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Original Research Article

Salinity Alters the Polyisoprenoid Alcohol Content and Composition of Both Salt-Secreting and Non–Salt-Secreting Mangrove Seedlings

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ABSTRACT

The effects of salinity on the polyisoprenoid alcohol content and composition of the salt-secreting mangrove species *Avicennia marina* and *Sonneratia alba* and the non–salt-secreting species *Bruguiera gymnorrhiza* and *Kandelia obovata* were studied. The seedlings of mangroves were grown for 5 months under 0% and 3% salt concentrations. The occurrence, content, and distribution of four mangrove seedlings were analyzed by two-dimensional thin layer chromatography. The structural groups of the polyprenols and dolichols in the leaves and roots were classified into two types (I and II). In type I, dolichols predominated over polyprenols (more than 90%), whereas in type II, the occurrence of both polyprenols and dolichols was observed. Polyprenols were not detected in the leaves of *A. marina* and *B. gymnorrhiza* under 0% salt (control), but were detected in small amounts in *K. obovata* leaves; however, significant amounts were found in the 3% salinity group. This finding in *A. marina*, *B. gymnorrhiza*, and *K. obovata* leaves implies a change to the structural group: under 0% salt concentrations, the groups are classified as type I, but become type II under 3% salt concentrations. The occurrence of ficaprenol (C_{50-55}) was found only in the leaves of the non–salt-secreting species *B. gymnorrhiza* and *K. obovata* under 3% salinity and not in the salt-secreting species *A. marina* or *S. alba*. It is noteworthy that the polyisoprenoid type in the roots of the four species showed no change under salinity; the two salt-secreting species *A. marina* and *S. alba* contained type I under 0% and 3% salt concentrations. On the other hand, type II polyisoprenoids were identified in the non–salt-secreting species *B. gymnorrhiza* and *K. obovata* under 0% and 3% salinity conditions. This finding suggested that polyisoprenoids play a protective role against salinity in the mangrove leaves of both salt-secreting and non–salt-secreting species.

1. Introduction

Mangrove plants are distributed in the intertidal zone of tropical and subtropical regions and are defined physiologically by their ability to grow under various levels of salinity, ranging from freshwater to hypersaline environments (Tomlinson 1986). Mangrove plants fall into three groups based on their approach to managing salinity tolerance: salt secretors, non–salt-secretors, and salt accumulators (Basyuni *et al.* 2012a; Tomlinson 1986). The species of salt secretors include *Avicennia marina* (Acanthaceae) and *Sonneratia alba* (Sonneratiaceae), which have either salt glands or salt hairs to remove excess salt. In contrast, non–salt-secretors, exemplified by *Bruguiera gymnorrhiza* and *Kandelia obovata* (Rhizophoraceae), do not have such morphological features for the excretion of excess salt. Salt accumulators, such as *A. marina*, *B. gymnorrhiza*, and *S. alba*, can cope with high salt concentrations in their cells (Basyuni *et al.* 2012a). *A. marina*, *B. gymnorrhiza*, *K. obovata,* and *S. alba* are common mangrove species on Okinawa Island, Japan, and are considered representatives of each group in terms of salt management strategies.

Mangroves are well known to produce secondary metabolites, including polyisoprenoids whose physiological roles remain

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unclear (Basyuni *et al.* 2016, 2017a; Skoczylas *et al.* 1994). There are two main types of polyisoprenoid alcohols in plants: polyprenols and dolichols (Figure 1). It is believed that polyisoprenoids may play a significant role in the adaptation of plants in response to adverse external stresses. The concentration of polyisoprenoids has been reported to change on biotic and abiotic stresses (Baczewska *et al.* 2014; Bajda *et al.* 2009; Zhang *et al.* 2008). Our previous studies have also demonstrated that levels of triterpenoids and triterpenoid synthase gene expression in salt-secreting and non-salt-secreting mangrove roots and leaves increase with increasing levels of salt (Basyuni *et al.* 2009, 2011, 2012a). This salt-dependent change in triterpenoid content is reversible on transfer to fresh water (Basyuni *et al.* 2012b). Aside from these metabolic shifts to overcome environmental stresses, the present study aimed to describe the effects of salinity on the polyisoprenoid alcohol content and composition of the salt-secreting mangroves *A. marina* and *S. alba* in comparison with that of the non-salt-secreting species *B. gymnorrhiza* and *K. obovata*.

2. Materials and Methods

2.1. Chemicals

A mixture of dolichol (C_{90}–C_{105}) and polyprenol (C_{90}–C_{100}) standard compounds was used to identify the polyisoprenoids that were detected in this study, as previously described (Basyuni *et al.* 2016). Silica gel 60 thin layer chromatography (TLC) glass plates and reversed-phase silica RP-18 high-performance thin-layer chromatography (HPTLC) glass plates were purchased from Merck (Darmstadt, Germany). All other chemicals and solvents were of reagent grade and obtained from Merck. The identification of the family corresponding to polyprenols or dolichols was performed for at least three experiments. The bombiprenone family (Figure 1), as described previously (Basyuni *et al.* 2016), was purified using silica gel chromatography of nonsaponifiable lipids (NSLs) from the $CHCl_3/CH_3OH$ (2:1) extracts of dry *Perilla* leaves. The purified fractions were confirmed by ESI-MS (Bruker Daltonics solariX, Manning Park Billerica, MA, USA) to have an m/z value $[M+Na]^+$ of 625.53183, which corresponded to $C_{43}H_{70}O$ (bombiprenone).

