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Guidance for mangrove replanting: 1. Interspecific variations in responses of mangrove saplings to two contrasting salinities

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The early growth of seven species of true mangroves representing all the categories relevant to viviparity (i.e. true viviparous species, crypto viviparous species and non-viviparous species) and including pairs of species which are closely related as well as species commonly used in replanting, was studied in response to two contrasting salinity regimes, low saline (i. e. 3-5 ppt) and high saline (i.e. 25-27 ppt). Growth performance of the seven species (i.e. *Avicennia marina*, *A. officinalis*, *Bruguiera gymnorrhiza*, *B. sexangula*, *Rhizophora apiculata*, *Rhizophora mucronata*, and *Sonneratia caseolaris*) in terms of plant dry weight, Relative growth rate (RGR), leaf area and shoot height was assessed. The percentage water content of plants under the two salinity levels was also assessed. Performances of all the aspects of *A. marina* under the low saline and high saline conditions were not significantly different implying that this species has the highest salinity tolerance among the seven species. *S. caseolaris* did not survive under high saline conditions proving that it is the lowest in salinity tolerance. The performances of the other five species were in between these two ends, and showed considerable variation. The RGR of each of *A. officinalis*, *B. gymnorrhiza*, *B. sexagula* and *R. apiculata* was significantly lower under high saline conditions, with reductions in growth compared with low salinity conditions of 51%, 40%, 64% and 32% respectively. By considering variations in the performance of all the factors assessed, it was possible to arrange the seven species in descending order of salinity tolerance as *A. marina* > *R. mucronata* > *B. gymnorriza* and *R. apiculata* > *A. officinalis*, and *B. sexangula* > *S. caseolaris*, showing that even taxonomically similar species may be distant in salinity tolerance. The percentage water content of the least saline tolerant mangrove species, i.e. *A. officinalis*, *B. sexangula*, *Rhizophora apiculata*, was higher when they were grown under low saline conditions, implying that species with less tolerance to salinity may opportunistically absorb and keep more water when the salinity is low. As salinity of the habitat appears to be a primary factor controlling the survival and growth of seedlings planted, these interspecific variations in salinity tolerance of species should be taken into consideration in mangrove replanting.

Key words: Salinity tolerance, Mangrove replanting, Interspecific variation, Growth

1. Introduction

Mangroves are woody shrubs and trees that are salt and flood tolerant and hence dominate intertidal areas of lagoons, estuaries and sheltered bays along tropical and subtropical coastlines (Ball 2002; Tomlinson 1986; Tuffers et al. 2001). These tidal forests are of enormous ecological and economic importance (Bandaranayake 1998; Bandaranayake 2002). Despite the importance of mangroves in providing ecosystem goods (food, medicines and timber) and services (such as fisheries nurseries and erosion control) to local communities living behind and within the forest, reportedly 50% of the world's mangrove forests have been destroyed in the second half of the 20th century, and current loss rates vary from 1 to 20% of total forest area per year (Alongi 2002). Hence the conservation and restoration of mangrove ecosystems deserves higher priority.

The increasing awareness of sea level rise due to global warming, which threatens to entirely inundate much land and render other low lying areas suitable only for salt tolerant plants (Dahdouh-Guabas et al. 2005), has raised the profile of mangroves as potential coastal protection belts. The massive tsunami that hit South-East Asia on December 26^{th} 2004, killing over 400,000 people and leaving millions homeless, was a dramatic and tragic reminder of this ecological function. In the aftermath of the killer tsunami, the common-sense view that mangroves can act as living dykes against ocean surges was taken seriously and received empirical support (Clarke 2005; Dahdouh-Guebas et al. 2005; Danielsen et al. 2005 ; Liu et al. 2005; Williams 2005). Concurrently, governments across the Indian Ocean have announced a plethora of new schemes to protect and replant mangroves and thereby attempt to rectify the widespread losses of mangroves during the last decades.

When a destroyed mangrove area is going to be replanted, ecological aspects of mangroves should be taken into consideration particularly in choosing the species suitable for the selected site. Salinity and hydrology (i.e. period and frequency of flooding) in selected habitats are some of the primary factors which determine the survival and growth of replanted mangroves and, hence the success of the replanting projects, because different true mangrove species vary in tolerance to such ecological factors (Allen et al. 2003; Hwang and Chen 2001; Ye et al. 2005).

