroxidase (Asada and Takahashi, 1987). The present results on SOD activity clearly showed that among the Casuarina species *C. junghuhniana* had superior activity of SOD and should be better tolerant to salinity compared to *C. cunninghamiana* and *C. equisetifolia*.

Catalase (CAT), another important antioxidant enzyme showed increase activity in all the Casuarina species in response to salinity stress (Figure 25). In the present work, the diverse responses of the CAT enzyme activity to salinity stress suggest that oxidative stress is influential component of environmental stresses on Casuarina species. In the present study, CAT activity in *C. junghuhniana* significantly increased by 46% in CSIRO 19491 and by 45% in CSIRO 19489 with 150mM salinity treatments when compared to the respective control plants while in *C. equisetifolia* CAT activity increased by 27% in CSIRO 18355 and by 26% in CSIRO 18271 with 150mM salinity when compared to respective control plants. In *C. cunninghamiana* CAT activity increased by 35% in 150mM salinity compared to the control plants.
CAT also is an efficient antioxidant enzyme that detoxify the H$_2$O$_2$ (Brisson et al., 1998; Acevedo et al., 2001). It has been reported that insufficient CAT activity also change the biochemistry of leaves with, because excess H$_2$O$_2$ may rapidly decarboxylate ketoacids such as hydroxypyruvate and glyoxylate to generate additional CO$_2$ (Zelitch, 1992). Thus additional loss of assimilated CO$_2$ might be avoided with higher CAT activity, thereby reestablishing the stoichiometry of CO$_2$ loss closer to 25% and increasing the net photosynthesis (Brisson et al., 1998). It is also suggested that in addition to the CAT reaction, CAT can use H$_2$O$_2$ to oxidize organic substrates such as ethanol to acetaldehyde. The latter represents the peroxidatic activity of CAT. Reduced catalase levels increased photorespiration and elevated CAT levels apparently reduced photorespiration (Brisson et al., 1998). These data suggest that catalase activity play a significant role in providing generated during salinity stress.

An antioxidant enzyme, Glutathione reductase (GR) showed increase in activity in response to salinity stress in all the three casuarina species (Figure 26). GR could play a critical role in protection against oxidative stress (Madamanchi et al., 1992). Certain studies have documented the accumulation of GSH (reduced glutathione) in plants exposed to oxidative stress conditions (Mahan and Burke, 1987). It has been suggested that that GR is involved in the maintainance of a high cellular GSH/GSSH ratio and scavenging of hydrogen
peroxide through the ascorbate-GSH cycle (Halliwell and Foyer, 1978). GSH is also an important cofactor, both for enzyme activities and for synthesis, as well as being control to the metabolism of reduced sulfur (Loggini et al., 1999). In the present study, our data showed that GR activity (Figure 26) significantly increased in *C. junghuhniana* (CSIRO 19491) by 24%, 37%, 46% in 50mM, 100mM and 150mM salinity-treated plants respectively, while in *C. equisetifolia* (CSIRO 18271) GR activity showed an increase by 26% with 150mM salinity treated plants. *C. cunninghamiana* showed an increase of GR activity with 100mM salinity by 23% and 150mM salinity by 35% compared to the control plants (Figure 26). It was previously suggested that increased GR levels have been correlated with tolerance to low temperature stress (De Kok and Oosterhuls, 1983) and drought (Gamble and Burke., 1984). During dehydration in plant cells, a significant increase in glutathione reductase has recently been reported (Loggini et al., 1999). In the present study, very much enhanced activities of glutathione reductase with all the salinity concentrations in *C. junghuhniana* species indicate that GR plays a key role in salinity tolerance through maintaining the levels of reduced glutathione used for scavenging the reactive oxygen species.

Ascorbate specific peroxidase (Apx) is the key enzyme for the scavenging hydrogen peroxide in chloroplasts, and protects the enzymes of the carbon
dioxide fixation cycle from inactivation by hydrogen peroxide (Kaiser, 1979; Asada and Takahashi, 1987; Yoshimura et al., 2000). Among the casuarina species from our study, *C. junghuhniana* (CSIRO 19491, 19489) showed significant increase in the activity with 50mM salinity (both 36%), 100mM salinity (both 51%) and 150mM salinity (58%, 56% respectively) compared to the respective control plants, while *C. equisetifolia* (CSIRO 18355, 18271) showed significant increase of Apx activity with 150mM salinity (both 28%) compared to the respective control plants. In *C. cunninghamiana* increase in Apx activity was noticed with 100mM salinity (29%) and with 150mM salinity (35%) compared to the control plants (*Figure 27*). It has been reported that the scavenging of hydrogen peroxide by ascorbate peroxidase in chloroplasts to be indispensable to photosynthesis (Asada and Takahashi, 1987) and also APX play a role in maintaining low levels of hydrogen peroxide in the cell. We conclude that the activity of Apx is also important for scavenging H$_2$O$_2$. The Apx activity could also serve as an indicator for stress tolerance. Certainly *C. junghuhniana* proved to be superior in possessing high activity of Apx under salinity stress.

