Effect of long-term salinity on the growth and biomass of two non-secretors mangrove plants *Rhizophora apiculata* and *Ceriops tagal*

M Basyuni1*, A Nuryawan1, Yunasfi1, L A P Putri2 and S Baba3

1 Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Jl. Tri Dharma Ujung No. 1 Medan, North Sumatera 20155, Indonesia
2 Department of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, North Sumatera 20155, Indonesia
3 International Society for Mangrove Ecosystems (ISME), Faculty of Agriculture, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

*E-mail: m.basyuni@usu.ac.id

Abstract. The present study describes the effect of long-term salinity on morphological character and biomass content of two non-secretors mangrove plants *Rhizophora apiculata* and *Ceriops tagal*. Two mangrove seedlings were grown for six months in 0%, 0.5%, 1.5%, 2.0% and 3.0% salt concentration. The growth of *R. apiculata* was significantly enhanced by salt with maximal stimulus at 1.5% (equal to 50% natural seawater), and this increase appeared to be attenuated by increasing the salinity concentration above 1.5%. By contrast, the growth of *C. tagal* thrived up to 0.5% salt concentration. Our findings, therefore, suggested that within the range of treatments used, 1.5% and 0.5%, respectively were the optimal salinity of *R. apiculata* and *C. tagal* for growth. The highest leaf area of *C. tagal* was obtained at 1.5% salinity concentrations and, on the other hand, *R. apiculata* showed much greater extent. The wet and dry weight of the two seedlings was changed in the same manner as the height of plants upon salt treatment. Our results indicated that *R. apiculata* was more salt tolerant than *C. tagal*, which may provide valuable information for mangrove rehabilitation in North Sumatra, Indonesia.

1. Introduction

Mangrove plants comprise a heterogeneous group of independently derived lineages that are denoted ecologically by their location in inter-tidal zones of tropical and sub-tropical areas and physiologically by their ability to withstand high concentrations of salt or low levels of soil aeration [1]. Indonesia is one of the world’s great mangrove nations, covering over 300,000 square kilometers, which is 22.6% of the global total [2]. According to their morphological characteristics in salt management, mangrove plants fell into two broad groups [3]. The first group is the salt-secreting species that have either salt glands or salt hairs to remove excess salt. The second is non-secreting species that do not have such morphological features for excretion of excess salt [3-4]. *Rhizophora apiculata* and *Ceriops tagal* (Rhizophoraceae) are common mangrove species in Pulau Sembilan, North Sumatra, Indonesia and belonging to the latter group.

Mangroves are one of the most threatened ecosystems all over the world today due to direct and indirect deforestation and degradation [5]. Conservation of mangrove implicates not only to protect the
coastal areas and communities from seawater intrusion and potential changes in sea level rise but also to ensure the availability of resources for future use through adaptation to changing environments.

Studies on the salt tolerance of North Sumatran mangroves is rare, and this information is vital to the success of rehabilitation and conservation effort in the region. Our previous studies shed light on the triterpenoid content and gene expressions of triterpenoid synthases in salt-treated and re-adapted mangrove plants [6-8]. It is important to obtain more insight into the morphological aspects of mangrove seedlings to cope with salinity, which may experience temporary periods of exposure to high salt concentrations. The present study, therefore, aimed to investigate the response of two non-secretors mangrove species, *R. apiculata* and *C. tagal* under various salt concentrations with the emphasis on seedling growth and biomass.

2. Materials and methods

2.1. Salt tolerant experiment
Viviparous mature and healthy propagules of two mangrove species *Rhizophora apiculata* BL. and *Ceriops tagal* (Perr.) C.B. Robinson (Rhizophoraceae) were collected from Sembilan Island, Langkat, North Sumatra, Indonesia, and planted in bottle pots with exposure to natural sunlight in a glass house, Faculty of Agriculture, University of Sumatera Utara. A seawater solution was prepared by dissolving commercial salt powder (Tropical Marine Reef Sea Salt, Thailand) to produce a salt concentration of 0%, 0.5%, 1.5%, 2.0% and 3% (equal to the level in sea water) by the manufacturer's protocol. Salinity in this study was defined as the mass of salt powder/weight of solution [9]. Salt concentration in the pots for each treatment was checked once weekly during the experiments using a salinity Refractometer S/Mill-E (Atago Co. Ltd., Tokyo, Japan), and adjusted accordingly. Nine to twelve plants per treatment grew for six months. After six months of cultivation, the two mangrove plants were harvested, washed, and the leaves and roots were stored at −4 °C for further analysis.

