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Structural and functional responses of xylem in *Rhizophora mucronata* Lam. seedlings under drought and hypersaline conditions

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Abstract. Water translocation in mangrove seedlings is often affected by water stress conditions such as drought, hyper-salinities and their frequent variations. This study was therefore aimed at studying the wood anatomical responses of xylem tissue and hydraulic conductivity of *Rhizophora mucronata* Lam., a common species in mangrove planting, under different levels of drought [25%, ~50% and ~100% of water holding capacity (WHC)] and soil salinity [high salinity (35 psu), moderate salinity (15 psu) and freshwater (0 psu)]. As wood anatomical responses, significantly higher vessel density, vessel grouping ($P<0.001$) along with narrow vessel elements ($P<0.001$) were observed in plants grown in the 25% and 50% WHCs and high salinity treatments. All these anatomical responses are more directed towards avoidance of vessel cavitation which is commonly found under water deficit conditions. The results showed that *R. mucronata* plants failed to maintain efficient transportation of water when the field capacity was 50% of WHC or lower and the level of salinity was 35 psu or greater, as evident by the reduction of water conductive areas, vessel areas and hydraulic conductivity ($P<0.05$). Overall, water use efficiency of *R. mucronata* seedlings under the imposed water stress conditions has remarkably reduced and it further indicated that such imposed stress conditions directly affect the survival of planted seedlings as depicted by the significantly low survival in 25% and 50% of WHCs and high salinity. Therefore, in-depth study on lagoon hydrology including inundation levels, water depth, salinity and the selection of correct tidal positioning is highly recommended as prerequisites in mangrove planting.

Keywords. Hydraulic architecture, hydraulic conductivity, mangroves, restoration, water stress.

1 Introduction

Mangrove forests are unique plant communities that grow in extreme environmental conditions such as high and changing salinity, frequent

inundation with associated hypoxia, low air humidity and high temperatures. Mangrove ecosystems are restricted to intertidal areas of lagoons, estuaries, and sheltered bays in tropical and sub-tropical areas worldwide (Tomlinson 1986; Spalding *et al*. 2010; Mukherjee *et al*. 2015).

However, the destruction of mangrove ecosystems is increased at an alarming rate and hence, attempts for mangrove replanting are taking place as a common activity in such areas. Mangrove planting in Sri Lanka has received a higher attention particularly after realizing the unprecedented mangrove loss in previous decades (Polidoro *et al*. 2010; Richards and Friess 2016) and safeguarding action of mangroves against the tsunami in 2004 (Dahdouh-Guebas *et al*. 2005b; Lee *et al*. 2014; Jayatissa *et al*. 2016; Satyanarayana *et al*. 2017). However, majority of the planting attempts showed higher failure rates (Ahmad 2012; Kodikara *et al*. 2017a) as they were not supported by sound scientific knowledge. Among the relevant causes for the failures, improper site selection without studying site history and planting inappropriate species have been identified as two major causes for the failures (Primavera and Esteban 2008; Lewis 2009; Kathiresan 2011; Ahmad 2012; Kodikara *et al*. 2017a). In many cases, mangrove planting practitioners tend to establish mangrove plantations out of intertidal range (i.e. natural mangrove growing area) of lagoon which is commonly known as "inappropriate tidal positioning" in mangrove planting (Samson and Rollon 2008; Brown *et al*. 2014a). This often brings about inappropriate ecological conditions, for example hard soil, low water content, high salinity and hypoxia which are detrimental for the survival of mangrove plants (Brown *et al*. 2014a). Therefore, planted mangrove propagules/seedlings are vulnerable for drought stress (also known as physical water stress) and salt stress (also known as physiological water stress), in case of planting beyond intertidal zone (supra-littoral) where soil water content is low (particularly in dry zone and dry season) and more deeper areas in lagoon water (infra-littoral) where high salinity (during dry season) and hypoxic (prolonged submergence) conditions are applied, respectively (Hoppe-Speer *et al*. 2011).

However, in general, mangrove plants have robust adaptions to overcome prevailing extreme conditions (Tomlinson 1994; Shi *et al*. 2005) and it is therefore important to study their plasticity of tolerance under these created extremities. Under the above stressed conditions, the water movement could be negatively affected which results in numerous variations in vessel anatomy as adaptations to ensure safe water movement (Hacke *et al*. 2006). In case of water limiting conditions, air bubbles could be formed inside vessel elements (cavitation) which breaks the continuity of water column (Cochard 2006; Robert *et al*. 2009) disturbing the movement of water. In response to these conditions, anatomical adaptations like high vessel density, high vessel grouping index, small and short vessels have been recorded for some

mesophytes (Baas *et al*. 1983). However, such studies for mangroves are scarce. Schmitz *et al*. (2006) and Robert *et al*. (2009) studied the anatomical traits like vessel density, vessel grouping index and vessel size of *Avicennia marina* and *Rhizophora mucronata* in relation to prevailing environmental conditions with particular attention on water relations. Several variations in size, density, and grouping of vessels have been observed. Nevertheless, the wood anatomical studies, water translocation behavior (functional response) and their plasticity under created abiotic stress conditions such as drought, hypersaline conditions and permanent submergence have not been adequately addressed.

