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Physiological and biochemical responses of *Ceriops roxburghiana* Arn. seedling under salt stress conditions

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ABSTRACT

Salt stress is one of the sternest ecological factors that reduces and confines expansion and improvement of plants. In the current exploration, the effect of different concentration of NaCl on growth and biomass accumulation, photosynthetic characteristics and biochemical determinations of *Ceriops roxburghiana* a mangrove plant has been studied. The superior boundary for the continued existence of this species to NaCl salinity was 600 mM. Consequences of the current study indicated that the optimal salt concentration for the overall better performances of the seedlings of *C. roxburghiana* was 300 mM NaCl. The growth parameters such as shoot and root length, fresh and dry weight increased with increasing salinity up to 300 mM NaCl. Sodium chloride salinity stimulated the chlorophyll and carotenoid content increased up to 300 mM NaCl concentration. The biochemical determinations such as amino acid and sugar decreased with increasing salinity up to the 300 mM levels and still at higher salinity levels and increased in the content of these two compounds was noticed. On the further hand, protein and starch contents were increased up to 300 mM NaCl and decreased at higher concentration. Proline and glycine betaine contents were randomized increased up to 600 mM NaCl.

KEY WORDS: Biochemical determinations, *Ceriops roxburghiana*, growth, NaCl, photosynthetic pigments

INTRODUCTION

Mangroves are halophytic plants flourishing in intertidal zones establish in temperate and sub-temperate climates (Tomlinson, 1986). The capability to utilize salt water is the individual outstanding quality of the mangrove plants. Similar to other halophytes, they have mechanisms to avoid unnecessary expand of Na^+ and Cl^- (Munns, 1988). Consequently, most of the salt in the outside elucidation is barred by the roots of these genuses and only a minute portion reaches the vegetation parts. On the other hand, mangrove plants having foliage with secreting glands, transport moderately more salt in the xylem. Thus, overload salt passed to the leaves is maintained surrounded by physiologically suitable levels by salt secretion (Ball, 1988). Specificity of salt secretion in halophyte plants is not healthy documented, but other ions can also be secreted by Na^+ and Cl^-, such as K^+, Ca^{2+}, Rb^+, SO_4^{2-}, and Zn^{2+} (Boon and Allaway, 1986). Ecophysiological processes play a key role in the mangrove forest composition (Ball and Sobrado, 1999). 7% of the land's surface and 5% of sophisticated lands are affected by salinity with the salt stress life form one of the most severe ecological factors restrictive the productivity of crop plants (Liu *et al.*, 2010). India alone, about 30 million hectares of coastal soil is lying unproductive and uncultivable since of earth affected by salinity. Stresses related with warmth, salinity, and drought only or in mixture are possible to improve the harshness of troubles in the future decades (Claussen *et al.*, 1985). The focus of the NaCl in the saline atmosphere is generally calculated as the Cl^- concentration, and it is regarding 35 g/L. Na is the foremost cation with concentration of 480 mM in the salt H_2O and in the earth. As the mangrove environmental conditions affect the survival and the productivity of the colonizing plants species, plant structures and physiological features explain their ecological success under harsh conditions (Smith *et al.*, 1989).

Salinity is the one of the most common abiotic stress factors affecting crop production drastically. According to

recent reports the universal region of salt pretentious soils together with saline and sodic soils is 831 meter hectares (Martinez Beltran and Manzur, 2005), mainly restricted to dry and semidry regions, where land degradation, H_2O deficiency and population growth are a major concern (Geissler *et al.*, 2010). Lots of the physiological adaptations of plant life to saline circumstances are as to the adaptations exposed by plants to aridity pressure, and it has been recommended with the intention of plants presentation drought resistance would as well show salinity broad-mindedness (Munns, 2002). On the other hand in a few halophytes, the salt broad-mindedness mechanisms are not satisfactory for broad-mindedness of drought or cold (Ueda *et al.*, 2003).

A lot of latest studies have focused on the morphological, physiological, biochemical, and molecular attributes that are linked with the reaction of adult plants to salinity. Coping with salt stress involve problematical mechanisms so as to consist of developmental, morphological, physiological and biochemical strategies (Taji *et al.*, 2004). Seed germination is a necessary process in plant improvement to get hold of most favorable seedling information that results in higher seed yield. Germination and seedling growth decreased with a lot of abiotic factors such as salt and drought stress that are perhaps two of the mainly significant stranded abiotic stress that limit amount of seedling and seedling growth development (Ansari and Sharif-Zadeh, 2012; Ansari *et al.*, 2013). In additional, salt stress-regulation genes are articulated, which leads toward change into the protein outline to facilitate plants to get used to salt accretion (Parker *et al.*, 2006). Salt tolerant plants have a lot of defense mechanisms which manage with stress. In stressed plant cells, a variety of compatible solutes i.e., polyamine and proline are generally accumulated and function as osmotic alteration. In higher plants, proline is biosynthesized by whichever the glutamate or the ornithine pathway. The glutamate pathway is measured the main route, particularly in reaction to osmotic stress (Kishor *et al.*, 2005). Commonly, proline protect plants from stress during altered processes, together with by the modification of cellular water, detoxification of relative O_2, defense of membrane reliability and stabilization of enzymes and proteins, thus it can be advantageous to plants in adapting to stress (Trovato *et al.*, 2008). *Ceriops roxburghiana* Arn., are representative woody mangrove shrubs with opposite leaves belonging to the family Rhizophoraceae.

In the current examination, a challenge has been completed toward study the effect of Sodium Chloride on different biochemical parameters were determined, in addition to photosynthetic pigment stabilization and growth presentation in salt stressed seedling of *C. roxburghiana*.

MATERIALS AND METHODS

Seedlings of *C. roxburghiana* were collected from Pichchavaram mangrove forest, Chidambaram, Tamil Nadu and were identified by Kumudranjan Naskar (2004) and Banerjee, *et al.* (1989). The experimentation was laid out in a completely randomized block design. Polythene bag cultures and the treatment procedures were carried out in the Botanical Garden and the biochemical analysis was conducted in Department of Botany, Arignar Anna Government Arts College, Villupuram, Tamil Nadu, India. The bags were filled with soil containing mixture of red soil, sand and farm work area compost at 1:2:1 ratio. Six concentrations of NaCl used for the treatment were 100 mM, 200 mM, 300 mM, 400 mM, 500 mM, 600 mM, and 0 mM served as control. For every treatment five replicates were maintained. Treatments were imposed on the plant on 30 and 60 days after sowing (Figures 1 and 2).