2.2. Growth measurement
The growth of *R. apiculata* and *C. tagal* seedlings was determined under varying salt concentrations. The stem heights and diameter of *R. apiculata* and *C. tagal* after six months of cultivation were the indices of growth used in this study. Measurement of the leaf area, the leaves were digitally scanned to quantify leaf area using ImageJ software [10].

2.3. Biomass measurement
Mangrove seedlings were separated into root, shoot, and leaf components to weight before drying. Dry weights of leaves, shoots, and roots of each plant were determined separately on balance, after drying in an oven at 75 ºC for 48 h. From these measurements, we calculated the wet and dry weight of leaves, shoots, and roots of individual seedling of both species.

2.4. Statistical analysis
Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s test for comparisons of all treatments against the control. The value of \( P < 0.05 \) was selected as a limit of statistical significance. All statistical analyses were performed using the SAS 9.1 statistical software program (SAS Institute Inc. Cary, NC, USA).

3. Results and discussion

3.1. Effect of salinity on the seedling growth
Seedling growth was measured by the height and diameter of the plants as described in Materials and Method. The growth of *R. apiculata* enhanced in the presence of salt with maximal stimulation at 1.5%. This increase appeared to be attenuated when the salinity was elevated more than 1.5% (Figure 1A). By contrast, the growth of *C. tagal* thrived up to 0.5% salt concentration (Figure 1A).
The salinity affected the secondary growth of diameter seedlings significantly in 0.5-2.0% salt concentration of *C. tagal* and only 1.5% in *R. apiculata* (Figure 1B). Effect of salinity on the number of leaves and leaves area was shown in Figure 1 (C and D). Salinity influenced the number of leaves of both species (Figure 1C) and leaves area only in *C. tagal* (Figure 1D). No significant change was noted in the leaves are of *R. apiculata* (Figure ID).

Mangrove species often show growth stimulation at low salinity (25% seawater/0.5% salt concentration) and moderate salinity (50% seawater/1.5% salinity) and then a decline in growth with further elevates in salinity [11-15]. However, the range of salinity in which the plant can survive varies according to the species [16]. This study suggested that mangrove seedlings survived to adapt either in saline or fresh water tolerance.

**Figure 1.** Effect of salinity on plant height (A), diameter (B), some of the leaves (C), and leaf area (D) in the leaves in *R. apiculata* (●) and *C. tagal* (○) seedlings. Data presented are the mean ± SE (n=9-12) and (n=5) for growth measurements and many leaves and leaf area, respectively. The asterisk indicates a statistically significant difference from 0% at *P*< 0.05 using Dunnett’s test.

Our current results also well agree with our previous reports on the optimal growth at 0.5% salinity in *Kandelia candel* and *Bruguiera gymnorhiza*, respectively [6]. The development of both species
slightly increased after removal to salinity [8]. Furthermore, the optimum growth at 1.5% salt concentration found in *Avicennia marina* and *Rhizophora stylosa* grown in a glass house [7].

Patel et al. [15] studied that *C. tagal* is a salt-tolerant mangrove with the high ability to develop in hyper saline and poorly inundated sites. It has been reported that *C. tagal* from Pakistan showed an optimal growth at 50% seawater salinity [17]. Growth and survival of halophytes are reliant on the high levels of ion accumulation in its tissue for the maintenance of turgor and osmotic alteration [18]. Seedling growth and establishment would extend mangrove distribution and increase mangrove rehabilitation as well as the land creation.

![Figure 2](image_url)

**Figure 2.** Effect of salinity on wet (A and C) and dry weight (B and D) in *C. tagal* and *R. apiculata* seedlings, respectively. Data presented are the mean ± SE (n=5) in biomass measurements. The asterisk indicates a statistically significant difference from 0% at *P*<0.05 using Dunnett’s test.

3.2. Effect of salinity on the seedling growth

Seedling biomass was studied concerning to different salt concentration in two non-secretors species *R. apiculata* and *C. tagal*. As shown in Figure 2, salinity changed the wet and dry weight of both seedlings. No statistical difference was noted in wet and dry weight of *R. apiculata* (Figure 2 C and D), on the other hand, statistically different was found only in *C. tagal* seedling (Figure 2 A and B).

Biomass production of *C. tagal* and *R. apiculata* was stimulated at 0.5 and 1.5% salt concentration. Similar results were obtained in *Avicennia marina*, *Ceriops tagal* and *Rhizophora mucronata* [16]. Our study indicates that *C. tagal* and *R. apiculata* growing along the aridest region of East Sumatra coast are more tolerant to salinity.
Our present findings supported our previous results on the salt tolerance of four mangrove seedlings studied in our laboratory increases in the order of B. gymnorrhiza<K. candel<C. tagal<R. apiculata<R. stylosa<A. marina, which is in agreement with their habitat zonation. A. marina grows closest to sea, whereas B. gymnorrhiza distributed rather inland than in the coastal area compared to C. tagal, R. apiculata, R. stylosa and K. candel, indicating B. gymnorrhiza was less tolerant to salt stress [1,7].