Further, in most of the studies, plant behavior under such stress conditions, are only discussed in term of vessel anatomy. However, we argue that hydraulic conductivity, i.e. the ease of water moving through a water transporting system, is a more reliable measure which reflects physiological response on the functional behavior of plants under water limiting conditions and it is more trustworthy, when accompanied with compatible anatomical data. Thus, the following aspects were addressed in this study, a) anatomical responses of *Rhizophora mucronata* plants under drought and hypersaline conditions, b) variations of hydraulic conductivity (Kh) of *R. mucronata* seedlings under above conditions and c) the plasticity of *R. mucronata* plants in modifying their vessel anatomy and water translocation behavior to secure their survival under water stress conditions.

2. Methodology

The research was conducted in a plant-house, in the Department of Botany, University of Ruhuna, Matara, Sri Lanka. *Rhizophora mucronata* Lam. was selected for the study as the most commonly used mangrove species in planting programmes (~97%) in Sri Lanka (Kodikara *et al*. 2017a).

2.1 Experimental details

In the plant-house experiment, mature propagules of *Rhizophora mucronata* collected from the natural mangrove forest in Pambala (07º31´N–79º49´E) in July, 2016 were kept floating in low saline (i.e. 2-3 psu) water for about one month. Later, the propagules were transferred from the water-filled containers to the plant-house conditions and maintained in a nursery to be used as the planting material. The soil mixture was prepared using garden top soil, mangrove top soil, sand and compost in the proportion of 1:1:1:1. The pots were prepared using black polythene of 23 cm x 25 cm and filled with about 3.5 kg of the prepared soil mixture. Thereafter, seedlings with the first two

unfurled leaves (i.e. same vigor) were selected for the experiment. For the two separated experiments (drought stress and salt stress), three treatment levels namely 25% WHC (Water Holding Capacity), 50% WHC and 100% WHC (control) were used for drought stress experiment (physical water stress) while another set of treatments for salt stress experiment (physiological water stress), high salinity (35 psu), moderate salinity (15 psu) and freshwater (0 psu) were used, keeping moderate salinity (15 psu) as the control since it is reported that *R. mucronata* performs its best under moderate salinity condition (Jayatissa *et al*. 2008). The water holding capacity was calculated based on the volume of the water held in the oven-dried, 100 g of soil sample when 100 ml water is added and the retained water volume was considered as the field capacity (100% WHC). Half and quarter of the field capacity volume were taken as 50% WHC and 25% WHC of the soil respectively. The individual pots were treated with the respective water volume daily to keep the imposed stress levels. In addition, several soil samples, taken randomly from the pots, were tested for the water level by using oven-dried method for further confirmation (NCRS, 2010). Three replicates for each treatment level were used with 12 seedlings per replicate. A total of 216 seedlings were subjected for the plant-house study. Low saline water (i.e. 5 psu) that was prepared separately by mixing sea water and aged tap water (i.e. water kept in open containers for few days before the use in order to remove excess chlorine), was added to maintain the respective WHCs and the same procedure was followed in preparation of moderate saline condition. Pots with seedlings were placed individually in well-separated tanks according to a completely randomized design (CRD) and this was done separately for the two experiments. Based on our preliminary measurements, the salinity of water in the tanks was checked once in every two days using a hand refractometer (ATAGO S/Mill-E, Japan) and adjusted the salinity when necessary (added aged tap water when the level of salinity was higher than the expected and seawater was added when the salinity was lower). Commercially available fertilizer was also applied (in the form of pellets) once a month by providing the same amount per pot (Adopted from Jayatissa *et al*. 2008; Dissanayake *et al*. 2014; Kodikara *et al*. 2017b).

2.2 Data collection

Data on level of survival of *Rhizophora mucronata* seedlings in each treatment were recorded. As growth parameters, cumulative height (CH) (summation of the height of main stem and lengths of branches) of the *R. mucronata* seedlings, was measured once a month using a scaled ruler. Average number of leaves was also recorded and total average leaf area

(TALA) of the seedlings in all treatments were measured once in a 2-week period by using a millimeter graph sheet.

Stomatal conductance (SC) was measured with the help of Steady State Porometer (SC-1; Decagon Devices, USA). Two measurements were taken on the same day as one at the late morning (10:00-11:00 hrs) and the other in early afternoon (13:00-14:00 hrs). The youngest, fully expanded leaf exposed to full sunlight was selected and abaxial conductance was recorded. The same leaf was subjected to measure the stomatal conductance of *R. mucronata* plants to avoid the variability among the different leaves.

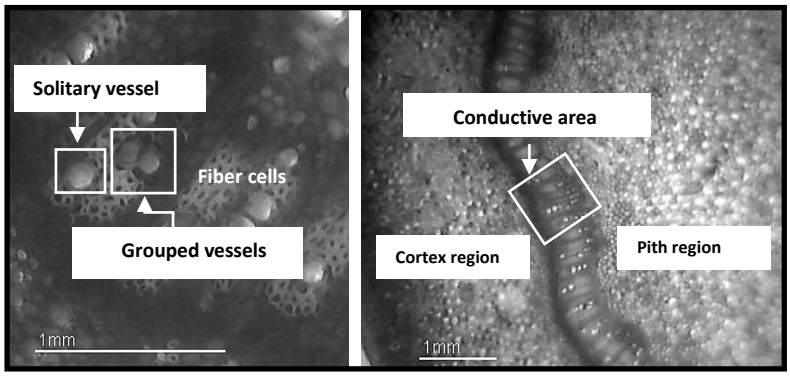

Fig.1 Solitary and grouped vessels in a cross section of *Rhizophora mucronata* stem (X 400). Conductive area of a cross section of *R. mucronata* stem (under low power), considered for the calculation of the hydraulic conductivity