Measurement of Growth and Biomass Accumulation

The effect of salt stress on growth was measured in terms of shoot and root length (cm/plant), whereas biomass accumulation was recorded in the form of the fresh weight (FW) and dry weight (DW) of shoots and roots (g/plant). The FWs were recorded immediately after harvesting the

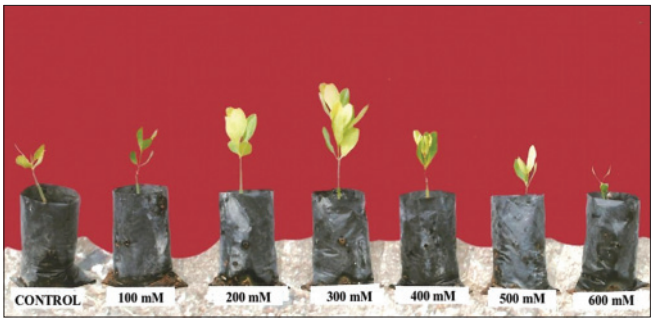


Figure 1: Effect of NaCl on the growth of *Ceriops roxburghiana* seedling (30th day)

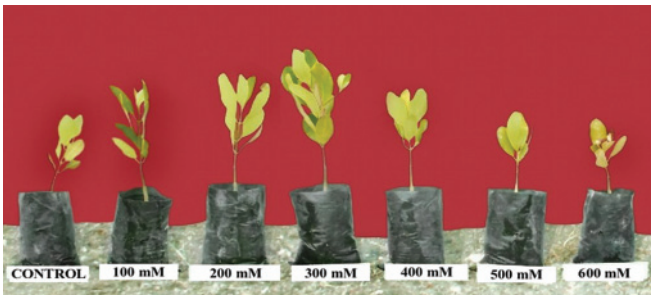


Figure 2: Effect of NaCl on the growth of *Ceriops roxburghiana* seedling (60th day)

plants; DWs was recorded after drying the tissue at 80°C in a hot air oven for 48 h.

Photosynthetic Pigment Determination (Lichtentaler, 1994)

80% acetone extracts were prepared from the freshly collected leaves. Absorbance was measured at 470 nm, 645 nm, and 663 nm using a spectrophotometer and concentrations of chlorophyl *a*, *b*, the total chlorophyll and carotenoids were determined.

Determination of Total Free Amino Acid (Moore and Stein, 1948)

The leaf, stem and root tissues were treated with 80% boiling ethanol for taking extract (5 ml extract demonstrating 1 g tissue). One ml of ethanol extract was taken in 25 ml test tube and neutralized with 0.1 N sodium hydroxide using methyl red indicator. One ml of ninhydrin reagent was added (800 mg stannous chloride in 500 ml citrate buffer pH, 5.0, 20 g ninhydrin in 500 ml methylcellosolve, both solutions were mixed). The contents were boiled in a water bath for 20 min and 5 ml of diluting solution (distilled water [d.H_2O] and n-propanol mixed in equal volume) was added, cooled and diluted to 25 ml with d.H_2O. The absorbance was measured at 570 nm in a spectrophotometer.

Determination of Soluble Protein (Lowry *et al*., 1951)

Five hundred mg of plant sample was macerated with a pestle and mortar with 10 ml of 20% trichloroacetic acid. The homogenate was centrifuged for 15 min at 600 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged. The supernatant was taken and made up to 5 ml with 0.1 N NaOH. This extract was used for the estimation of protein. Five ml of copper solution was added to tubes containing 0.1 ml of the protein extract. The copper solution composed of 100 ml of sodium chloride (0.1 N) in which were dissolved, 2 g of anhydrous sodium carbonates and 1 ml of sodium tartrate (2.7%), 1 ml of copper sulfate (1%) were mixed immediately before use and the tubes were left for 15 min, 0.5 ml of Folin-phenol reagent [Folin–Ciocalteu and d.H_2O were mixed in the ratio 1:2 (v/v)] was added to this solution and kept at room temperature (30°C) for 10 min, then the optical density (OD) was measured at 570 nm. The same steps were repeated with the standard solution (of known concentration) of bovine serum albumin. Steps (1 and 2) were repeated thrice, and the mean value of the three readings was compared with the standard curve of bovine serum albumin.

Determination of Total Soluble Sugar (Jayaraman, 1981)

Total soluble sugars were determined with anthrone reaction. Each 1.0 g of fresh leaf tissue was extracted with 25 ml of d.H_2O. The extract was incubated for 30 min at 100°C. The extract was used for the detection of total soluble sugars. Plant extract was mixed with 5 ml of anthrone, heated in a boiling water bath for 10 min, then cooled on ice for 10 min and incubated for 20 min at room temperature (25°C). The absorbance (OD) was read under the visible light with wavelength 625 nm. Spectrophotometer was used for all the following spectrophotometric reading unless specified. The soluble sugars concentration was determined from a standard curve.

Determination of Starch (Thayumanavan and Sadasivam, 1984)

Fresh roots and shoots (250 mg each) were independently homogenized in hot 80% ethanol (v/v) to eliminate sugars. Residue was retained after centrifugation at 5000 × *g* for 15 min at room temperature. The starch was extracted by 52% perchloric acid at 0°C for 20 min. Starch was estimated by using anthrone reagent spectrophotometrically at 630 nm wavelength on a ultraviolet-visible spectrophotometer and calculated from graph plotted using glucose as a standard.

Determination of Proline (Bates *et al*., 1973)

Each 1.0 g of fresh leaf tissue was homogenized with 5 ml of 3% sulfosalicylic acid and was extracted at 100°C for 10 min. 2.0 ml of supernatant with 2.0 ml of d.H_2O, 2.0 ml of glacial acetic acid, 4.0 ml of acid ninhydrin solution (0.75 g ninhydrin in 30 ml of glacial acetic acid) and 2.0 ml of sulfosalicylic acid was extracted for 1 h at 100°C. The reaction was terminated on an ice bath. The reaction mixture was extracted with 4 ml of toluene mixed vigorously by passing a continuous stream of air for 2 min. Chromophore containing toluene was aspirated from the aqueous phase; 1.0 ml of chromophore containing toluene was warmed to room temperature and absorbance were read under the visible light with wavelength 520 nm. The proline concentration was determined from a standard curve.