Although the salinity is often considered as a “stress”, NaCl may also be a resource for halophytic species (Ball 2002). The classic growth response of halophytes, including mangroves, to increasing salt concentration is similar to that shown for nutrients, with variation in the shape of the response curve reflecting concentrations which are deficient, saturating and toxic to growth (Ball 2002). However, the concentration at which salt becomes toxic depends on the species. Although mangroves are a group of salt tolerant plant species, the degree of tolerance varies depending on the species (Tomlinson 1988; Ye et al. 2005). This range of degrees of tolerance to salinity is one of the factors thought to generate zonation patterns in mangrove communities in the intertidal zone (Macnae 1968).

Although the salinity tolerances of some mangrove species have been studied, much information on salt tolerance that would be of use to restoration efforts remains to be discovered. The salinity tolerance or water use characteristics of the same species may vary depending on climatic and edaphic factors (Youssef and Saenger 1998). Hence field studies of mangrove distribution and growth correlated with salinity will usually be confounded by many other pertinent variables. Growth and mortality in the field is likely to be controlled by the most severe conditions the tree experiences. Therefore, in the present study, the performance of eight true mangrove species, including species commonly used in replanting, were tested under conditions representing extreme, but naturally occurring, salinity levels.

Viviparity may also affect the salinity tolerance of mangrove saplings (Ye et al. 2005). The distribution and composition of mangrove species in Sri Lanka, a small island with one third of the worlds true mangrove species (Jayatissa et al. 2002), show that the co occurrence of two species of the same genus in the same habitat is unlikely (Jayatissa, unpublished data), implying different mangrove species in the same genus also may have different tolerance levels for edaphic factors. Therefore, mangrove genera with different categories of vivipary were selected for this study, with replicate species within in genera selected where possible.

2. Objectives

The main objective of this project was to study the interspecific variations in the salinity tolerance of common mangroves in Sri Lanka, at their early growth. As a specific objective, particular attention was paid to differences between taxonomically more related species in the same genera in their responses to high saline conditions, because such variations are apparently neglected in many replanting programs, particularly in which experts are not involved, thus leading to failures.

3. Materials and Methods

3.1. Selection of species

Out of the fourteen mangrove genera in which 20 species of true mangroves are reported to occur in mangrove communities along the coastline of Sri Lanka, four genera include more than one species in each genus (Jayatissa et al. 2002). The responses of a mangrove species to harsh environmental conditions in its early growth may vary depending on whether the species is viviparous or not (Ye et al. 2005). Therefore vivipary was considered during the selection of species for this study. By considering these two facts, eight common species were selected for the study as four species from viviparous genera, i.e. *Bruguiera gymnorrhiza*, *B. sexanguila*, *R. epiculata*, and *Rhizophora mucronata*, two species from cryptoviviparous genera, i.e. *Avicennia marina*, and *A. officinalis*, and two species from the non-viviparous genera, *Sonneratia caseolaris* and *S. alba*. However, mature seeds of *S. alba*, a comparatively rare species, were not available during the study period, hence the study was restricted to the rest of the seven species.

3.2. Culture and experimental design

Mature propagules or seeds of the selected species were collected from natural mangrove sites and used as planting materials. A sandy soil was prepared by mixing sieved loam soil with sand and organic matter (i.e. degraded mangrove litter) in 1:1:1 proportion. Initially propagules and seeds of all the species were planted in plastic pots (with 5 cm diameter and 15 cm height) filled with the prepared soil mixture and kept in a nursery irrigated with fresh water until the establishment of seedlings. Seedlings with the first two leaves unfurled were considered as established seedlings; eight seedlings from each species, all of similar size, were transferred individually to larger plastic pots (15 cm diameter and 40 cm height) filled with the same mixture of soil. Pots with seedlings were placed individually on 7 cm deep plastic trays to get extra water collected after watering and four replicate pots from each species were assigned to each salinity treatment. The experiment was started with these established seedlings in order to minimize masking of salinity effects by other effects (i.e. effects of seed or propagule quality).

Two salinity regimes, 'low saline' (i.e. 3-5 ppt) and 'high saline' (i.e. 25-27 ppt), were selected for the experiment. Low saline and high saline water was prepared by mixing seawater and tap water, left in tanks and used to irrigate seedlings in pots. Each pot was irrigated twice a day by the water with the salinity assigned to each pot. Excess water accumulated in trays was returned to tanks every other day and the salinity of the water in tanks was checked by a hand refractometer (ATAGO S/Mill-E, Japan) and adjusted by adding tap water when necessary once every four days. Commercially available fertilizer was also applied once a month by dissolving a recommended dose in high saline and low saline water before used to irrigate seedlings in pots. Seedlings in pots were distributed and left in the green house according to a completely randomized design.