Vessel density (VD), vessel diameter (Vdia), vessel grouping index (VGI), number of solitary vessels (SV) and conductive area (CA) and vessel area (VA) were measured as anatomical parameters, by following the procedure given; the stems from the middle part of *R. mucronata* plants were collected and hand sectioned to obtain transverse sections of the stems. In case of hard stems, (about 4 months after planting), the collected samples were stored in a softening solution i.e. copenhague mixture: 70% ethanol; 28% de-ionised water and 2% glycerol in a 1:1:1 ratio) for about one month and then transverse sections were obtained using a microtome. Sections were stained using Safranin for 5 minutes and were then dehydrated by transferring through an ethanol series of 50%, 70%, 96% and 100% keeping 3 minutes in each. Sections were mounted on glass slides using Canada balsam. Microscopic images of the sections were taken at different magnifications (25x, 40x, 100x) with an Olympus microscope (BX60F-3, Tokyo, Japan) equipped with a digital camera (RUH-CAM, University of Ruhuna, Sri Lanka). Vessel dimensions were measured using IMAGEJ software, a public

domain, Java-based image processing program. Afterwards, Vdia was directly measured by IMAGE software while VD (number of vessels per mm^2) and VGI (mean number of vessels per vessel group) and CA were calculated based on the vessel counts and area measurements. Percentage conductive area was calculated by taking percentage of total conductive area to the total area of the cross section whereas percentage vessel area was obtained by taking percentage of total vessel area to the total conductive area (Fig. 1). Hydraulic conductivity of the *R. mucronata* plants was measured at the 6th month after planting, using manually designed "Choatometer" [See Fig. 2 adopted from Choat *et al*. 2005].

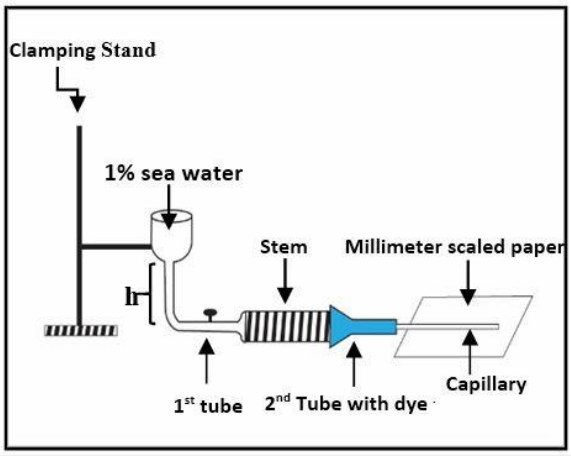

Fig.2 Diagrammatic illustration of the manually designed Choatometer used in the experiment [adopted from Choat *et al*. 2005] ("h" = height of the pressure head).

Segments of about 15 cm long and about 1 cm diameter were cut under water, from the stems (at the middle part), for hydraulic measurements according to Choat *et al*. (2005). A seawater solution (1%) filtered with a syringe filter of 0.22 μm, was used as perfusion solution for the experiment. The ends of the branch segments were cut smoothly with a razor blade and the mass of the branch segments i.e. mass start (m_0), was recorded, before wrapping the segment ends with Parafilm. Thereafter, the branch segments were placed in the tubing system, connected at one end to a solution reservoir creating a pressure head (h) and to a pipette, placed on millimeter paper and filled with coloured liquid at the other end. The movement of the liquid in the pipette was followed by a camera taking pictures with a 3 seconds time interval (in total 30 seconds) and small video clips were also kept for further confirmation. After taking ten pictures, the mass of the branch segments was

weighed again i.e. mass end (m1). Furthermore, the length of the branch, the diameter of the branch, the number of nodes per branch segment, the air temperature and the relative humidity during the experiment were also recorded. The bark, pith and xylem area were measured on pictures of a transverse section of the branch segments, using ImageJ software. Hydraulic conductivity (Kh) was then calculated using the equation (1).

$$Kh = \frac{F \times L}{P} \quad \text{……………………………………………} (1)$$

where Kh is hydraulic conductivity, F is flow rate, L is branch length, and P is pressure drop.

2.3 Statistical analyses

Cumulative height (CH), total average leaf area (TALA), stomatal conductance (SC), hydraulic conductivity (Kh), and vessel diameter (VDia) were measured and treated as the continuous variables while vessel density (VD), vessel grouping index (VGI), conductive area (CA), vessel area (VA) number of solitary vessels (SV) was taken as the count data (proportional and percentage). Mean and standard deviation were calculated as descriptive statistics. Three hypotheses, normality (Shapiro test; $P>0.05$), linearity (scatter-plot) and homogeneity of variance (Levene's test; $P>0.05$) were tested and all conditions were met for the data.

Parametric tests were, therefore, performed. The mean differences of the aforementioned continuous variables were checked using one-way ANOVA test (at a significance level of $P<0.05$), taken the "levels of stress" as the "factor" (for the same species *Rhizophora mucronata*) followed by the Tukey-Kramer multiple comparison test to check all pairwise differences. Chi-square test was conducted for the count data. All statistical analyses were performed using the R-3.2.2 statistical software.