Determination of Glycine Betaine Content (Grieve and Grattan, 1983)

The dried plant material was finely ground, mechanically shaken with 20 ml deionized water for 24 h at 25°C. The samples were then filtered, and the filtrates were

diluted (1:1) with 2 N sulfuric acid (H_2SO_4). Aliquots (0.5 ml) were taken into centrifuge tubes and cooled in ice for 1 h. Cold KI-I_2 reagent (0.20 ml) was added, and then reactants were gently stirred (KI-I_2 reagent: 15.7 g of iodine and 20 g of potassium iodide were dissolved in 100 ml of d.H_2O). The tubes were stored at 4°C for 16 h and then centrifuged at 10,000 rpm for 15 min at 0°C. The supernatant was carefully aspirated with a fine tipped glass tube. The iodide crystals were dissolved in 9.0 ml of 1, 2-dichloroethane and mixed vigorously. After 2 h the absorbance was measured at 365 nm by using a spectrophotometer.

RESULTS

Growth Parameters

Root and shoot length

Sodium chloride treatment increased the shoot and root growth with increasing concentrations up to 300 mM and this concentration was found to be optimal and promoted the maximum growth (Graph 1). Concentration of NaCl beyond 300 mM showed a gradual reduction in the root (5.8 and 11.5 cm/plant) and shoot growth (15.6 and 17.3 cm/plant). The 30th and 60th day seedlings at an extreme salinity of 600 mM showed a highly retorted growth.

FW

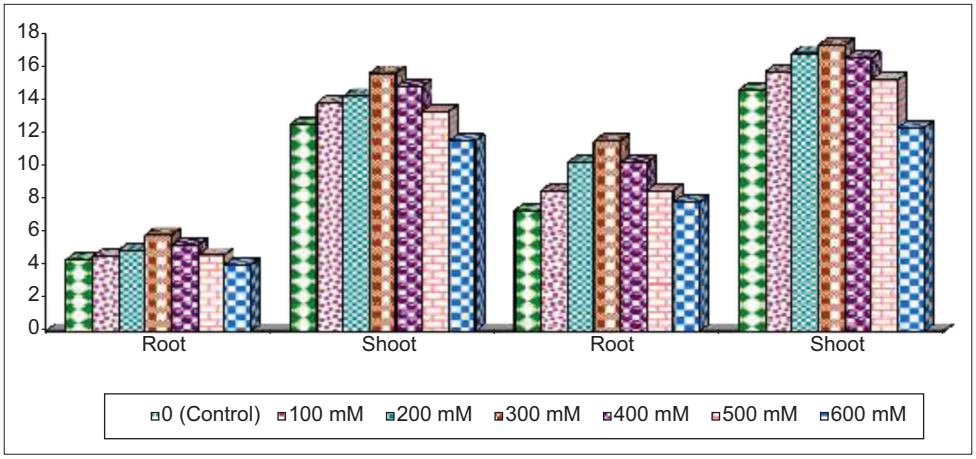
The results on the effect of NaCl on the FW of leaf and root of *C. roxburghiana* are given in Graph 2. Increase in the FW of the leaf (15.7 and 17.8 g/plant) and root (8.10 and 9.90 g/plant) up to an optimum concentration of 300 mM NaCl on 30th and 60th day seedlings.

DW

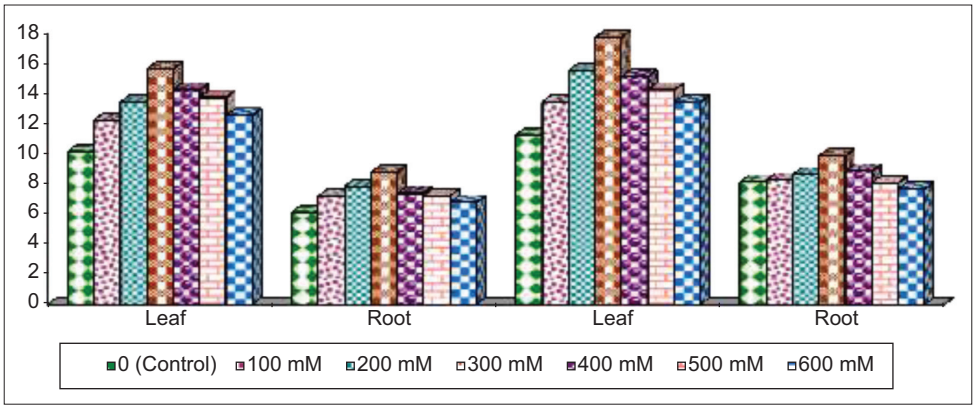
The results on the effect of NaCl on the DW of shoot and root of *C. roxburghiana* are given in Graph 3. The DW of the leaf (6.3 and 7.8 g/plant) and root (3.00 and 3.80 g/plant) increased with increasing NaCl salinity up to 300 mM on all the sampling days. At higher concentrations, there was a continuing decrease in the DW of seedlings.

Photosynthetic pigments

The data on the changes in the chlorophyll and carotenoid content in the leaf of saline treated plants showed that



Graph 1: Effect of NaCl on root and shoot length (g/plant) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment



Graph 2: Effect of NaCl on fresh weight content (g/plant) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment

NaCl stimulated the chlorophyll synthesis up to optimal concentration of 300 mM (Graph 4) on all the sampling days. At higher concentration there was decrease in the chlorophyll and carotenoid content. chl "*a*," chl "*b*," total chlorophyll and carotenoid value ranges between 0.162 and 1.135; 0.525 and 0.928; 1.137 and 2.243; 0.188 and 0.412 mg/g fr. wt. at 300 mM NaCl concentration on 30th and 60th day, respectively.

Biochemical Determinations

Total free amino acid

Changes in the total free amino acid at different levels of NaCl salinity were observed, and the results are presented in Graph 5. The amino acid content in leaf (2.01 and 3.5 mg/g fr. wt.) and root (3.43 and 4.75 mg/g fr. wt.) decreased with increasing salinity up to 300 mM on all the sampling days and the maximum was observed at 600 mM on 30th and 60th day, respectively.