3.3. Data collection

The shoot height of each sapling (starting from the propagule end in the case of the viviparous species) was measured once a week. Plants or saplings were harvested after three months of growth. The plastic pots were removed, and the soil carefully washed away by tap water to get the intact root system. Cleaned plants were blotted dry and separated into roots, hypocotyls, stem and leaves. The fresh weights of these four parts of each plant were measured and leaf area was quantified manually using millimeter paper on which the exact size and shape of leaves were marked. Then all parts were oven dried at 60℃ for dry weight. The difference between the dry weight and fresh weight of individual plants was taken as the water content. Relative growth rate (RGR) for each plant was calculated for individual plants according to;

$$RGR = W/t$$

where W is the dry weight of each plant without the hypocotyl of viviparous species and t is the growth period, i.e. 13 weeks (Ye et al. 2005).

It was observed that hypocotyl part of planted propagules of *Rhizophora* and *Bruguiera* were enlarged slightly during the 13-week growth period implying that hypocotyls also were grown. It can be assumed that their dry weight have also been increased during that growth period. If it is true, the initial dry weight of the propagule should be subtracted from the dry weights of each of the three months old saplings to get the growth of the sapling to calculate RGR of each sapling. In order to calculate the initial dry weight of propagules of three months old saplings, a regression of dry weight over fresh weight of mature propagules at the initial stage is needed. But when the mean dry weight of propagule parts of three months old saplings, were compared with the mean dry weight of mature propagules at the initial stage, it was revealed that the dry weight of propagule parts of saplings was lower, although the propagule parts of saplings have been grown. It is possible as a large amount of foods are stored in propagules at the initial stage and those food reserves are used for the early growth of the plants converting storage tissues of the propagule into structural tissues of the sapling. Therefore, in the calculation of RGR, the propagule parts of three months old saplings were omitted and the dry weight of other parts, i.e. roots, newly grown stem, and leaves, were considered as the net growth of the total sapling during the experimental period.

3.4. Data analysis

Mean and standard deviation values of total dry weight, mean leaf size, percentage water content and RGR were calculated separately for quadruplicate pots of each species grown under each salinity level. Two sample t-tests (Zar 1984) were carried out for individual species to learn whether the plants grown under two different salinities are different in each of the above parameters.

One-way ANOVA with Tukey-Kramer HSD test (Zar 1984) was used to test significant interspecific differences in dry weight, mean leaf size, water content and relative growth rate among the seven mangroves. For this purpose, data under high saline condition and low saline condition were considered and used as one set of data, neglecting the salinity levels.

4. Results

4.1. Interspecific variations irrespective of salinity effects

The comparison of the seven mangrove species for interspecific variations in dry weight, percentage water content, RGR and mean leaf size revealed that *R. mucronata* was significantly different from all the other species showing the highest values in three factors, dry weight, RGR, and mean leaf size, and the lowest value in percentage water content (Table 1). For all the species studied, the higher values of percentage water contents were recorded when they are grown under low saline conditions and the highest water content was recorded from *S. caseolaris* under low saline conditions. When differences between the two species in each of the same genus are considered, *R. apiculata* and *R. mucronata* show significant differences in RGR, dry matter accumulation and percentage water content whilst the two species in the genus *Avicennia*, i.e. *A. marina* and *A. officilanis*, show a significant difference only in percentage water content (Table 1). *B. gymnorrhiza* and *B. sexangula* were not significantly different in RGR and percentage water content, but in mean dry weight and mean leaf size. The highest diversity among species was recorded in percentage water content.

The growth rate in terms of the increment of shoot height also shows a remarkable interspecific variation. The lowest and highest growth rates were recorded from *B. sexangula* and both species of *Avicennia* respectively. The growth rates of the rest of the species were at intermediate levels (Figure 1).

4.2. Interspecific variations in response to salinity

The negative effect of salinity on the growth of saplings was most pronounced for *S. caseolaris* as all the individuals under high saline conditions died within the first two weeks period. However the growth curve of *S. caseolaris* saplings under low saline conditions shows the highest growth rate after the first five weeks (Figure 1g). Growth curves of the other species show that the growth of saplings of *A. officinalis* and *B.sexagula* under high saline conditions was greatly reduced whilst that of *B. gymnorrhiza* and *R. apiculata* was moderately reduced. Salinity effects on the height growth of shoots of *A. marina* and *R. mucronata* were negligible (Figure 1). Figure 2 corroborates this result. It shows that RGR values of saplings of each of *A. marina* and *R. mucronata*, grown under low saline and high saline conditions are not significantly different whilst those of the other species are significantly different. At the high saline condition, decreases in RGR of *A. officinalis*, *B. gymnorrhiza*, *B.sexagula* and *R. apiculata* were 51%, 40%, 64% and 32% respectively.