3 Results

The level of survival of *Rhizophora mucronata* seedlings decreased over time in all treatments except the control treatments. At the end of 18th week, 61% of *R. mucronata* plants survived in the 50% WHC level while all the seedlings died in the 25% WHC level by the end of 16th week (Fig. 3). In salt stress experiment, 21% of seedlings out of the initial number was able to survive in the high salinity level and only a few seedlings died in both moderate and

freshwater treatments showing no significant difference in level of survival between two treatments (Fig. 3).

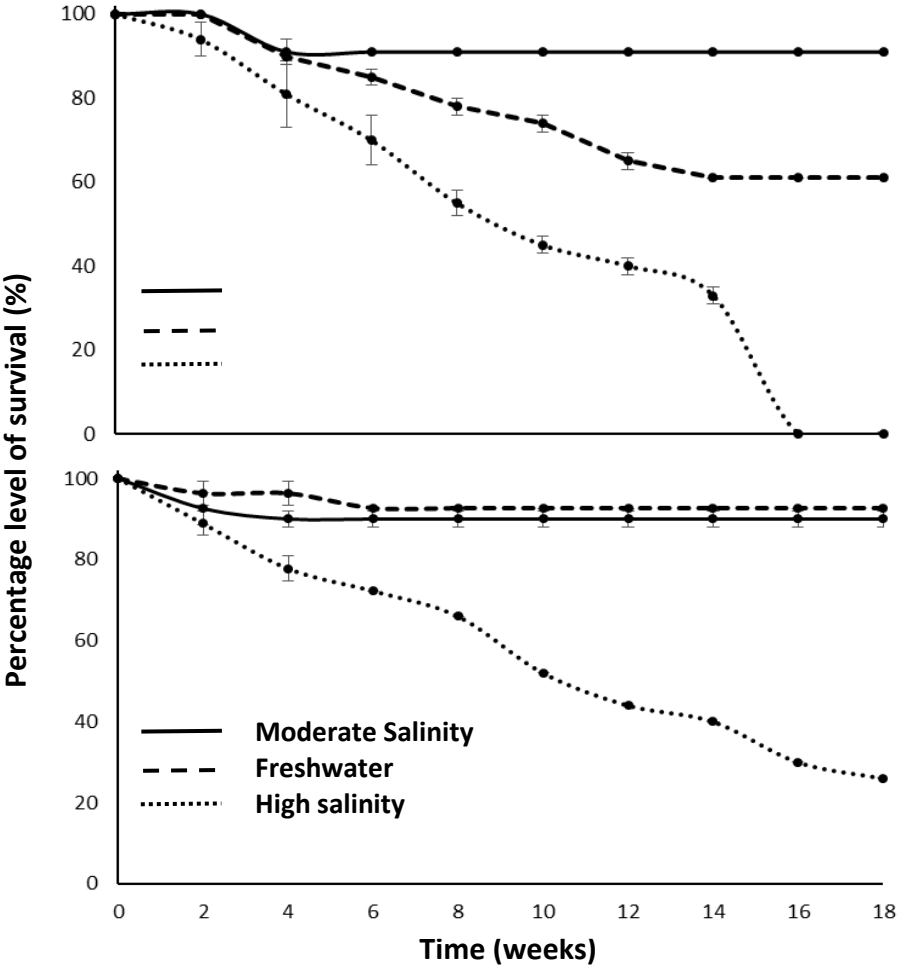

Fig. 3 Percentage survival of *Rhizophora mucronata* seedlings over the study period. (Top) drought stress experiment and (bottom) salt stress experiment. Error bars indicate the standard deviation (±).

In drought stress experiment, both CH and TALA of *Rhizophora* seedlings grown in the 50% WHC level was significantly lower ($P<0.05$) than that of the seedlings in the 100% WHC (Fig. 4 & Fig. 5). Interestingly, there was no significant difference in growth performances of the seedlings grown in freshwater and moderate saline condition whilst CH and TALA of the *R.*

mucronata seedlings in high salinity were remarkably lower ($P<0.05$) when compared with that in the freshwater and the moderate saline conditions (Fig. 4 & 5). The SC also showed noteworthy variation among the treatments. It was significantly lower ($P<0.05$) in the 50% WHC treatment level when compared with that of the control treatment (Fig. 6). However, there was no significant difference in SC between the moderate saline and freshwater conditions. It was significantly lower ($P<0.001$) in the high salinity treatment (Fig. 6).