Soluble protein

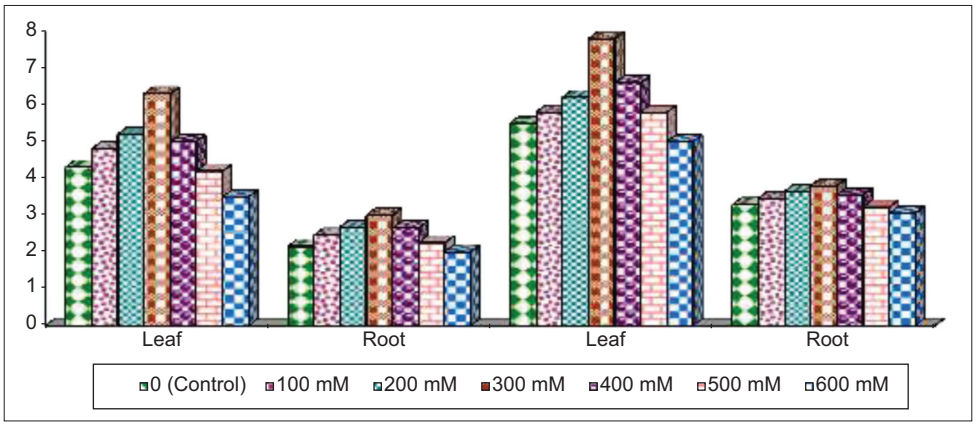
The results on the effect of NaCl on the protein content in leaf (5.10 and 5.68 mg/g fr. wt.) and root (3.59 and 4.35 mg/g fr. wt.) are presented in Graph 6. The protein content increased with increasing salinity up to 300 mM and all the sampling days, with the maximum increase on the 30th and 60th day. The leaf had more protein than the root.

Total soluble sugar

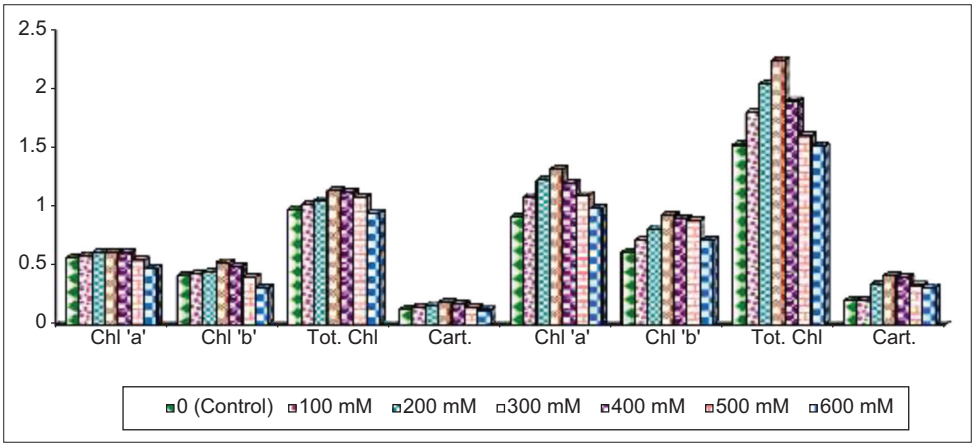
Changes in the total sugar content in leaf (6.50 and 7.50 mg/g fr. wt.) and root (2.96 and 5.18 mg/g fr. wt.) in response to different concentrations of NaCl are given in Graph 7. There was a gradual decrease in the total sugar content with increasing salinity up to the optimal level (300 mM) in leaf and root on 30th and 60th day, respectively. At higher salt concentration the total sugar content also increases.

Starch

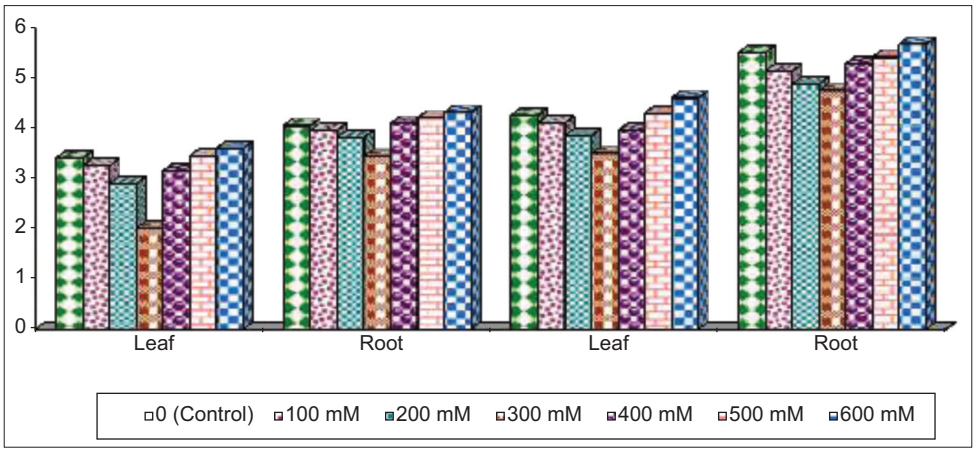
The data on the starch content of the leaf (5.12 and 6.15 mg/g fr. wt.) and root (4.10 and 5.95 mg/g fr. wt.) at different salinity levels are presented in Graph 8. The starch content increased with increasing salinity up to 300 mM in leaf and root on 30th and 60th day, respectively. At higher concentrations, there was a gradual decrease in the starch.