The patterns of salinity effects on the dry weight of saplings of the seven mangroves are similar to those shown for RGR. The mean dry weight of *A. marina* saplings grown under high saline conditions was not significantly different from that of saplings grown under low saline conditions. It is true for *R. mucronata* also. Sapling dry weights of each of *A. officinalis*, *B. gymnorrhiza*, *B.sexagula* and *R. apiculata* under low saline and high saline conditions were significantly different (Figure 3).

The percentage water content in three month old saplings of each of *A. officinalis*, *B. sexangula* and *R. apiculata* grown under low saline conditions were significantly different from those grown under high saline conditions. Saplings of *A. marina*, *B. gymnorrhiza* and *R. mucronata* did not show such a difference in their water content (Figure 4).

Apart from *A. marina*, in which leaf size under low saline conditions and high saline conditions was not significantly different, leaves produced by all the other species under high saline conditions differed significantly in size from those produced under low saline conditions (Figure 5).

5. Discussion

In planning the rehabilitation and reconstruction efforts after the recent killer tsunami that hit South-East Asia on December 26^{th}, 2004, many countries gave a high priority for the re-establishment of natural barriers against tsunami and other ocean surges. Concurrently, mangroves were subjected to extensive discussions as a potential natural barrier against tsunami and some other ocean driven disasters (Clarke 2005; Dahdouh-Guebas et al. 2005; Danielsen et al. 2005; Liu et al. 2005; Williams 2005). Mangrove replanting programs were initiated and supported with the help of governmental and non-governmental organizations, particularly in tsunami affected countries. However, the success of such programs depends on the selection of suitable species based on the prevailing edaphic conditions at the replanting site. Although true mangrove species show extreme adaptations to harsh environmental conditions in general, most of the species require specific conditions