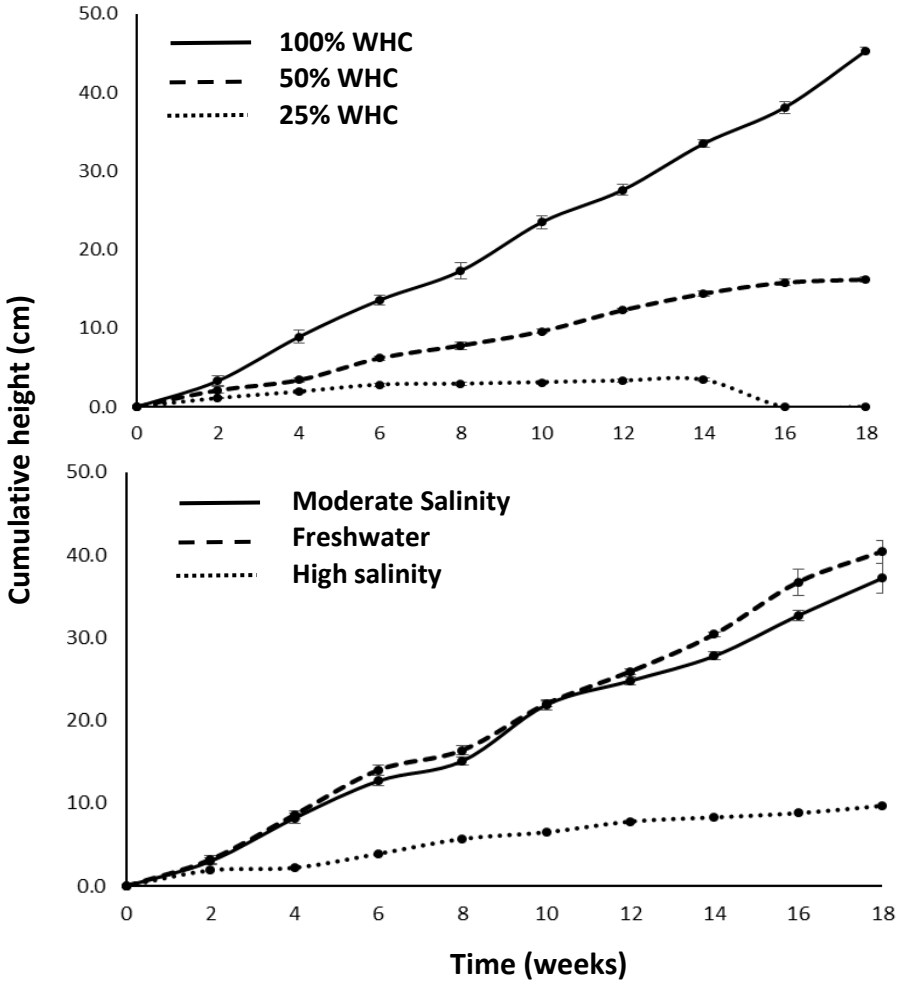

Fig. 4 Cumulative height of *Rhizophora mucronata* seedlings over the study period, in drought stress experiment (top) and salt stress experiment (bottom). Error bars indicate the standard deviation (±).

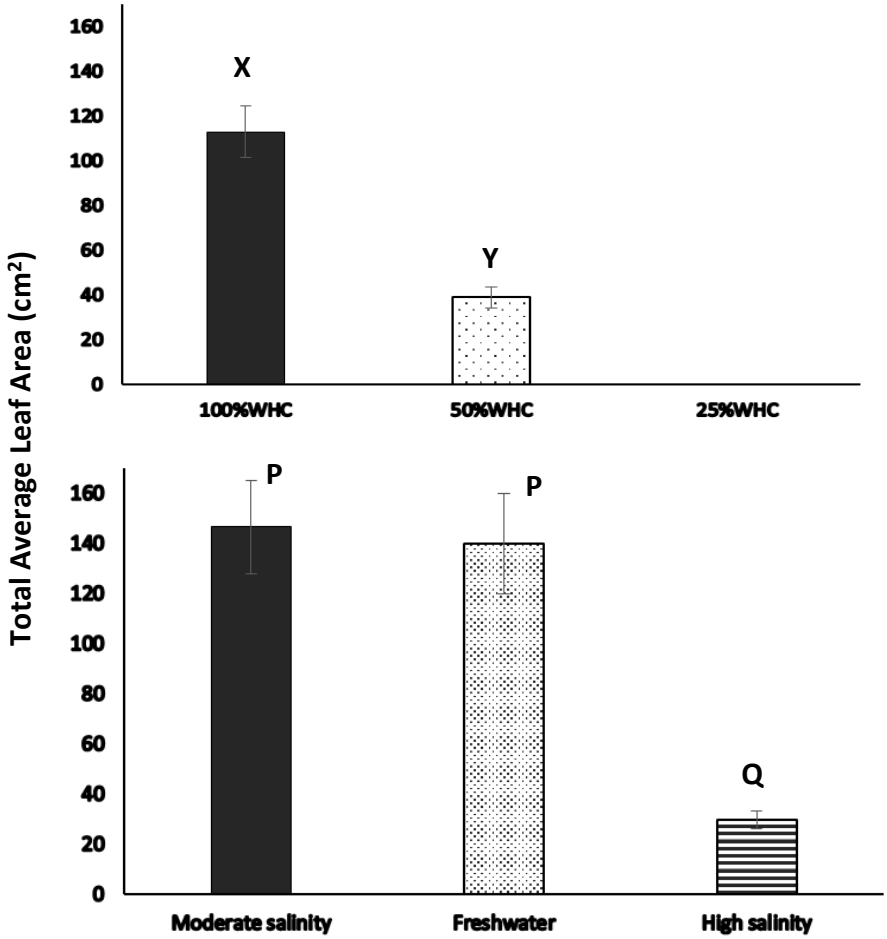

Fig.5 Total average leaf area (cm^2) of *Rhizophora mucronata* seedlings at the end of the study period in (top) drought stress experiment and (bottom) salt stress experiment. Vertical bars indicate the standard deviation (±) while significant level by different letters at 95% confidence level.

Anatomical responses and hydraulic conductivity

Rhizophora mucronata seedlings showed remarkable variation in their anatomy and Kh under the given stress conditions which indicated that all the stress factors tested had a significant effect on vessel anatomy and Kh.