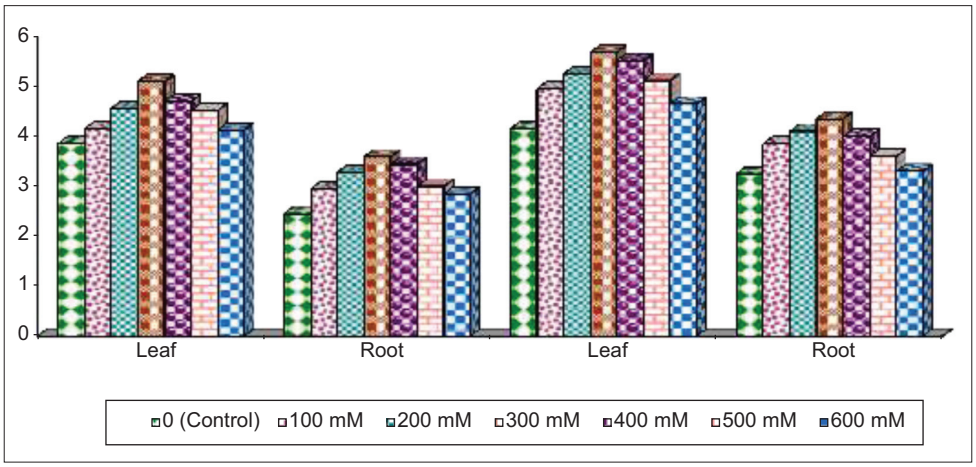
Graph 3: Effect of NaCl on dry weight content (g/plant) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment



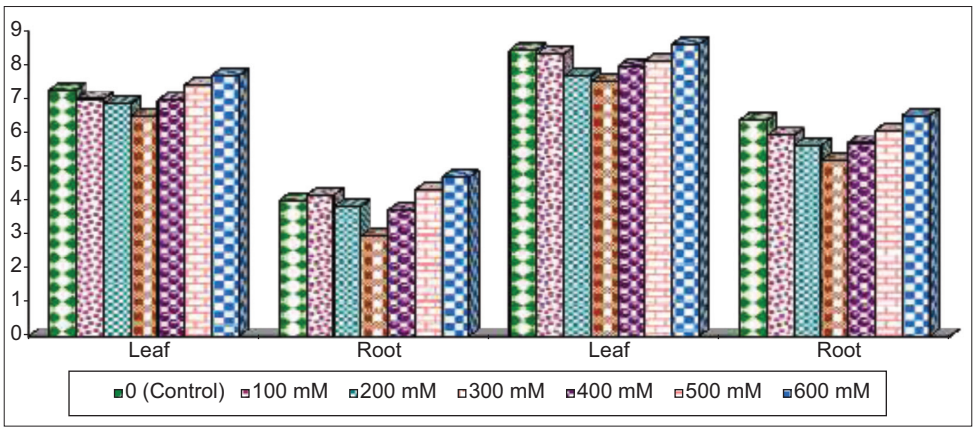
Graph 4: Effect of NaCl on photosynthetic pigment (mg/g fr. wt.) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment



Graph 5: Effect of NaCl on total free amino acid content (mg/g fr. wt.) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment



Graph 6: Effect of NaCl on soluble protein content (mg/g fr. wt.) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment



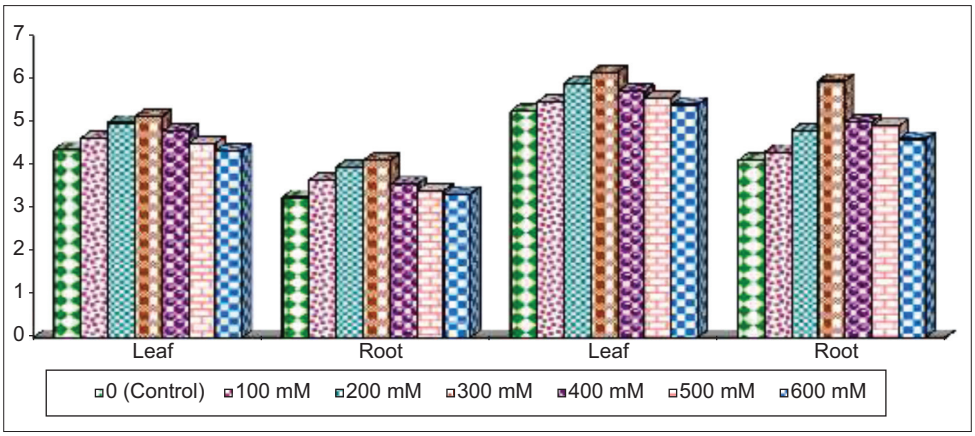
Graph 7: Effect of NaCl on total soluble sugar content (mg/g fr. wt.) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment

Proline

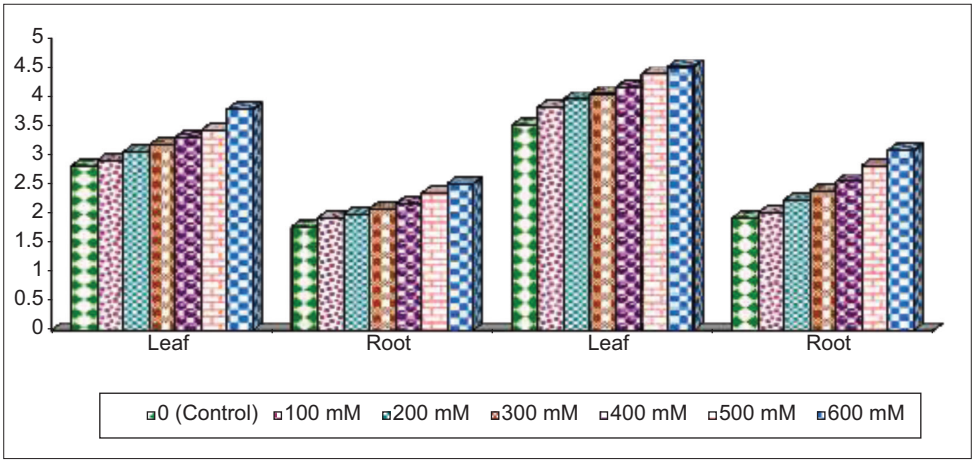
In general, proline accumulation increased significantly under various concentrations of NaCl and was maximum at higher salt stress (600 mM) in leaf (3.78 and 4.57 mg/g fr. wt.) and root (2.51 and 3.08 mg/g fr. wt.) on 30th and 60th day seedlings (Graph 9).

