

DIFFERENTIAL EXPRESSION IN MORPHOLOGY AND PHOTOSYNTHETIC PIGMENT COMPOSITION OF *CERIOPS DECANDRA* AFTER NaCl STRESS

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Summary: The effect of sodium chloride on growth and pigment composition of *Ceriops decandra* were investigated. Thirty days old *Ceriops decandra* propagules were grown in polythene bags and treated with sodium chloride solution on 30 Days after planting (DAP) at the concentration of 200, 400, 600, 800, and 1000 mM by soil drenching method. The shoot and root length, number of leaves and roots, fresh and dry weight of leaves and roots were studied on the 60th, 90th and 120th DAP. The results showed that the maximum growth and biomass were observed in plants treated with 400 mM concentration. The pigment analysis showed no significant changes in chlorophyll and carotenoid content.

Keywords: NaCl; morphology; pigments; leaf area; pichavaram; *Ceriops*.

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INTRODUCTION

Mangroves are woody plants that inhabit intertidal zones with high salinity and can tolerate a wide range of salinity under natural conditions (Shan et al., 2008). Mangroves display a range of adaptive responses to their specific habitat, including salt exclusion by root ultra-filtration (Scholander, 1968) and salt secretion (elimination of substances not metabolically changed) via glands (Roth, 1992). The mechanism of salt tolerance of mangrove plants at organ level has been reported by Ball and Farquhr (1984). High salinity causes

severe damage to plants, including growth inhibition, impaired metabolism, necrosis and loss of production and quality. Spatial and temporal changes in salinity could affect the growth and physiology of plants (Naidoo, 1985). In general, the growth of mangrove plants usually declines at high salinity, but nevertheless an optimal growth was obtained at moderate salinity (Clough, 1984). As a function of tolerance, salinity stress decreases the leaf water potential of the plant (Clough, 1984), retarded the growth of plants and

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lowered the water potential resulting in variation in sap osmotic pressure, salt exclusion at the root level and active salt excretion through leaves (Hutchings and Saengar, 1987). Stress might occur as a complex mechanism of several interacting environmental factors that cause variations in plant phenotype, as plants respond to complex growth conditions (Shao et al., 2007). The plant cell and tissue culture methods could be useful in studying the salinity tolerance mechanism in plants and their effects on crop production when they are not evidently known (Akinici et al., 2004). The level of salinity required for optimal growth varies from 10 to 50% seawater (Downton, 1982; Clough, 1984; Naidoo, 1985) and a decline in growth occurs with a further increase in salinity. The plant ability to adapt to the salt stress includes alterations at leaf level, associated with morphological, physiological and biochemical characteristic where by many plants adjust to high salinity and low soil water availability (Cicek and Cakirlar, 2008). Many studies have shown that the fresh and dry weights of the shoot and root system are affected, either negatively or positively, by changes in salinity concentration, type of salt present, or type of plant species (Taffouo et al., 2009; Memon et al., 2010). Photosynthesis is one of the most important biochemical pathways by which plant prepare their food material and grows. As a matter of fact, there has been knowledge on increase of chlorophyll content in saline environment depending on salt concentration. Many studies confirm that the inhibitory effect of salinity on biochemical processes, of which photosynthesis is the most

important. The effect on photosynthesis can be gauged from the effect on the photosynthetic pigments. The results of specific studies (Taffouo et al., 2010) clearly indicate that salinity reduces the content of photosynthetic pigments in treated plants. This study is to assess the effect of different concentration of NaCl on total length of the shoot and root, number of leaves and root, total leaf area, fresh and dry weight of leaf, stem and root and pigment composition of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content in *Ceriops decandra*.

MATERIAL AND METHODS

Collection of propagules

Propagules of *Ceriops decandra* belongs to the family *Rhizophoraceae* were collected from Pichavaram mangrove forest situated at 11° 27' N latitude and 79° 47' E longitude, in the East coastal region of Tamilnadu, India.