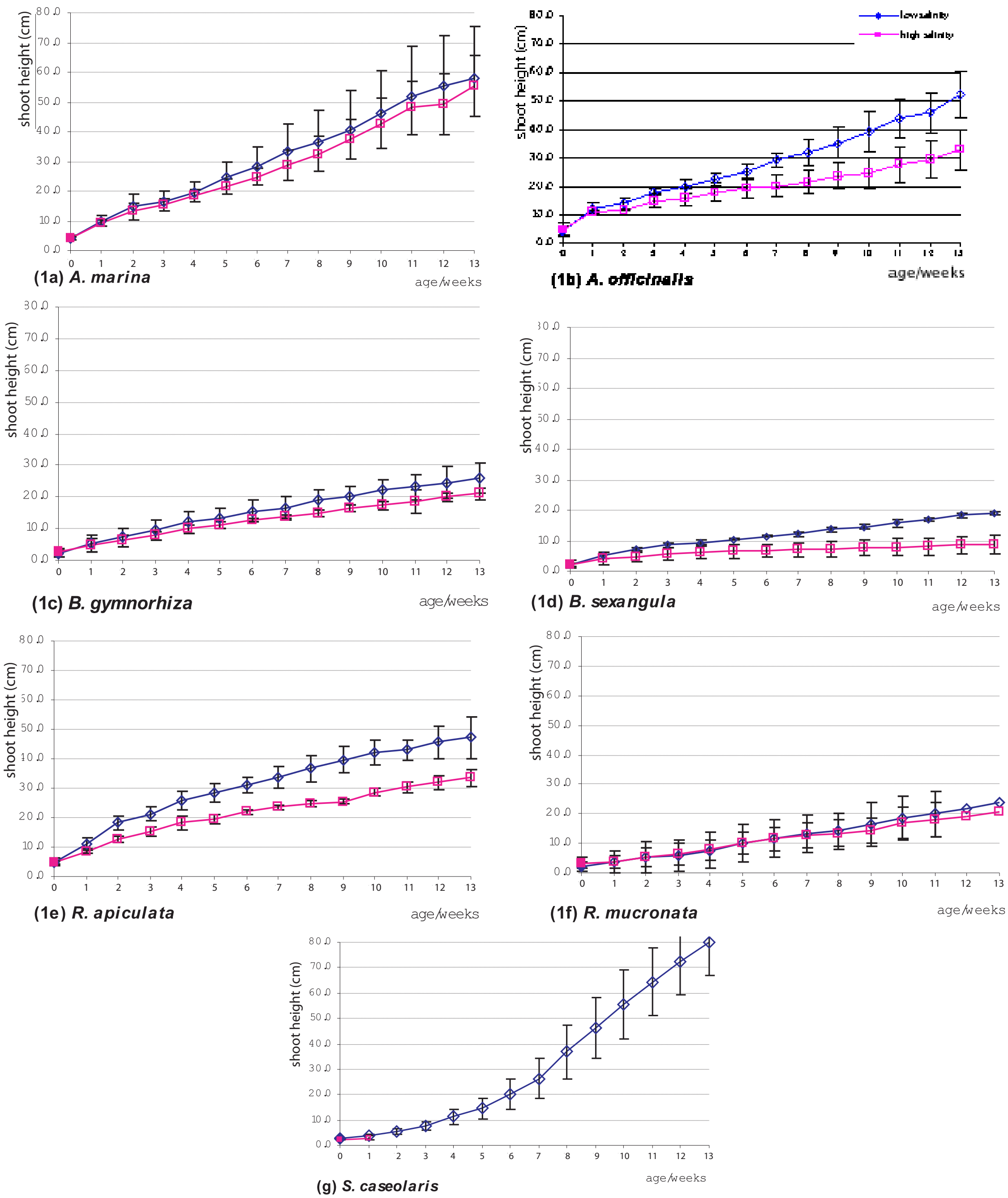

Figure 1 Variations of the mean shoot (i.e. stem height) growth of mangrove saplings grown under two different salinity regimes (Low saline = 4-5 ppt; High saline = 25-26 ppt.).

Note. Longitudinal bars indicate standard deviations

Table 1 Interspecific differences in mean dry weight, mean leaf size, % water content and Relative growth rate of seven mangrove species, as resulted by one-way ANOVA. (The data from S. caseolaris was not included to the ANOVA as the available data were from plants grown under low salinity level only.)

Species	Mean dry weight (g)	Mean leaf size (cm^2)	Water content (%)	RGR
A. marina	5.97^{d}	9.39^{c}	76.31^{b}	0.4587^{bc}
A. officinalis	7.09^{d}	20.02^{bc}	79.99^{a}	0.5437^{bc}
B. gymnorrhiza	12.09^{c}	23.39^{b}	71.75^{cd}	0.440^{bc}
B. sexangula	5.01^{d}	10.70^{c}	73.93^{cd}	0.2337^{c}
R. apiculata	16.49^{b}	36.58^{a}	71.20^{d}	0.5662^{bc}
R. mucronata	23.39^{a}	40.10^{a}	66.86^{e}	1.4962^{a}
S. caseolaris	11.65	16.8	84.32	0.8961

* Different superscripts with each value denote significantly different (p< 0. 05) groupings according to Tukey-Kramer HSD test.

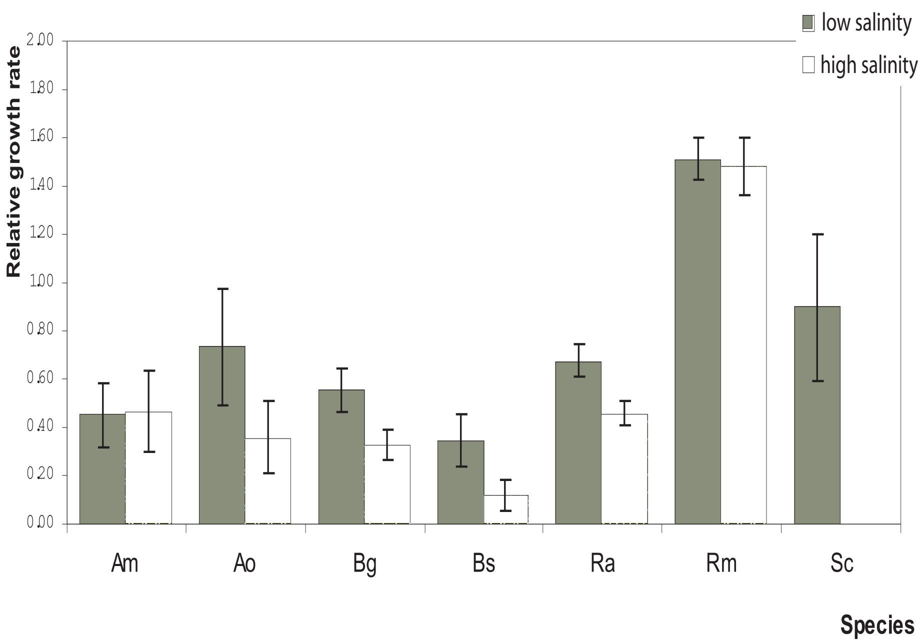

Figure 2 Variations of Relative Growth Rate (RGR) of 90 day old saplings of six mangrove species (Am, *Avicennia marina*; Ao, *Avicennia officinalis*, Bg, *Bruguiera gymnorrhiza*; Bs, *Bruguiera sexangula*; Ra, *Rhizophora apiculata*; Rm, *Rhizophora mucronata*) grown under two different salinity regimes(Low saline = 4-5 ppt; High saline = 25-26 ppt) for three months.

