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Effect of Salt and Fresh Water Concentration on Polyisoprenoid Content in *Bruguiera cylindrica* Seedlings

Mohammad Basyuni[1, 2*], Santi Sari Nainggolan[1], Taufiq Qurrahman[3], Poppy Anjelisa Zaitun Hasibuan[3], Sumaiyah Sumaiyah[3], Sumardi Sumardi[4], Etti Sartina Siregar[5], Arif Nuryawan[1]

[1]Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan, 20155, Indonesia; [2]Center of Excellence for Mangroves, Universitas Sumatera Utara, Medan, 20155, Indonesia; [3]Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia; [4]Faculty of Pharmacy, Universitas Tjut Nyak Dhien, Medan, 20123, Indonesia; [5]Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan 20155, Indonesia

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***Correspondence:** Mohammad Basyuni. Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan, 20155, Indonesia; Center of Excellence for Mangroves, Universitas Sumatera Utara, Medan, 20155, Indonesia. E-mail: m.basyuni@usu.ac.id

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Abstract

BACKGROUND: Mangrove forest is a typical forest found along the coast or river mouth which is affected by tides and salinity. Although polyisoprenoid was widespread in the plant kingdom, the physiological roles of these compounds are not well understood, especially from mangrove plants. It is therefore essential to characterize the polyisoprenoid content under abiotic stress.

AIM: This study aimed to determine the effect of salinity and subsequent fresh water change on polyisoprenoids concentration in *Bruguiera cylindrica seedlings*.

METHODS: *Bruguiera cylindrica* planted in a greenhouse for three months under various salinity concentrations. After three months grew under variable salinity, these seedlings were then divided into two treatment groups, and grown for another three months: one continuously in a salt solution and another in fresh water to relieve salt stress. The leaves and roots of *B. cylindrica* seedlings were harvested after six months of cultivation. The leaves and roots of *B. cylindrica* seedlings were extracted for polyisoprenoids content and composition analyzed using two-dimensional thin layer chromatography.

RESULTS: Polyisoprenoids composition under salinity and subsequent fresh water with dominating dolichols (more than 90%) were found in leaves and roots of *B. cylindrica* seedlings referring type I of polyisoprenoid composition. The carbon chain length of dolichols located in the leaves and roots were ranging from C_{75}–C_{100} and C_{75}–C_{105}, respectively.

CONCLUSION: Dolichol dominated over polyprenol both in *B. cylindrical* leaves and roots under salinity and subsequent relief supported the previous finding on the predominance dolichols over polyprenols in mangrove plants. The present study suggested the significance of dolichols in the adaptation to cope with salt stress and or water stress.

Introduction

The mangrove forest is a typical forest found along the coast or river mouth which is affected by tides and salinity [1]. Each species of mangrove plant has different adaptability to environmental conditions such as soil conditions, salinity, temperature, rainfall, and tides [1]. This condition causes the structure and composition of mangrove plants with distinctive boundaries, ranging from zones close to land to zones close to the ocean, and causes differences in the formation of mangrove plants from one region to another [2], [3]. Information about the adaptability of mangroves to environmental influences is still minimal. Salinity is one of the factors that determine the development of mangrove forests.

Although polyisoprenoid was widespread in the plant kingdom, the physiological roles of these compounds are not well understood, especially from mangrove plants [4], [5], [6]. Our previous studies have reported the salinity elevated the triterpenoid content and triterpenoid synthase genes in mangrove plants [3], [7], [8]. These studies suggested the involvedness and diversity among mangrove species. Investigating the salt tolerant mechanism of species is essential for mangroves [9]. The present study, therefore, extends our works [9] and we now report the effect of salinity and subsequent fresh water on polyisoprenoids distribution of *Bruguiera cylindrica* seedlings.