Plant materials and culture conditions

Ceriops decandra propagules were raised in the green house with non-saline and non-brackish water under Green shade net in Botanical garden, Department of Botany, Annamalai University. 30 days old healthy seedlings were selected for NaCl treatment. The preliminary experiments were carried at different concentrations of NaCl (200, 400, 600, 800, 1000 mM) solution by soil drenching method. Above 800 mM NaCl concentrations the seedlings did not survived. The experimental plants were treated with various concentrations of NaCl solution by soil drenching

method. The treatment was given on one in two days. 200 mM, 400 mM, 600 mM and 800 mM NaCl treated seedlings were alone maintained in the experimental plot. Each treatment has five replicates. The experimental yard was roofed with transparent polythene sheet at a height of 3m from the ground in order to protect the plants from rain.

Morphological studies

During each and every sampling day, samples were randomly collected, washed thoroughly with tap water followed by distilled water. The total length of the shoot and root, number of leaves and roots per plant were calculated. Five plants were collected from each concentration and used for studying the morphological parameters.

Total leaf area

The total leaf area was calculated by measuring the length, width and number of leaves and multiplied by a correlation factor (0.66) derived from the method of Yoshida et al. (1972).

$$\text{Leaf area (cm}^2\text{)} = L \times W \times 0.66.$$

Fresh and dry weight (g plant⁻¹)

For the estimation of fresh weight, leaf, stem and root portions were separated and weighed. They were dried in a hot air oven at 80°C for 24 hours. Then, the dry weight was taken by using an electronic balance.

Photosynthetic pigment determination

One gram of fresh tissue, taken from the third and fourth leaf, was extracted by grinding in a mortar using 20 ml 80% acetone, a small amount of pure (Silica Quartz), and 0.5 g calcium carbonate

to equalize the cellular sap acidity. The extract was filtered using a glass funnel (Sentered glass funnel G4) and collected in a conical flask. The residue was re-extracted using the same method, until it became devoided of color. All the filtrate was collected in a standard flask and the volume completed to a specific amount by adding 80% acetone. The optical density (O.D.) of the extract was measured at wave lengths 663, 645, and 440.5 nm to estimate chlorophyll 'a' and 'b', and carotenes respectively, using a Spectrophotometer (Spectronic 21D) and a vitreous cell (thickness of photo route 1 cm). Three replicates were used for each treatment, and the amount of pigment present in each sample was calculated according to the following equations:

$$\text{Chl 'a'} = X = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$\text{Chl 'b'} = Y = 22.9 \times A_{645} - 4.68 \times A_{663}$$

$$\text{Chl 'a'/'b'} = X/Y$$

$$\text{Carotenoids (}\mu\text{g/ml)} = A_{480} + (0.114 \times A_{663} - 0.638 \times A_{645})$$

RESULTS

Effect of NaCl on shoot and root length

Inhibition of shoot length was observed in increasing concentrations of NaCl on the 60th days after planting (DAP) of *Ceriops decandra* (Figure 1). However the maximum shoot length was obtained at 400 mM NaCl on 120 DAP. At higher concentrations of NaCl treatment the shoot length was decreased when compared to control plants. The root length increased marginally in NaCl salinity up to 400 mM concentrations. At concentration of 800 mM NaCl, plants showed decreased root length to a larger extent on 120 DAP.

Number of leaves and roots

The total number of leaves per plant increased in all regimes of NaCl concentration on the 60th DAP (Figure 2). On the 90th and 120th DAP moderate increase was registered at 200 mM and 400 mM NaCl treatment but there was no any further changes at 600 mM and 800 mM NaCl concentrations in all growth stages. Prominent increase in the number of roots was observed at 200 mM and 400 mM NaCl treatments on the 60th and 90th DAP.