Note. (Longitudinal bars indicate standard deviations. Different superscripts with two bars relevant to each species, indicate that RGR values under high saline and low saline conditions are significantly different at p<0.05).

and a narrow range of many ecological factors for their optimum growth (Kathiresan and Bingham 2001). Salinity is one of the major environmental factor controlling

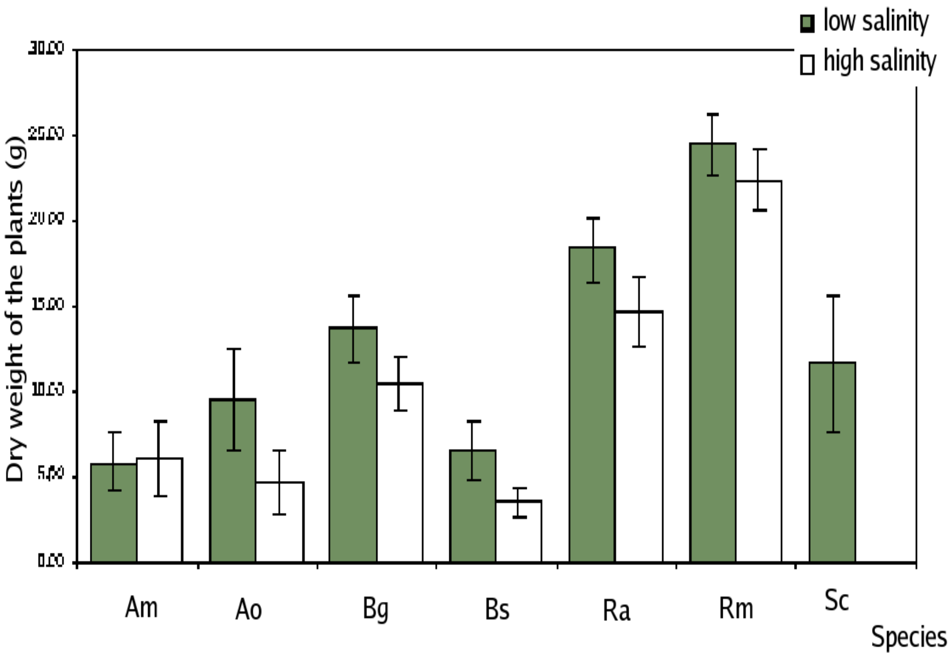

Figure 3 Variations of the mean dry weight of saplings of different mangrove species (Am, *Avicennia marina*; Ao, *Avicennia officinalis*, Bg, *Bruguiera gymnorrhiza*; Bs, *Bruguiera sexangula*; Ra, *Rhizophora apiculata*; Rm, *Rhizophora mucronata*; Sc, *Sonneratia caseolaris*) grown under two different salinity regimes (Low saline = 4-5 ppt; High saline = 25-26 ppt.) for three months.

Note. (Longitudinal bars indicate standard deviations. Different superscripts with two bars relevant to each species, indicate that dry weight values under high saline and low saline conditions are significantly different at $p<0.05$).

the growth and survival of mangrove plants (Allen et al. 2003; Hwang and Chen 2001; Ye et al. 2005). Effects of such factors could be crucial particularly at the early growth of saplings, although the effects of salinity on seedling establishment were not considered here since most replanting programs are started with seedlings established in a nursery where fresh water or low saline water is used for irrigation.

In this study, responses of mangrove saplings under low saline and high saline conditions were studied for only 13 weeks. Some studies may suggest that such a short period may be insufficient to reveal slowly developing responses (Ball et al. 1997). Nevertheless, rapid responses to soil salinity during early growth of saplings may be important determinants of their survival. As an example, as *B. sexangula* under high saline conditions shows a severe inhibition (i.e. 64%) of its RGR after three months of growth, further growth and development is also likely to be impaired, particularly once the initial food reserves in the propagule are exhausted. It is reported that viviparous species perform better than non-viviparous species in seedling establishment, implying the beneficial effects of propagules in early growth (Ye et al. 2005)

Out of the seven species tested in this study, *S. caseolaris* proved that it is strictly a low saline species, as all the seedlings under high saline conditions died within