Material and Methods

Mature and healthy propagules of *Bruguiera cylindrica* Blume (Rhizophoraceae) were collected from Sembilan Island, Langkat, Sumatera Utara, Indonesia in September. Pests and diseases do not attack propagules. Mature propagules of *B. cylindrica* were recognized as hypocotyl slightly curved cylindrica fruit, 10-15 cm long and 0.5-1.0 cm diameter. The surface propagule was smooth. The propagule color is green to purplish green. Selection Propagules *B. cylindrica* is derived from parent trees aged five years or more. Identification of *B. cylindrica* was conducted at Herbarium Medanense, Medan, North Sumatra. A specimen voucher has been deposited there.

B. cylindrica propagules were planted in bottle plastic, then filled with sand media which has been sterilized beforehand and given a salinity that varies according to the study. Artificial seawater (marine salt) was prepared by dissolving 5 g, 15 g, and 20 g of a powdered salt commercial for 1 liter of water [3], [7], [8] to make salinity concentration of 0%, 0.5%, 1.5%, and 2%.

After three months, the seedlings were divided into two treatment groups and were grown for another three months: one group continuously in a saline solution and the other in fresh water to remove salt stress. In this step, all the seedlings were washed with fresh water to remove salts [8]. Leaves and roots of *Bruguiera cylindrica* seedlings were harvested from the greenhouse of Faculty of Agriculture, Universitas Sumatera Utara. All fresh samples were stored until the plant materials were used for analysis.

Leaves and roots are dried at 60-75°C for 1-2 days. Dried leaves and roots tissue (every 5 grams) crushed to a fine powder and soaked in 30 ml of chloroform/methanol (2: 1, vol / vol) for 48 hours as earlier described [5], [6]. Lipid extract from the leaves and roots were saponified at 65°C for 24 hours in 0:45 g KOH concentration, 2 ml of ethanol, and 2 ml of distilled water. The non-saponifiable lipids of leaf and root of *B. cylindrica* seedling then diluted with n-hexane and ready to be analyzed [4].

The first dimension TLC was performed for 60 minutes on silica-gel (20 x 3 cm) with solvent system toluene-ethyl acetate (9: 1) [6]. The longitudinal edge of the first dimension TLC with a width of 1 cm and a concentration zone of reverse phase C-18 TLC are clamped by using two magnetic bars (4.0 x 1.1 x 0.8 cm) to confront each phase gel. TLC plate-bound later developed perpendicular to the first dimension to transfer polyprenol and dolichol to concentration zone reverse phase TLC [7].

The second-dimension phase reverse RP-18 silica-gel TLC performed with acetone for about 30 minutes. Position polyisoprenoid alcohol and developed separated by two-dimensional silica-gel TLC, then identified and visualized with iodine vapor. The chromatogram was obtained and scanned. Polyprenol and dolichol concentration detected in HPTLC RP-18 was measured using ImageJ with dolichol and polyprenol standards as a reference [10].

Results

Table 1 shows the total lipid of *B. cylindrica* seedlings extract on leaf tissue ranged from 0-781.17 mg/g with the most significant total lipid in the treatment of 0.5% to 0% and the smallest in treatment 2% and 2% to 0%.

Table 1: Total lipid and polysioprenoids in *B. cylindrica* under salinity and freshwater