Glycine betaine

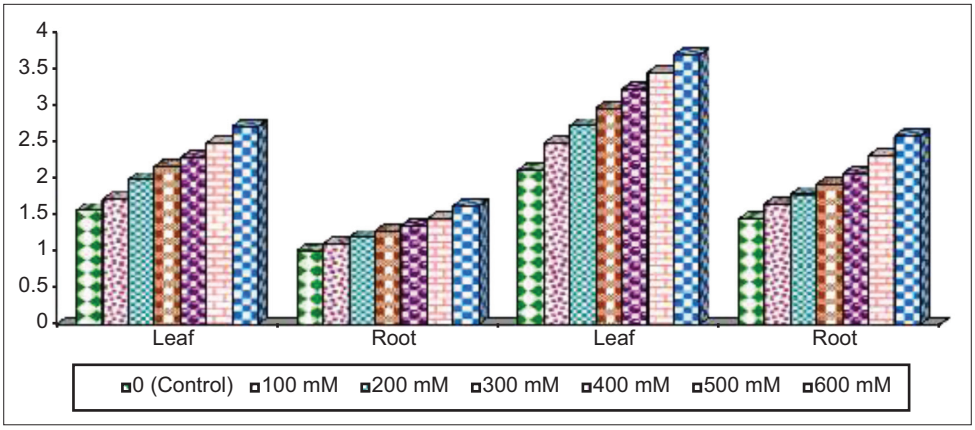
Similarly, the glycine betaine content was also increased significantly at all concentrations on 30th and 60th day seedlings (leaf 2.71 and 3.69 mg/g fr. wt. and root 1.62 and 2.58 mg/g fr. wt.) of NaCl compared with 0 mM NaCl (Graph 10).



Graph 8: Effect of NaCl on starch content (mg/g fr. wt.) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment



Graph 9: Effect of NaCl on proline content (mg/g fr. wt.) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment



Graph 10: Effect of NaCl on glycine betaine content (mg/g fr. wt.) of *Ceriops roxburghiana* on 30th and 60th day after salt treatment

DISCUSSION

In the present investigation, *C. roxburghiana* was found to survive in NaCl concentration up to 600 mM. However, the favorable effect for maximum growth and development was noticed at 300 mM NaCl). A stimulation of growth in response to moderate levels of NaCl salinity has been reported for several halophytic plants. The growth of *Sesuvium portulacastrum* showed positive result to NaCl concentrations up to 600 mM and the upper limit for survival of this species was 900 mM (Ashraf, 1999). Then again KCl was exposed to be a lesser amount of effectual within promoting growth and in several instances was poisonous. There is confirmation intended for synergism

between NaCl and KCl (Duncan, 2000). The decline in leaves quantity at high salt concentrations was due to the leaf fall because of senescence. Salinity has been shown to be one of the outdoor factors that influence the process of senescence and the consequential flaking of leaves (Shah *et al.*, 2002). Seedling FWs and DWs in *C. roxburghiana* increased drastically at 300 mM NaCl and declined slowly up to 600 mM. The seedling of species is still able to grow in the presence of 300 mM NaCl and remains alive when confronted to 600 mM NaCl that is a higher dose than salt concentration of seawater. Similar results were previously reported in other halophytic plants (Moghaieb *et al.*, 2004; Amor *et al.*, 2005; Heidari-Sharifabadi and Mirzaie-Nadoshan, 2006). The increase in FW of the plant leaf tissues can be accredited toward the increase in leaf thickness and the accumulation of ions and H_2O in the tissues (Khan *et al.*, 2005).

Sodium chloride salinity stimulated chlorophyll synthesis up to optimum concentration of 300 mM and at upper concentrations the pigment content demonstrates gradually decreased. The NaCl salinities favored chlorophyll mixture in the leaves of *C. roxburghiana* equal to the optimal concentrations of the respective salts. However, at a high level of salt content concentrations, the salt content had decreased the chlorophyll content. A constructive result of NaCl salinity on chlorophyll pigment synthesis in the halophytic plants has been reported (Rajaravindran and Natarajan, 2012). On the other hand, reduce in the chlorophyll content above high salinity have been reported in an amount of halophytic plants (Khan *et al.*, 2000). Along with the increase in total chlorophyll content, there was increase in the carotenoid pigment up to 300 mM in *C. roxburghiana*. Sabra *et al.* (2012) reported so as to the uppermost salt concentration condensed Chl *a*, Chl *b* and carotenoid contents in *Echinacea purpurea* and *E. angustifolia* and generally in mutual species, this decline was correlated with shoot Na^+ content rather than Cl^-, suggesting that Na^+ was the major ion causing pigment reduction. Nevertheless, in other plant genus like *Vicia faba*, the reduction in the leaf Chlorophyll was rigorously accredited to Cl^- accumulation in the leaves (Tavakkoli *et al.*, 2010).

Free amino acids in the leaf and root of *C. roxburghiana* significantly decrease with increasing concentrations of NaCl up to 300 mM. Beyond this concentration an increase in amino acids was observed. Increasing concentrations of NaCl up to 300 mM had decreased the free amino acid and sugar content and at higher salt concentration, readily available was a continuing increase in the amino acid and sugar content. Buildup of a number of amino acids viz., asparagines, aspartic acid as well as reversible trend was observed (Shah *et al.*, 2002). Increased content of amino acids through increase in salinity was observed in many other crops (Khan *et al.*, 2000). Superior accumulation of the amino acids was also observed in young plants in reaction to increased seawater salinity in growth medium (Shah *et al.*, 2002). The accumulation of free amino acids in salt stressed plants may be due to a reduction in the incorporation and conversion of amino acid into protein as observed by Silambarasan and Natrajan (2014). The pattern of the alterations in soluble protein demonstrated a reverse tendency to that of free amino acids implying that the increase in protein content perhaps at the expenditure of the amino acids and that the NaCl salinity changes influenced the interconversion of these compounds. Similar findings were observed in various halophytic species such as *Heleochloa setulosa* (Joshi *et al.*, 2002). Increase in protein was associated with the decrease in the amino acids content under moderate salinity and a reverse trend was noticed at higher salinity ranges. Salinity was shown to reduce the protein content accompanied by a considerable increase in the pigment and amino acid content (Silambarasan and Natrajan, 2014). A few reports are extremely decreased protein contents of leaves in glycophytes (Wang and Han, 2009) even in a non-secreting mangrove *Bruguiera parviflora* (Parida *et al.*, 2002) in response to salinity. Proteins perhaps synthesized in reaction to salt stress or perhaps present constituently at low concentration and increase as soon as plants are uncovered to salinity stress (Bartles and Sunkar, 2005).