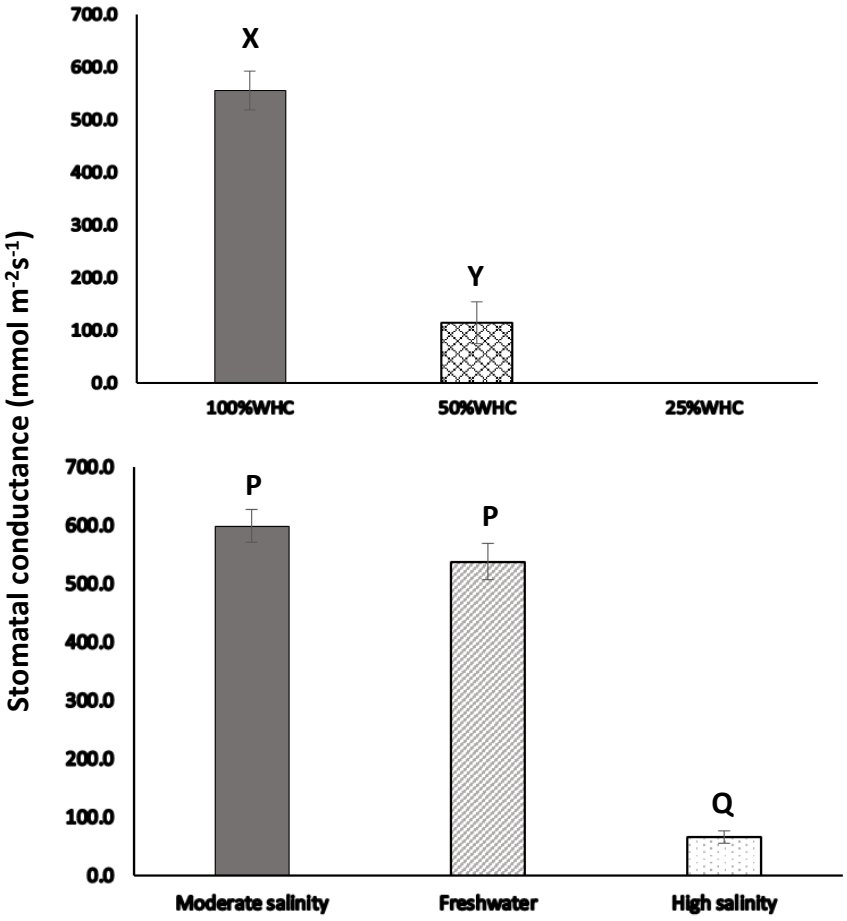

Fig.6 Stomatal conductance (mmol $m^{-2}s^{-1}$) of *Rhizophora mucronata* seedlings over the study period in (top) drought stress experiment and (bottom) salt stress experiment. Vertical bars indicate the standard deviation (±) while significant level by different letters at 95% confidence level.

According to the results, percentage cover of CA of the stems of *Rhizophora mucronata* in both 50% WHC and high salinity treatments were significantly lower ($P<0.001$) when compared with those of the stems, harvested from their respective controls i.e., 100% WHC and moderate saline condition (Table 1). However, no significant difference in the percentage cover of CA was observed among freshwater and moderate saline conditions. When considering the vessel characters, both VD and VGI were significantly higher

(P<0.001) in the mangrove plants grown in the 50% WHC when compared with that of the respective control, 100% WHC (Table 1).

Table 1. Anatomical responses of vessel elements of *Rhizophora mucronata* seedlings under drought and salt stress conditions (Tukey multiple comparison; 95% confidence level, P<0.05; significant differences among treatments within drought stress and salt stress experiments separately for the same parameter are shown by different superscript letters) (n: number of cross sections & n*: number of measurements).

Parameter	Drought stress experiment			Salt stress experiment		
	100% WHC	50% WHC	25% WHC	Moderate saline	Fresh water	High saline
Conductive area (%) (n= 84)	11.7±2.2^{a}	4.1±1.7^{b}	All plants died after 14 weeks	12.9±3.1^{a}	13.1±2.4^{a}	6.1±1.5^{b}
Vessel area (%) (n=84)	12.8±1.1^{a}	5.2±0.9^{b}		13.8±2.6^{a}	14.4±1.9^{a}	6.7±1.8^{b}
Vessel density (mm^{-2} xylem area) (n= X) (n= 84)	25.8±1.6^{a}	66.2± 3.9^{b}		24.5 ±2.9^{a}	25.9± 1.7^{a}	68.2 ±4.1^{b}
Vessel grouping index (n=84)	2.0 ±0.5^{a}	5.1± 0.6^{b}		2.4± 1.8^{a}	1.8± 1.0^{a}	6.1± 1.4^{b}
Solitary vessels (%) (n=84)	72.4±4.2^{a}	33.4±3.1^{b}		75.6±1.9^{a}	83.2±2.2^{a}	38.9±4.1^{b}
Vessel diameter (μm) (n*=1680)	82.6±13.6^{a}	37.2±2.7^{b}		88.7±11.8^{a}	92.1±12.8^{a}	34.8±3.8^{b}

In contrast, Vdia of *R. mucronata* plants in the 50% WHC showed significant decrease (P<0.05) when compared that of the 100% WHC treatment. In salt stress experiment, increased VD and VGI were found in the high salinity treatment (P<0.05) while the Vdia was significantly lower (P<0.05) as compared to that of the moderate saline condition. Interestingly, vessel anatomy of *R. mucronata* seedlings grown in the freshwater condition showed the pattern as same as the control plants i.e. low VD and VGI while having larger Vdia. Percentage of SV elements in conductive area of *R. mucronata* seedlings grown in the 50% WHC was significantly low (P<0.001) when compared with the control. In the same way, percentage of SV in *R. mucronata* stems grown under the high salinity condition was remarkably low (P<0.001) as compared to the moderate saline condition.