2.2. Plant material

Viviparous mature and healthy propagules (seeds) of two non-salt-secreting mangrove species, *B. gymnorrhiza* (L.) Lam. and *K. obovata* (S., L) Yong (formerly as *Kandelia candel*) (Rhizophoraceae), as well as crypto-viviparous mature and healthy seeds of two salt-secreting species, *A. marina* (Forssk.) Vierh (Acanthaceae) and *S. alba* J. Sm. (Sonneratiaceae), were collected from Iriomote Island, Okinawa, Japan, and planted in Wagner pots under varying salinity levels in a glass house. The characteristic indicators of *B. gymnorrhiza* propagule maturity were reddish-brown or greenish-red hypocotyls, a 1.7–2.0 cm diameter, and a 20–30 cm length; mature *K. obovata* propagules had yellowish-green hypocotyls and greenish-yellow cotyledons and were 20–30 cm long and 1.5–2.0 cm wide in diameter. Mature seeds of *A. marina* were green in color (similar to the pericarp color), 1.5–2.5 cm long and 1.5–2.0 cm wide. Mature *S. alba* fruit were characterized by a size of 40 mm or more, and mature fruits contained 150–200 seeds.

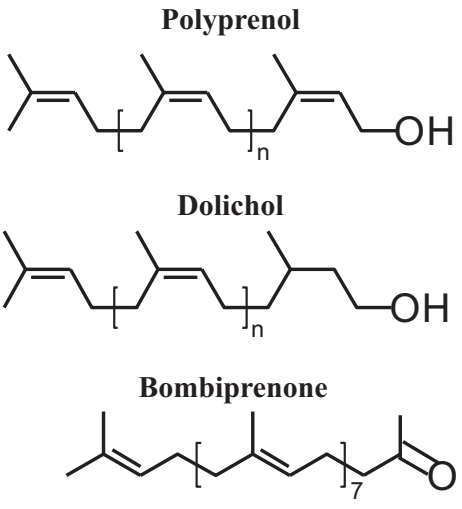

Figure 1. Structure of polyprenol, dolichol, and bombiprenone. n shows the number of internal isoprene residues.

The germinated seedlings grew for 5 months under exposure to natural temperature and sunlight in an uncontrolled glass house. The maximum irradiance in the glass house was 950 μmol m^{-2} s^{-1}, and the average temperature was 24.1°C. A seawater solution was prepared by dissolving commercial salt powder (Red Sea Salt, Houston, TX, USA) to make 0% and 3% (equal to seawater level) salinity concentrations in accordance with the manufacturer's protocol. Salinity in this study was measured as the mass of salt powder/weight of solution (Basyuni *et al.* 2012b). The salt concentration in each pot treatment in this study was checked weekly during the experiments by an S/Mill-E Salinity Refractometer (ATAGO Co., Ltd., Tokyo, Japan) and was adjusted by adding tap water for the control (0%) or pure water (salt treatments) to compensate for water lost because of evapotranspiration. Three plants in a separate pot, that is, five portable pots per species per salinity treatment, were grown for 5 months. After 5 months of cultivation, the four species of mangrove plants were harvested and washed, after which the leaves and roots were flash frozen in liquid nitrogen and then stored at −20°C for further analysis.

2.3. Isolation of polyisoprenoid alcohols

The procedure for the isolation of polyisoprenoids was performed, as previously described (Basyuni *et al.* 2017a). The leaves and roots of *A. marina*, *B. gymnorrhiza*, *K. obovata*, and *S. alba* seedlings under 0% and 3% salt concentrations were dried at 75°C for 1–2 days. The dried tissue (2–4 g each) was crushed to a fine powder, and then immersed in 30 mL of chloroform/methanol (2:1, v/v) solvent for 48 h. The lipid extract of the leaves and roots was saponified at 65°C for 24 h in 50% ethanol containing 2 M KOH. The NSLs of each tissue were extracted with hexane, and the organic solvent was evaporated and redissolved in hexane. The leaf (≈100 μg) and root (≈150 μg) extracts were added to each TLC plate.

2.4. Investigation of polyisoprenoids by two-dimensional thin layer chromatography

First-dimensional TLC was carried out for approximately 60 min on a silica gel glass plate (20 × 3 cm) with a solvent system of toluene-ethyl acetate (9:1), as previously described (Basyuni *et al.* 2016; 2017a). Two-dimensional reversed-phase C-18 silica gel HPTLC was performed with acetone as the solvent for approximately 40 min. The position of the separated polyisoprenoid alcohols by two-dimensional silica gel TLC was identified and visualized with iodine vapor. To determine whether the family corresponded to dolichols or polyprenols, dolichol or polyprenol reference standards were added to the sample line of the first-dimension TLC and developed with a solvent system, as previously described (Basyuni *et al.* 2016). The developed chromatographic images were obtained and digitally scanned with a PIXMA G2000 Canon Series Printer (Canon Singapore Pte. Ltd.). The polyisoprenoid family was identified via the comparison of mobility in TLC with that of authentic