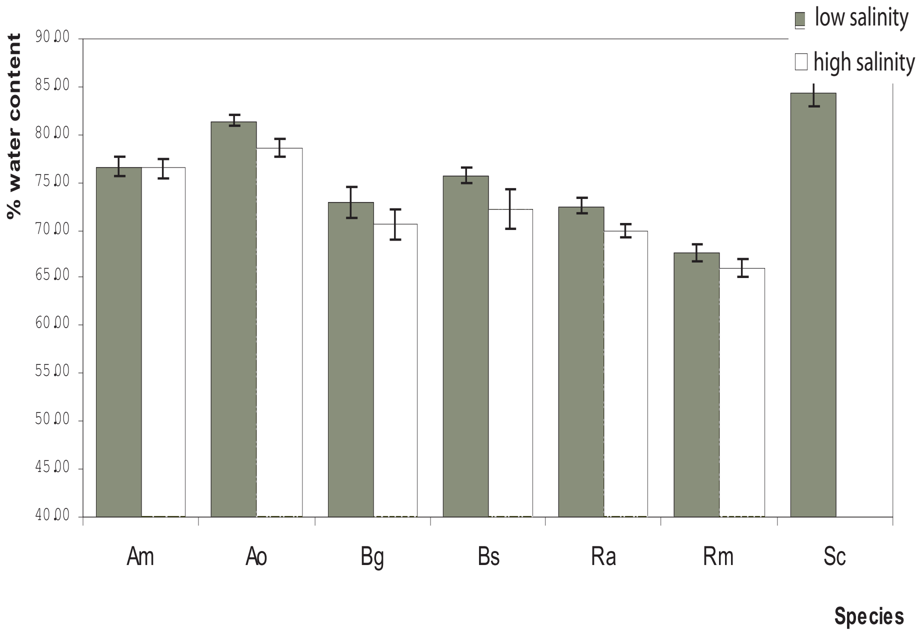

Figure 4 Variations of the percentage water content of 90 day old saplings of six mangrove species (Am, *Avicennia marina*; Ao, *Avicennia officinalis*, Bg, *Bruguiera gymnorrhiza*; Bs, *Bruguiera sexangula*; Ra, *Rhizophora apiculata*; Rm, *Rhizophora mucronata*) grown under two different salinity regimes (Low saline = 4-5 ppt; High saline = 25-26 ppt) for three months.

Note. (Longitudinal bars indicate standard deviations. Different superscripts with two bars relevant to each species, indicate that % water content values under high saline and low saline conditions are significantly different at $p<0.05$).

the first two weeks. This suggests that *S. caseolaris* is not suitable for high saline areas, although mangrove dwellers may favor this species over the other species due to economic uses of the species (Jayatissa et al. 2006). The distribution of this particular species in Sri Lanka is mainly restricted to river estuaries of the wet zone of the country (Jayatissa et al. 2002; Jayatissa et al. 2006) supporting the fact that it is a low saline species. However, salt tolerance of mature individuals of *S. caseolaris* could be higher than that of saplings (Bhosale 1994).

The rest of the six species survived under high saline conditions but showed some differences in growth performances, with the exception of *A. marina*. The reduction of the leaf size was the first apparent sign of the salt stress in this study, indicating that the leaf size is the most sensitive factor for salinity (Parida and Das 2005). Out of the mangroves tested in this study, *A. marina* is the only species that did not show a significant reduction of the Leaf size under high saline conditions. Therefore, *A. marina* proved to be the species with the highest tolerance to high saline conditions among the species tested in this study. Many other studies have also shown that *A. marina* has a better salinity tolerance (Clough 1984; Downton 1982; Ye et al. 2005).

Dry matter accumulation, RGR, and water content of *R. mucronata* saplings grown under high saline condition were not significantly different from those grown