Leaf area

The total leaf area increased with the age in control as well as in NaCl treated plants (Figure 3). On the 90th DAP the 800 mM NaCl treated plants manifested an increased leaf area, but at maturation stage (120th DAP) the leaf area was not significantly changed.

Fresh and dry weight

A prominent gain in fresh weight was observed in the leaves of *Ceriops decandra* subjected to NaCl stress from control to 400 mM of NaCl concentration (Figure 4). At 200 mM and 400 mM NaCl concentrations showed significant increase in the fresh weight on the 60th and the 90th DAP. The stem fresh weight increased with the age in control and treated plants up to 400 mM NaCl concentration in all the growth stages. But it decreased when salt concentration was increased. The maximum fresh weight of roots was observed at 400 mM NaCl concentration on the 90th DAP. At 600 mM and 800 mM NaCl concentrations the root fresh weight was decreased when compared to other treatments and increased when compared to control in all growth stages.

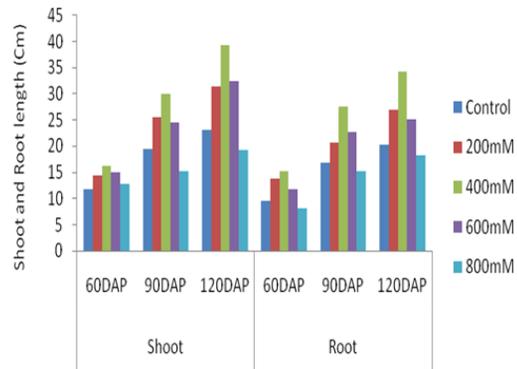


Figure 1. Effect of NaCl on shoot and root length of *Ceriops decandra* on 60DAP, 90DAP, and 120DAP.

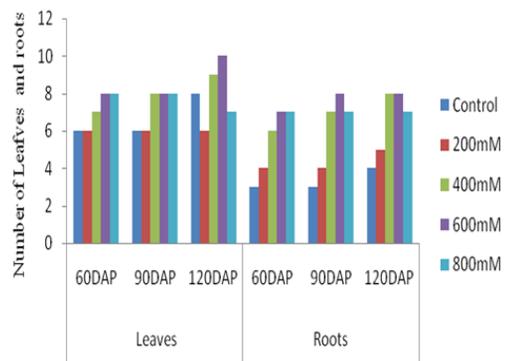


Figure 2. Effect of NaCl on number of leaves and roots of *Ceriops decandra* on 60DAP, 90DAP, and 120DAP.

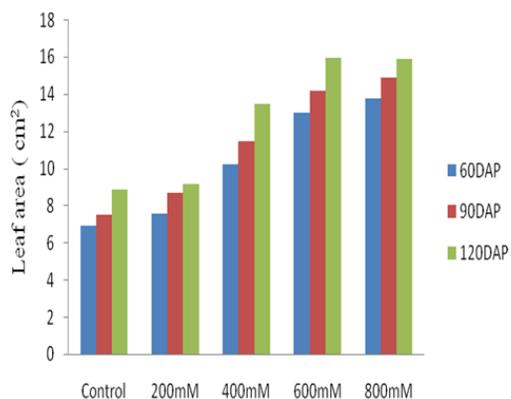


Figure 3. Effect of NaCl on leaf area of *Ceriops decandra* on 60DAP, 90DAP, and 120DAP.

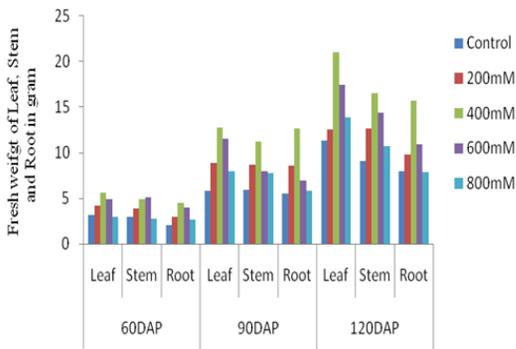


Figure 4. Effect of NaCl on fresh weight of leaves, stems and roots of *Ceriops decandra* on 60DAP, 90DAP, and 120DAP.