Salinity stress also affects the peroxidase activity (*Figure 28*) in Casuarina provenances. Peroxidases contribute to the scavenging of cytosolic H$_2$O$_2$ (Mandal, 2000; Jan et al., 2001). The results presented in this study clearly...
show that peroxidase activity increased in *C. equisetifolia* (CSIRO 18355, 18271) with 150mM salinity (33%, 30% respectively) compared to the respective control plants, while *C. junghuhniiana* (CSIRO 19491, 19489) showed increase with increasing salinity: 50mM salinity (39%, 38% respectively), 100mM salinity (52%, 51% respectively) and 150mM salinity (61%, 60% respectively) compared to the respective control plants. In *C. cunninghamiana* significant increase in peroxidase activity was noticed at 100mM salinity (33%) and with 150mM salinity (44%) compared to the control plants. In the present study increased activity of peroxidase might be considered as an evidence for enhanced scavenging of free oxygen radicals in Casuarina generated during stress. It has been reported that peroxidase activity increased during the growth-restricted states of senescence and cold acclimation (Van Huystee and Cairns, 1980). Guaiacol peroxidases are assumed to play a key role in broad spectrum of biological activities, such as biosynthesis of lignin, biosynthesis of ethylene, degradation of indole-3-acetic acid, wound healing and defense against pathogens (Chen et al., 1992; Sakharov et al., 2001). Adaptation to salinity only depend on different mechanisms, including the capacity to maintain high levels of antioxidants.

It is clear that the response of antioxidants and antioxidant enzymes to salinity stress depends on the severity of stress and it varies among the species as well.
as among provenances of Casuarina (Tsugane, 1999). The present data on antioxidants have shown that salinity stress tolerance of *C. junghuhniana* (CSIRO 19491, 19489) appears to involve an ability to maintain higher antioxidant enzyme activities at all the salinity concentrations (50, 100, 150) than *C. equisetifolia* and *C. cunninghamiana*. Increased activity of antioxidant enzymes and antioxidants can significantly reduce the oxidative damage caused by environmental stresses. It could allow *C. junghuhniana* to withstand more to the salinity stress compared to *C. equisetifolia* and *C. cunninghamiana*.

In higher plants, the sucrose status of a tissue play a crucial role in the regulation of photosynthetic 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 is related to sucrose synthesis (Geiger and Fondy, 1991) and, therefore, to some extent the sucrose phosphate synthase (SPS) 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). SPS activity in *C. junghuhniana* (CSIRO 19491, 19489) showed a decrease with 150mM salinity (13%, 15% respectively) compared to respective control plants, while *C. equisetifolia* (CSIRO 18355, 18271) showed significant decrease with 50mM salinity (17%,
18% respectively), 100mM salinity (35%, 34% respectively) and 150mM salinity (42%, 46% respectively) compared to their respective control plants. *C. cunninghamiana* showed decrease in SPS activity with 100mM salinity (14%) and 150mM salinity (22%) compared to control plants. A major control point for the partitioning 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; Burleigh and Harrison, 1999). SPS activity in mature leaves was reduced in plants subjected to water stress (Vassay and Sharkey, 1989; Vassey et al., 1991; Castrillo, 1992). We believe that metabolism of sucrose and its transport are highly essential for the proper growth and metabolism of *Casuarina*. SPS is also an important cytosolic enzyme that is known to control the flux of carbon fixation into sucrose and starch (Cheng et al., 1996). The photosynthetic efficiency as seen in the case of *C. junghuhniana* might be attributed to the efficient SPS system.

Amylases (α and β amylases) are important enzymes for degradation of starch (Beck and Ziegler, 1989). It is assumed that the above starch degrading enzymes may also participate in the mobilization of transitory starch chloroplasts and thus contribute for regulation of photosynthesis (Vally and Sharma, 1995). α-amylase activities in *C. junghuhniana* (CSIRO 19491, 19489) slightly decreased with 150mM salinity (9%, 10% respectively) compared to
their respective control plants, while *C. equisetifolia* (CSIRO 18355, 18271) showed decrease with 50mM salinity (both 12%), 100mM salinity (19%, 20% respectively) and 150mM salinity (24%, 30% respectively) when compared to their respective control plants. *C. cunninghamiana* showed decreased activity of α-amylase with 100mM salinity (8%) and 150mM salinity (13%) compared to control plants (Figure 30). Similar trend in all Casuarina species (Figure 31). It is note that the quantity of starch in Casuarina needles was inversely related to the starch metabolism enzymes (amylases). The Casuarina species with high starch content like *C. equisetifolia* (Figure 12) possessed low activity of enzymes (Figure 30, 31) while those with low starch content like *C. junghuhniana* exhibited high activity of both the enzymes. The data in this study have clearly shown that amylases are well related to the quantity of starch in the leaves of all Casuarina species in response to different salinity concentrations (Figure 30, 31).

One of the most extensively characterized stress responses in higher plant at the molecular level is the synthesis of stress shock proteins. The proteins are synthesized under a variety of stresses such as high salinity, desiccation, drought, heavy metals, chilling and anoxia (Uma et al., 1995). Many of these proteins are suggested to protect the cell against the adverse effects of stress. The significance and relevance of these stress proteins in various plants is a
matter of great significance. The ability of induced systems to tolerate severe levels of stress signifies the importance of stress proteins. However, information on differential synthesis of stress proteins in tree species associated with stress tolerance is inconclusive. In this study we examined, the accumulation of some stress proteins induced by salinity in the two Casuarina species case as studies. Quantitatively, the salinity stressed plants had accumulated extra proteins with increasing salinity concentrations (50, 100, 150) in both the species of Casuarina (Plate 6 and 7). Our data show that in *C. junghuhmannana* (CSIRO 19491), the proteins of apparent molecular weight such as 14 KDa, 43KDa, and 68KDa accumulared more with increasing salinity concentrations compared to the control plants, while in *C. equisetifolia* (CSIRO 18271), accumulation of such proteins was low. Our present study demonstrates that the accumulation of more quantity of stress proteins might protect the plant subjected to salinity stress. *C. junghuhmannana* proved to be superior in accumulating certain stress proteins in more quantities compared to *C. equisetifolia*.