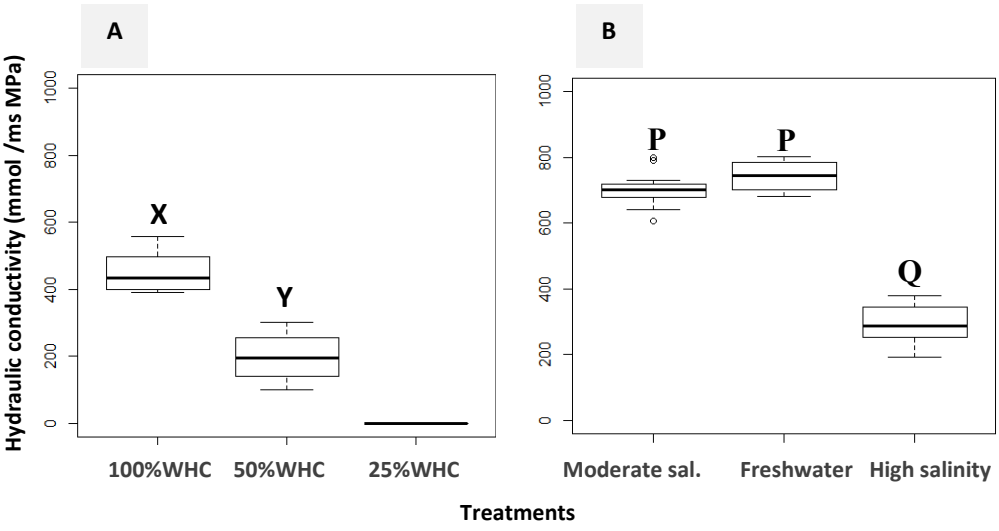

Fig.7 Boxplot graphs of hydraulic conductivity (mmol/ms MPa) of *Rhizophora mucronata* plants in (A) drought stress experiment and (B) salt stress experiment. Tukey-Kramer multiple comparison; 95% confidence level, P<0.05. Significance level shows by different letters and were used separately for two treatments.

Further, we did not observe significant difference in the percentage of SV between the plants grown under the freshwater and moderate saline conditions. In spite of a high variability in vessel anatomy, Kh also varied among the treatments. The Kh of *R. mucronata* seedlings grown in the 50% WHC level and the high salinity treatment were significantly lower (P<0.05) as compared to their respective controls (Fig. 7) i.e. 100% WHC and moderate saline conditions respectively. The highest Kh was observed in the freshwater treatment which was not remarkably different from the control plants.

4 Discussion

Selection of incorrect tidal positioning in mangrove planting due to ignorance of the accepted technical guidelines such as EMR (Ecological Mangrove Restoration) methodology (Lewis 2005; Lewis and Brown 2014) and modified protocols (Bosire *et al*. 2008), often leads to create stress conditions like drought (or negative soil water potential) and hypersaline conditions for planted seedlings (Kodikara *et al*. 2017a). Mangrove seedlings have therefore no choice rather than facing to such stress conditions. Although, mangrove

plants are well-armed with various strategies to overcome extreme conditions such as high saline conditions and frequent inundation (Alongi 2002), the level of tolerance secondarily depends on the type and intensity of stress (Hsiao 1973; Larcher 2003).

We further emphasize that created stress conditions may have relatively more deleterious effects on planted mangrove seedlings, particularly in the early life stage, than naturally prevailing extreme conditions. Under natural conditions, both level of stress and exposure time are relatively low since the effects of stresses are naturally moderated, for example, effect of mangrove soil salinity increase is often diluted with periodic inundation.

However, according to the results, survival, growth performances, stomatal conductance, hydraulic traits and hydraulic conductivity of *Rhizophora mucronata* plants were affected in different ways and to different degrees under aforementioned two stress conditions. In this study, the stressed *R. mucronata* plants in both drought and salt stress conditions showed similar responses in many aspects.

The decreased level of survival and growth performances of the mangrove plants grown in the 50% WHC, 25% WHC and high salinity conditions, could be due to low water potential and osmotic stress (Munns and Tester 2008). Reduced cell turgor pressure is expected under both drought and hypersaline conditions due to which cell expansion is hampered (Naidoo 2006).

Also, accumulation of salt ions like NaCl in cell vacuoles further intensifies negative water potential under hypersaline condition (Werner and Stelzer 1990) where it needs prompt osmoregulation to cope up with such conditions (Naidoo 1985). Allocation of energy for osmoregulation may be prioritized over plant growth and development which may ultimately restrict energy allocation for growth, showing the reduction of growth parameters such as plant height, leaf production and total leaf area (Naidoo 2006; Hoppe-speer *et al*. 2011). When *R. mucronata* plants run out of metabolic energy in case of exposing to intense stress conditions for a longer period, the plant may end up with early mortality. In that sense, hypersaline condition has made more lethal effects on *R. mucronata* plants as the level of survival was reduced up to 26% whilst the survival was 61% under drought conditions (50%WHC).