4. Conclusions
The current study apparently showed that within the range of treatments used, 1.5% and 0.5% were the optimal salinity by R. apiculata and C. tagal for growth, providing necessary information for mangrove rehabilitation in North Sumatra. Furthermore, our study indicated that the salt tolerance of the two non-secretors mangrove seedlings increased C. tagal<R. apiculata, which is accordance with habitat zonation of mangrove.

References
[1] Basyuni M, Oku H, Tsujimoto E, Kinjo K, Baba S and Takara K 2007 Triterpene synthases from the Okinawan mangrove tribe, Rhizophoraceae FEBS J. 274 5028–5042.
[2] Giri C, Ochieng E, Tieszen L L, Zhu Z, Singh A, Loveland T, Masek J and Duke N 2011 Status and distribution of mangrove forests of the world using earth observation satellite data Global Ecol. Biogeogr. 20 154–159.
[3] Scholander P F, Hammel H T, Hemmingsen E and Garey W 1962 Salt balance in mangroves Plant Physiol. 37 722–729.
[4] Tomlinson P B 1996 the Botany of Mangroves Cambridge University Press London.
[5] Duke NC, Meynecke J O, Dittmann S, Ellison A M, Anger K, Berger U, Cannicci S, Diele K, Ewel K C, Field C D, Koedam N, Lee SY, Marchand C, Nordhaus I and Dahdouh-Guebas F 2007 A world without mangroves? Science 317 41–42.
[6] Basyuni M, Baba S, Inafuku M, Iwasaki H, Kinjo K and Oku H 2009 Expression of terpenoid synthase mRNA and terpenoid content in salt-stressed mangrove J. Plant Physiol.1661786–1800.
[7] Basyuni M, Baba S, Kinjo Y and Oku H 2012 Salinity increases the triterpenoid content of a salt secretor and a non-salt secretor mangrove Aquat. Bot.97 17–23.
[8] Basyuni M, Baba S, Kinjo Y, Putri LAP, Hakim L and Oku H 2012 Salt-dependent increase in triterpenoids is reversible upon transfer to fresh water in mangrove plants Kandelia candel and Bruguiera gymnorrhiza J. Plant Physiol.169 1903–1908.
[9] Fofonoff NP and Lewis EL 1979 A practical salinity scale J. Oceanogr.35 63–64
[10] Schneider C A, Rasband W S, Eliceiri K W 2012 NIH image to ImageJ: 25 years of image analysis Nature Methods 9 671–675
[11] Clough B F 1984 Growth and salt balance of the mangroves Avicennia marina (Forssk.) Vierh. and Rhizophora stylosa Griff. in relation to salinity J. Plant Physiol.11 419–430.
[12] Downton W J S 1982 Growth and osmotic relations of the mangrove Avicennia marina, as influenced by salinity Aust. J. Plant Physiol. 9 519–528.
[13] Naidoo G 1987 Effects of salinity and nitrogen on growth and plant water relations in the mangrove Avicennia marina (Forssk.) VierhNew Phytol.107 317–326.
[14] Burchett M D, Clarke C J, Field C D and Pulkwownik A 1989 Growth and respiration in two mangrove species at a range of salinities Physiol. Plant.75 299–303.
[15] Patel NT, Gupta A and Pandey AN 2010 Strong positive growth responses to salinity by Ceriops tagal, a commonly occurring mangrove of the Gujarat coast of IndiaAot PLANTS2010 plq011.
[16] Ball MC 1988 Salinity tolerance in the mangroves, Aegiceras corniculatum and Avicennia marina. Water use in relation to growth,carbon partitioning and salt balance Aust. J. Plant Physiol. 15 447–464.
[17] Khan MA and Aziz I 2001 Salinity tolerance in some mangrove species from Pakistan *Wetl. Ecol. Manag.* 9 219–223.

[18] Flowers TJ, Troke PF and Yeo AR 1977 The mechanism of salt tolerance in halophytes *Annu. Rev. Plant Physiol.* 28 89–121.

**Acknowledgment**

This work was supported in part by a Competence Grant (No. 017/SP2H/LT/DRPM/II/2016 to MB) from the Directorate for Research and Community Service, Ministry of Research, Technology and Higher Education, Republic of Indonesia. A Grant-in-Aid for a Community Service Research-Based of BOPTN grant (No.453C/UN5.2.3.2.1/PPM/2015 to MB) from University of Sumatera Utara 2015 is acknowledged.