Tissue	Salinity	TL (mg/g dw)	PI (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% TL			% PI		Type
						PI	Pol	Dol	Pol	Dol	
Leaves	0%	590.6 ± 11.6	68.0 ± 4.1	nd	68.0 ± 4.1	11.5	nd	11.5	nd	100	1
	0.5%	591.1 ± 63.6	182.4 ± 21.6	nd	182.4 ± 21.6	30.8	nd	30.8	nd	100	1
	0.5%→0%	781.2 ± 184.7	100.3 ± 24.4	nd	100.3 ± 24.4	14.1	nd	14.1	nd	100	1
	1.5%	611.6 ± 44.9	129.8 ± 15.4	nd	129.8 ± 15.4	21.2	nd	21.2	nd	100	1
	1.5%→0%	547.7 ± 16.1	102.3 ± 26.3	nd	102.3 ± 26.3	18.7	nd	18.7	nd	100	1
Roots	0%	693.3 ± 12.8	49.1 ± 6.3	nd	49.1 ± 6.3	76.7	nd	76.7	nd	100	1
	0.5%	714.6 ± 302	59.9 ± 15.5	nd	59.9 ± 15.5	8.4	nd	8.4	nd	100	1
	0.5%→0%	674.2 ± 72.7	63.5 ± 7.7	nd	63.5 ± 7.7	9.4	nd	9.4	nd	100	1
	1.5%	551.8 ± 13.8	55.6 ± 27.8	nd	55.6 ± 27.8	10.1	nd	10.1	nd	100	1
	1.5%→0%	634.3 ± 45.8	129.1 ± 16.4	nd	129.1 ± 16.4	20.4	nd	20.4	nd	100	1
	2%	657.9 ± 71.7	689.7 ± 63.1	nd	689.7 ± 63.1	104.8	nd	104.8	nd	100	1
	2%→0%	575.2 ± 38.4	382.9 ± 50.2	nd	382.9 ± 50.2	66.6	nd	66.6	nd	100	1

The arrow (→) denoted fresh water recovery treatment; Note: nd = not detected; TL = Total lipids; PI = Polyisoprenoids; Pol = Polyprenols; Dol = Dolichols; dw = dry weight; Data are stated as mean ± SD (n = 3) at least three independent experiment.

In the root tissue, the total value of lipids ranged between 575.23-714.57 mg/g with the most substantial total lipid in 0.5% treatment and the lowest in treatment 2% to 0%.

Table 2: The length of the carbon chain of seedlings *B. cylindrica*

Tissue	Salinity	Dolichol
Leaves	0%	80 85 90 95 100
	0.5%	85 90 95 100
	0.5% → 0%	75 80 85 90 95
	1.5%	75 80 85 90 95
	1.5% → 0%	85 90 95 100
Root	0%	80 85 90 95 100 105
	0.5%	85 90 95
	0.5% →0%	85 90 95 100
	1.5%	75 80 85 90 95
	1.5% →0%	85 90 95 100
	2%	85 90 95
	2% → 0%	80 85 90 95 100

The arrow (→) denoted fresh water recovery treatment.

Table 2, Figure 1, and Figure 2 describe the distribution of dolichol compounds in B. cylindrica seedling leaf and root tissue with carbon lengths of C_{75}-C_{95}, C_{80}-C_{100}, and C_{85}-C_{95}, C_{80}-C_{105}.

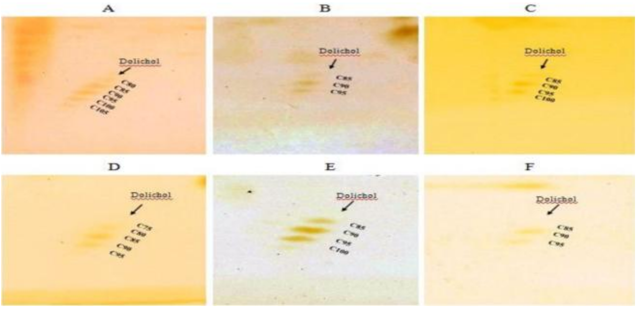

Figure 1: Analysis of two dimensions chromatogram of leaves of Bruguiera cylindrical seedlings at salinity 0% (A); 0.5% (B); 0.5% → 0% (C); 1.5% (D); 1.5% → 0% (E); and in the roots of Bruguiera cylindrica seedling at 0% (F) salinity; The arrow (→) denoted fresh water recovery treatment

Table 1 showed that the distribution of dolichol classified in type 1 due to the spread of dolichol which is found in many tissues of leaves and roots, so the existence of dolichol 100% of seedling B. cylindrica.