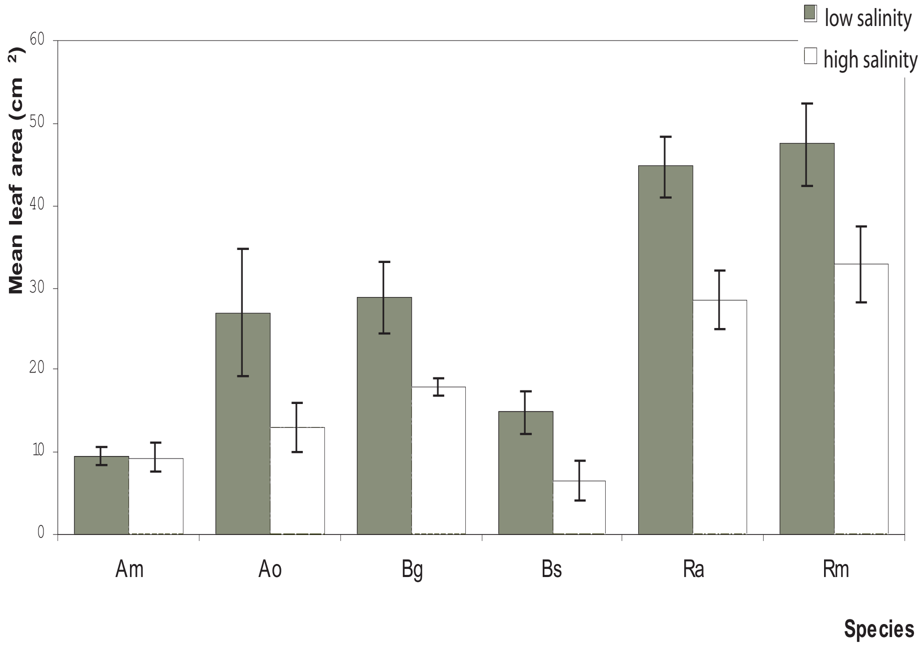

Figure 5 Variations of the mean leaf sizes of saplings of six mangrove species (Am, *Avicennia marina*; Ao, *Avicennia officinalis*, Bg, *Bruguiera gymnorrhiza*; Bs, *Bruguiera sexangula*; Ra, *Rhizophora apiculata*; Rm, *Rhizophora mucronata*) grown under two different salinity regimes for three months period (Low saline = 4-5 ppt; High saline = 25-26 ppt) for three months.

Note. (Longitudinal bars indicate standard deviations. Different superscripts with two bars relevant to each species, indicate that mean leaf sizes under high saline and low saline conditions are significantly different at $p<0.05$).

under low saline conditions. The leaf size was the only exception. Therefore, out of the seven species studied, *R. mucronata* appeared to be the second highest in salinity tolerance.

When the decreases or inhibitions of leaf size, RGR and dry matter accumulation under high saline conditions are considered, the species tested in this study can be arranged in descending order of the salinity tolerance as *A. marina* > *R. mucronata* > *B. gymnorriza*, & *R. apiculata* > *A. officinalis*, and *B. sexangula* > *S. caseolaris*. *S. alba* was not included in this study as it is a rare species in Sri Lanka. But documentary evidences say that *S. alba* can grow well under high saline conditions (Ball and Pidsley 1995).This grading shows that taxonomically more related species i.e. species in the same genera, may be distant in salinity tolerance. There may be some discrepancies in the optimal salinity for the better performances of mangroves, grown in the field versus in green house conditions (Hwang and Chen 2001). It could be due to the fact that the salinity level in the field could fluctuate and plants preferentially take up water when the salinity is low. But under the greenhouse conditions plants exposed continuously to the same salinity level. However, when the distribution of these species in Sri Lanka are considered, it was noted that these pairs of species, which are in the same genus but distant in salinity tolerance, rarely exist in the same micro habitat, although all the species are in the same community, and the distribution of the last three species of the above series, are restricted to mangrove communities with more riverine influence (Jayatissa et al. 2002; Jayatissa Unpublished data).

In general, plants growing in habitats with a deficit of water possess adaptations to store water. Mangrove plants also have to face to a physiological drought due to the higher soil salinity implying that mangroves growing under higher salinities should have higher water content. In contrast, this study reveals that the percentage water content in saplings of species less tolerant to salinity, i.e. *A. officinalis*, *B. sexangula*, *Rhizophora apiculata*, were higher when they were grown under low saline conditions. In a mangrove habitat, the soil salinity is not constant but fluctuates depending mainly on the fresh water inflow and, in the case of Sri Lanka, blocking of the lagoon mouth. Mangrove species, particularly those that are less tolerant to high saline conditions, could be opportunistically absorb and store more water when they are exposed to low saline conditions (Kathiresan and Bingham 2001). Hence, lower values of the water content in plants under high saline conditions may be considered as a sign of salt stress.

For a mangrove species, the capacity to invade intertidal habitats as well as the position occupied in a species zonation, depends on their salinity tolerance at early growth (Macnay 1968; Saurez and Medina 2005; Ye et al. 2005). As an example, *A. marina* may occupy a wider range of intertidal habitats as its salinity tolerance is higher (Dahdouh-Guebas et al. 2002). The tolerance of mangrove species to salinity should be taken into consideration in the selection of planting sites and suitable species for mangrove ecosystem rehabilitation. Many attempts to restore mangroves fail completely, as they are poorly planned and managed. Planting the wrong species in the wrong place is one of the main reasons for many failures in mangrove rehabilitation (Lewis 2005).

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