Dry weight

Dry weight of leaves was affected in varying pattern in *Ceriops decandra* under sodium chloride stress (Figure 5). Reduction in dry weight in the leaves was observed when NaCl concentration was increased. In 600 mM and 800 mM NaCl concentration the leaf dry weight was decreased when compared to other treatments and increased when compared to control. The dry weight of stem also showed maximum biomass at 400 mM NaCl concentration. Decreased dry matter was observed when were treated with higher concentration of NaCl. The decreased stem dry weight was recorded at 600 mM and 800 mM NaCl concentrations in all growth stages. The root dry weight increased in the plants when treated with 400 mM NaCl concentration. The root dry weight reached maximum level at 400 mM NaCl concentration on the 60th DAP. The root dry matter decreased when salt concentration was increased.

Photosynthetic pigments

Chlorophyll a

NaCl salinity affected the chlorophyll a content in *Ceriops decandra* at 400 mM

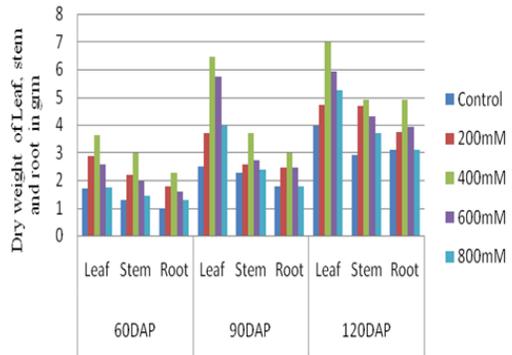


Figure 5. Effect of NaCl on dry weight of leaves, stems and roots of *Ceriops decandra* on 60DAP, 90DAP, and 120DAP.

NaCl concentration on 90thDAP (Figure 6). When salinity increased the chlorophyll a content decreased progressively. At 120th DAP the chlorophyll content was decreased at 600 mM and 800 mM NaCl concentrations when compared to other treatments.

Chlorophyll b

The chlorophyll b content was higher than that of chlorophyll a in the leaves of control and treated plant (Figure 6). Variations were observed in chlorophyll b content also but at 200 mM and 400 mM NaCl concentrations the chlorophyll b content increased. The chlorophyll b content was decreased at 600 mM and 800 mM in all growth stages.

Total chlorophyll

Increased content in total chlorophyll was observed with increasing salinity but above 400 mM NaCl the total chlorophyll was decreased. For example, the increment in chlorophyll concentration was noted at 200 mM and 400 mM NaCl concentrations but not after 600 mM and 800 mM NaCl treatment (Figure 6).

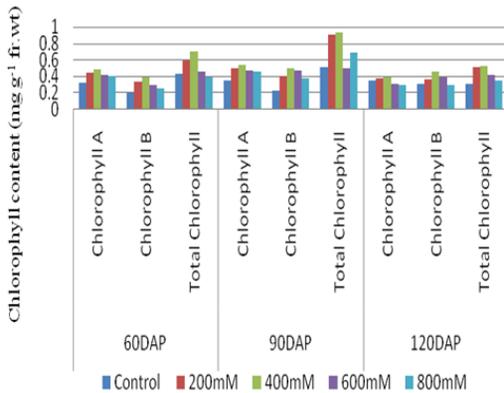


Figure 6. Effect of NaCl on chlorophyll content in leaves of *Ceriops decandra* on 60DAP, 90DAP, and 120DAP.

Carotenoids

The carotenoid content of *Ceriops decandra* increased at 200 mM and 400 mM NaCl concentrations (Figure 7). Significant results of carotenoid content were observed at 400 mM NaCl on the 60th, and 90th DAP. At 600 mM and 800 mM NaCl the carotenoid content decreased when compared to previous treatments during 60th, 90th, 120th DAP.