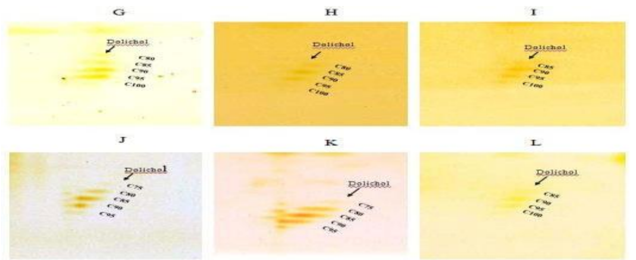

Figure 2: Analysis of chromatography of seedlings of Bruguiera cylindrica at 0.5% salinity (G); 0.5% → 0% (H); 1.5% (I); 1.5% → 0% (J); 2% (K); 2% → 0% (L); The arrow (→) denoted fresh water recovery treatment

This finding is consistent with the previous results [4], [5], [6] reported in type 1 is dolichol dominated compared polyprenol (over 90%) were observed. Therefore, the emergence of a higher number of dolichol even in a mangrove plant leaf, indicating that polyprenol does not play an essential role in some mangrove leaves.

Discussion

The amount of polyisoprenoid content in the leaves and roots of *B. cylindrica* was between 0-182.35 mg/g with the most significant amount in the treatment of 0.5% and the smallest in the treatment of 2% to 0% in leaf tissues. This finding is in accordance with [11], [12], [13] reported that the content of polyisoprenoid alcohol was significantly increased in tissues during aging, especially the accumulation of polyisoprenoid in old leaf. Interestingly, polyisoprenoid contents increased exclusively in tissues that are resistant but not in susceptible plants. Long chains dolichol varies from tissue to tissue even for the same species and appear to dominate families that are different from molecular species [14]. Dolichol occurs as a family with a predominance of carbon chains of C_{70}, C_{80}-C_{95} and C_{100}-C_{110} depending on the type of mangrove and tissue observed. These results support the previous findings responsible for the formation of polyprenol short chains, polyprenol and dolichol long chains in mangrove plants [14].

The difference in the length of the polyisoprenoid chain can be caused by several factors, including salinity stress factors, differences in the network of light effects and increased age or tissue aging [11], [12], [13], [14]. This circumstance may be part of the mechanism of the plant to survive salt stress [15]. In addition to metabolism shifting to overcome environmental challenges, plant membrane cells themselves are a fundamental obstacle to external factors. Lipids in cell membranes play an essential role in adaptation to different salinity through changes in the composition of sterols, lipids, and terpenoids [3], [7], [8], [16].

From these results also showed that dolichol dominated every leaf and root tissue of *B. cylindrica*. This result is consistent with previous research reports that dolichol dominated in mangrove plants [4], [5], [6]. The presence of undetectable polyprenol may be caused by the age of the plant which is classified as a young organ [11]. This result is similar to [5], [11], [14] that increased polyprenol accumulation had been reported in old leaves and aging.

Analysis polyisoprenoid in mangrove plant leaf showed that a significant component compound polyisoprenoid, was not polyprenol but dolichol [4], [5], [6], this is due to the activity of the lipid carrier dolichol as sugar biosynthesis N-glycoprotein and protein GPI [12], [17], [18]. Therefore, the amount and concentration of dolichol were more substantial than polyprenol compounds suggested the presence of polyprenol reductase which catalyzes the reduction of polyprenol to be dolichol, may be active in mangrove plant leaf tissue [7]. Every organism has a different tolerance level against environmental factors. Plants that have a wide tolerance range has resistance to unfavorable environmental conditions, which under certain circumstances is referred to as stress environment [19], [20], [21].

Dolichol dominated in *Bruguiera cylindrica* leaves and root tissue under salinity and subsequent relief to fresh water. This finding is consistent with previous studies have been reported that dolichol was significant polyisoprenoid composition in mangrove leaves and roots. The present study indicated the significance of dolichols in the adaptation to cope with salt stress and or water stress.

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