There was a decrease in the sugar content up to optimum salinity level of 300 mM NaCl. Beyond this level, it gradually increased. Under severe salinity stress, the decrease in sugar content possibly will be either because of high respiration or a reduction in photosynthetic activity accompanied by decrease in growth rate. A rising sugar content and matching reduce in the starch at higher salinities have been reported in few halophytes (Rajaravindran and Natarajan, 2012; Ashraf and Harris, 2004). Sucrose is considered to be the principal substrate for starch production. The starch content improved with rising salinity up to 300 mM and decreased with higher salinity. The increase in starch and reduce in total sugar under salinity has been accredited to the function of Na on the opening stomata. The starch content had increased with increasing NaCl and KCl salinity capable of most favorable levels. The reverse condition was noticed at upper salinity. The increase in starch content and reduce in total sugar had been recognized to the role of sodium on the stomatal opening (Shah *et al.*, 2002). It has moreover recognized that starch content increases below

the conditions anywhere sucrose content is decreased (Rajakumar, 2013).

The rising buildup of proline was established among increasing concentrations of salinity. The accretion of proline was additional in the leaves than in the stem and root tissues of salt treated plants. NaCl tolerance has been related with the capability of a species to build up proline and it acts as an intracellular osmotic (Ben Hassine *et al.*, 2008). The current investigations are in accordance with more than a few studies that proline content gradually augmented with high levels of NaCl in *S. portulacastrum* (Ramani *et al.*, 2006) and *Odyssea paucinervis* (Naidoo *et al.*, 2008). A considerable raise in proline content was originated only on over salinity (Rajaravindran and Natarajan, 2012; Wang, *et al.*, 2006). The substantial increase in the glycine betaine content was noticed with escalating NaCl concentrations up to 600 mM. The accumulation was comparatively more in the leaves. Glycine betaine is well thought-out to be a well-suited solute in salt tolerant species and it occurs primarily in the cytoplasm (Silambarasan and Natrajan 2014). The accumulation of glycine betaine was also assumed to have constructive functions in relation to the maintenance of membrane integrity and the constancy of other cellular structures under salt and drought stress has been reported in *Atriplex halimus* (Martínez *et al.*, 2005) and *Atriplex nummularia* (Silveira *et al.*, 2009). Despite the involvement of proline and glycine betaine in osmotic adjustment, entire osmotic balance flanked by the vacuole and the cytoplasm requires the accretion of supplementary organic compounds such as soluble sugars (Pagter *et al.*, 2009). Mangroves store high concentrations of proline and glycine betaine in their leaves (Mickelbart *et al.*, 2003).

CONCLUSION

The present study shows that *C. roxburghiana* is a moderately salt tolerant species. Sodium chloride salinity stimulated its growth, biomass, photosynthetic pigments and biochemical determinations up to the optimum concentration of 300 mM NaCl. Therefore, it is concluded that the species could be recommended for cultivation in salt affected soils to a lessen soil salinity level and the red mated soil can be utilized for cultivation of the crop.

REFERENCES

Amor NB, Hamed KB, Debez A. Physiological and antioxidant response of the perennial halophyte *Crithmum maritimum* to salinity. Plant Sci 2005;168:889-99.

Ansari O, Azadi MS, Sharif-Zadeh F, Younesi E. Effect of hormone priming on germination characteristics and enzyme activity of mountain rye (*Secale montanum*) seeds under drought stress conditions. J Stress Physiol Biochem 2013;9:61-71.

Ansari O, Sharif-Zadeh F. Osmo and hydro priming improvement germination characteristics and enzyme activity of Mountain Rye (*Secale montanum*) seeds under drought stress. J Stress Physiol Biochem 2012;8:253-61.

Ashraf M, Harris PJC. Potential biochemical indicators of salinity tolerance in plants. Plant Sci 2004;166:3-16.

Ashraf M. Breeding for salinity tolerance in plants. Crit Rev Plant Sci 1999;13:17-42.

Ball MC, Sobrado MA. Ecophysiology of mangroves: Challenges in linking physiological process with patterns in forest structure. In: Press MC, Scholes JD, Barker MG, editors. Advances in Plant Physiological Ecology. Oxford: Blackwell Science; 1999. p. 331-46.

Ball MC. Salinity tolerance in the mangroves *Aegiceras corniculatum* and *Avicennia marina* L. Water use in relation to growth, carbon partitioning and salt balance. Aust J Plant Physiol 1988;15:447-64.

Banerjee LK, Sastry AR, Nayar MP. Mangroves in India: Identification Manual. New Delhi: Publisher Botanical Survey of India; 1989.

Bartles D, Sunkar R. Drought and salt tolerance in plants. Crit Rev Plant Sci 2005;24:23-58.

Bates LE, Waldern RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil 1973;39:205-7.

Ben Hassine A, Ghanem ME, Bouzid S, Lutts S. An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. J Exp Bot 2008;59:1315-26.

Boon PL, Allaway WG. Rates of ionic specificity of salt secretion from excised leaves of the mangrove, *Avicennia marina* (Forsk.) Vierh. Aquat Bot 1986;26:143-53.

Claussen W, Loveys BR, Hawker JS. Comparative investigation on the distribution of sucrose synthase activity and invertase activity within growing mature and old leaves of some C_3 and C_4 plant species. Physiol Plant 1985;65:275-80.

Duncan RR. Plant tolerance to acid soil constraints: Genetic resources, breeding methodology and plant improvement. In: Wilkinson RE, editor. Plant Environment Interaction. 2nd ed. New York: Marcel Dekker; 2000. p. 1-38.

Geissler N, Hussin S, Koyro HW. Elevated atmospheric CO2 concentration enhances salinity tolerance in *Aster tripolium* L. Planta 2010;231:583-94.

Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 1983;70:303-7.

Heidari-Sharifabad H, Mirzaie-Nadoushan H. Salinity induced

growth and some metabolic changes in three *Salsola* species. J Arid Environ 2006;67:715-20.

Jayaraman J. In: Laboratory Manual in Biochemistry. Chennai: Wiley Eastern Limited; 1981. p. 51-3.

Joshi AJ, Sagar Kumar A, Heriglajia H. Effects of seawater on germination, growth, accumulation of organic components and inorganic ions in halophytic grass *Heleochaloa setulosa* (TRIN), Blatt et. Mccann. Indian J Plant Physiol 2002;7:26-30.