DISCUSSION

Morphological changes

It is well known that growth inhibition is a common response to salinity. The inhibitory effects of salt stress on growth parameters were also reported by other researchers using various plants (Shaddad et al., 1982; Soussi et al., 1998; Chaparzadeh et al., 2004). Almost all the studies in which mangrove was grown in a laboratory under controlled conditions confirm that maximum growth does not occur in fresh water (Flower et al., 1977; Downton, 1982; Clough, 1984; Zheng and Lin, 1992; Wang and Lin, 1999), and our present study was no exception.

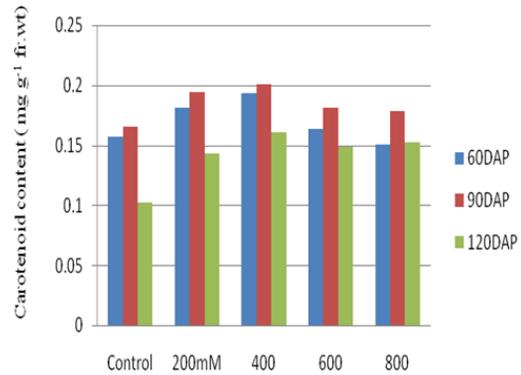


Figure 7. Effect of NaCl on carotenoid content of *Ceriops decandra* on 60DAP, 90DAP, and 120DAP.

Moderate levels of salinity always facilitate seedling growth but extreme levels (600, 800 and 1000 mM) arrest the growth and the same results were obtained in our study. We consider 1000 mM salinity as an extreme salinity because, in a sense, it is a stress as far as the growth of *C. decandra* seedlings is concerned. With prolonged exposure to salt stress, growth was poor at the extreme levels (600 and 800 mM) and better at moderate levels (400 mM). Downton (1982) found that the seedlings of *Avicennia marina* grew rapidly at salinity in the first few weeks but the growth of leaves and buds was affected later on and the accumulation of the dry matter was reduced thus these results correlated with our present results. Clough (1984) found inhibition in the leaf development (leaves were smaller and fewer in number) in *Avicennia marina* and *Rhizophora stylosa* when grown in fresh water, compared with 25, 50, and 75% of seawater salinity. Similar results were also obtained by Pezeshki et al., 1990, who found that the total dry weight of *Laguncularia racemosa*, *Rhizophora mangle* and

Avicennia germinans increased under conditions of salt stress and salt stress combined with water logging.

Photosynthetic pigments

Salinity caused an increase in the total chlorophyll, chlorophyll a, chlorophyll b and carotenoid content in *Ceriops decandra* plants. Photosynthetic pigments content decreased progressively as salt stress increased. Salinity had a stronger effect on Chl b content. The lowest pigments levels were observed in plants treated with 800 mM NaCl. Salt stressed plants contained less carotenoid than the control variant, however there was no significant difference in carotenoid content between various concentrations of NaCl. Similarly, Iyengar and Reddy (1996) have reported that salinity caused a decrease in chlorophyll and carotenoids level in other plants. Decrease in chlorophyll level under salt stress may be due to a reduction in the pigment biosynthesis or enzymatic chlorophyll degradation (Xu et al., 2008 and Yang et al., 2009). The elevation of chl a/chl b ratio under saline condition suggested salinity had the most adverse effect on chlorophyll content. Chlorophyll level is an index of the intensity in photosynthesis and the decrease in chlorophyll level leads to a reduction in plant growth parameters. Slight reduction in carotenoids contents may be due to their protective role against the reactive oxygen species. Salinity can lead to oxidative stress and causing significant decrease to photosynthetic systems. Carotenoids can protect photosynthetic system against reactive oxygen species generated under salt stress (Parida and Das 2005; Parviz and Satyawati, 2008).

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