Several anatomical responses could also be observed under the imposed stress conditions, and those responses could be considered as modifications which may be contributory in securing their plant functionality and survival. However, decreased CA in both 50% WHC and high salinity treatments is disadvantageous as it reduces water transporting area inside the plants. Cell shrinkage which has resulted in due to the reduced cell turgor pressure under drought and hypersaline conditions may be the cause for the reduced CA (Akram *et al*. 2002). The common observations i.e. increased VD and VGI as well as narrow vessel elements of the mangrove plants with the enhanced

drought (e.g., 50% WHC and 25% WHC) and salinity (>35 psu) can be explained in different ways. The formation of vessel conduits with different vessel diameters primarily depends on the differentiation rates of conduits from the cambium. It has showed that negative water potential under water stress conditions caused plants to form small cambial derivates resulting in narrow vessels (Arend and Fromm 2007). Moreover, Aloni (2004) has suggested that the formation of narrow vessels under water stress is a result of the elevated auxin concentrations in the plant body which induces the early secondary wall formation and lignification preventing further cell expansion. However, on the other hand, such anatomical modifications, have several associated functional significances.

As reported by Robert *et al*. (2009), the described anatomical features like increased VD, VGI and decreased Vdia are known to be the adaptations which support the plant to overcome risk of cavitation as air bubbles are easily formed inside the water filled vessel elements under negative water potential obstructing the flow of water. Therefore, high VD and VGI under the water stress conditions help to serve more efficient bypass (being closer) of embolized vessels which minimizes the number of dysfunctional vessel elements due to cavitation (Schmitz *et al*. 2006). Probably this could be the reason why higher number of solitary vessels was favored in non-stress conditions, for example, 100% WHC, freshwater and moderate saline conditions, since risk of cavitation is comparatively low under sufficient water availability. However, the chance for cavitation under negative water potential cannot be totally avoided by the anatomical modifications in conductive tissues (Robert *et al*. 2009). This is clearly evident from the fact that hydraulic conductivity of *R. mucronata* seedlings grown in the 50% WHC and high salinity conditions, has remarkably reduced even when occurring the suitable anatomical modifications in conductive tissue. Therefore, we propose that this would happen either due to the presence of a higher number of dysfunctional vessels through which a resistance to water flow is created or any internal adjustment to water flow, done by the plants themselves in case of higher water demand. The reduced hydraulic conductivity could secondarily depend on SC of the plants as SC of the *R* plants grown in the 50% WHC and high salinity was significantly lower while the highest was recorded in the plants grown in the freshwater treatment. Several studies reported that high stomatal conductance in *R. mucronata* was recorded in freshwater condition and it was reduced with the increase of salinity and similar results were recorded for other mangrove species as well (Naidoo 1985; Aziz and Khan 2000; Kodikara *et al*. 2017b). Moreover smaller leaf area is also a strategy to reduce water loss from plants under water deficit conditions (Ball 1988; Brugnoli and Lauteri 1991). Interestingly, Parker and Pallardy (1985) have observed a strong positive correlation of leaf area with vessel diameter, stem conductive area and total xylem area for seedlings of several species. We also observed

the reduced CA in both experiments of our study. However, this indicates that water use efficiency (WUE) decreases with the increased negative water potential under substrate drought and hypersaline conditions.

According to the observations, when the *Rhizophora mucronata* plants face water stress conditions (substrate drought or hypersaline condition), both anatomy of the xylem tissues and the hydraulic conductivity were modified as a response to stress. Further, the plants make anatomical modifications such as increased vessel density, vessel grouping index with narrow vessel elements which are more directed toward the avoidance of vessel cavitation. Parallel to these anatomical changes, stomatal conductance and leaf area also decreased, most probably as a measure of water conservation under water stress conditions. The trade-off between the energy allocation for the above tolerant mechanisms and plant growth is achieved through retarded growth of *R. mucronata* plants as evident by the plants grown in the 50% WHC, 25% WHC and high salinity treatments. At extreme level, *R. mucronata* plants ended up in early mortality (e.g. 25% WHC), more likely due to physiological imbalances that occur with increased water and energy demand under such prolonged water stress conditions. On the other hand, *R. mucronata* seedlings could build up some safety mechanisms inside the plants with anatomical modifications though, efficiency of water transportation was not well-maintained as reflected by decreased hydraulic conductivity of the plants under highly stressed conditions. Further, it clearly demonstrated that the water use efficiency of the *R. mucronata* plants also reduces under the stress conditions.

The results of the study demonstrate that, under abiotic stress conditions, *Rhizophora mucronata* seedlings are able to survive for a certain period (up to some critical level) through some structural and physiological adjustments. Nevertheless, if these highly stressed conditions prevail for a longer period (prolonged exposure), the *R. mucronata* plants are unable to secure their survival. This clearly depicts that the impacts of stress conditions, created on mangrove plants particularly in early life stage due to the inappropriate planting practices, are more detrimental when compared with the natural fluctuations of salinity and availability of water in mangrove ecosystems. This emphasizes the need of understanding the lagoon history with special attention to behavior of lagoon hydrology including inundation levels, water depth, salinity and selection of suitable tidal position accordingly for mangrove planting.

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