Khan MA, Ungar IA, Showalter AM. Salt stimulation and tolerance in an inter tidal stem-succulent halophyte. J Plant Nutr 2005;28:1365-74.

Khan MA, Ungar IA, Showalter AM. The effect of salinity on the growth water status and ion content of a leaf succulent perennial halophyte *Suaeda fruticosa*. J Arid Environ 2000;45:73-84.

Kishor KP, Sangam S, Amrutha RN, Sri Laxmi P, Naidu KR, Rao KR, *et al*. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr Sci 2005;88:424-38.

Lichtentaler HK. Chlorophyll and carotenoids pigments of photosynthetic biomembranes. Method Enzymol 1994;148:350-82.

Liu J, Guo WQ, Shi DC. Seed germination, seedling survival and physiological response of sunflowers under saline and alkaline conditions. Photosynthetica 2010;48:278-86.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.

Martinez Beltran J, Manzur CL. Overview of salinity problems in the world and FAO strategies to address to the problem. Proceedings of the International Salinity Forum California: Riverside; 2005. p. 311-3.

Martínez JP, Kinet JM, Bajji M, Lutts S. NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus* L. J Exp Bot 2005;56:2421-31.

Mickelbart MV Peel G, Joly RJ, Rhodes D, Ejeta G, Goldsbrough PB. Development and characterization of near isogenic lines of sorghum segregating for glycine betaine accumulation. Plant Physiol 2003;118:253-61.

Moghaieb RE, Saneoka H, Fujita K. Effect of salinity on osmotic adjustment, glycine betaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophyte plants, *Salicornia europaea* and *Suaeda maritima*. Plant Sci 2004;166:1345-9.

Moore S, Stein WH. Photometric ninhydrin method for use in the chromatography of amino acids. J Biol Chem 1948;176:367-88.

Munns R. Comparative physiology of salt and water stress. Plant Cell Environ 2002;25:239-250.

Munns R. Effect of high external NaCl concentrations onion transport within shoot of *Lupinus alba*. I. Ions on xylem sap. Plant Cell Environ 1988;11:283-9.

Naidoo G, Somaru R, Char PA. Morphological and physiological responses of the halophyte *Odyssea paucinervis* (Staph) *Poaceae* to salinity. Flora 2008;203:437-7.

Naskar K. Manual of Indian Mangroves. New Delhi: Daya Publishing House; 2004.

Pagter M, Bragato C, Malagoli M, Brix H. Osmotic and ionic effects of NaCl and Na_2SO_4 salinity on *Phragmites australis*. Aquat Bot 2009;90:43-51.

Parida A, Das AB, Das P. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove *Bruguiera parviflora*, in hydroponic cultures. J Plant Biol 2002;45:28-36.

Parker R, Flowers TJ, Moore AL, Harpham NV. An accurate and reproducible method for proteome profiling of the effects of salt stress in the rice leaf lamina. J Exp Bot 2006;57:1109-18.

Rajakumar R. A study on effect of salt stress in the seed germination and biochemical parameters of rice (*Oryza sativa* L.) under *in vitro* condition. Asian J Plant Sci Res 2013;3:20-5.

Rajaravindran M, Natarajan S. Effects of salinity stress on growth and biochemical constituents of the halophyte *Sesuvium portulacastrum*. Int J Res Biol Sci 2012;2:18-25.

Ramani B, Reeck T, Debez A, Stelzer R, Huchzermeyer B, Schmidt A, *et al*. *Aster tripolium* L. and *Sesuvium portulacastrum* L.: Two halophytes, two strategies to survive in saline habitats. Plant Physiol Biochem 2006;44:395-408.

Sabra A, Daayf F, Renaulta S. Differential physiological and biochemical responses of three *Echinacea* species to salinity stress. Sci Hortic 2012;135:23-31.

Shah SD, Tobita S, Shono M. Cation co-tolerance phenomenon in cell cultures of *Oryza sativa* adapted to NaCl. Plant Cell Tissue Organ Cult 2002;71:95-101.

Silambarasan N, Natarajan S. Biochemical responses of Sankankuppi (*Clerodendron inerme* L.) to salinity stress. Afr J Agric Res 2014;9:1151-60.

Silveira JA, Araujo SA, Lima JP, Viegas RA. Roots and leaves display contrasting osmotic adjustment mechanisms in response to NaCl-salinity in *Atriplex nummularia*. Environ Exp Bot 2009;66:1-8.

Smith JA, Poop M, Lutge U, Cram WJ, Diaz M, Griffith H, *et al*. Ecophysiology of xerophytic and allophytic vegetation of a costal alluvial plain in northern Venezuela. VI. Water relations and gas exchange of mangroves. N Phytol 1989;11:293-307.

Taji T, Seki M, Satou M, Sakurai T, Kobayashi M, Ishiyama K, *et al*. Comparative genomics in salt tolerance between

Arabidopsis and *Arabidopsis*-related halophyte salt cress using *Arabidopsis* microarray. Plant Physiol 2004;135:1697-709.

Tavakkoli E, Rengasamy P, McDonald GK. High concentrations of Na and Cl- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. J Exp Bot 2010;61:4449-59.

Thayumanavan B, Sadasivam S. Physicochemical basis for the preferential uses of certain rice varieties. Qual Plant Foods Hum Nutr 1984;34:253.

Tomlinson PB. The Botany of Mangroves. London: Cambridge University Press; 1986.

Trovato M, Mattioli R, Costantino P. Multiple roles of proline in plant stress tolerance and development. Rend Lincei 2008;19:325-46.

Ueda A, Kanechi M, Uno Y, Inagaki N. Photosynthetic limitations of a halophyte sea aster (*Aster tripolium* L) under water stress and NaCl stress. J Plant Res 2003;116:65-70.

Wang XS, Han JG. Changes in proline content, activity, and active isoforms of antioxidative enzymes in two alfalfa cultivars under salt stress. Agric Sci China 2009;8:431-40.

Wang Z, Yuan Y, Quan J, Hualin WO, Zhang MC. Glutamine synthetase and glutamate dehydrogenase contribute differentially to proline accumulation in leaves of wheat (*Triticum aestivum*) seedlings exposed to different salinity. J Plant Physiol 2006;164:695-701.