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Effects of Leaf Establishment on Shoot Growth and Hydraulic Characteristics of *Excoecaria agallocha* in Sathurukkondan, Batticaloa

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Abstract

Excoecaria agallocha (Family: Euphorbiaceae, Tamil: Thilla, Sinhala: Thela or Thelakiriya) is a semi deciduous true mangrove found in both inland (opposite site to Batticaloa lagoon) and shoreline areas in Sathurukkondan, Batticaloa, Sri Lanka. Leaf shedding in *Excoecaria agallocha* is a common phenomenon that occurs during September – November and leaves rejuvenate in January onwards. Preliminary ecophysiological experiments were conducted using adult plants coppices in an inland of Sathurukkondan mangrove site from December 2005 to March 2006. Above ground hydraulic and growth parameters were investigated at leaf establishment stages of early unfolded (December – January, 2005) and fully unfolded (March, 2006), whether any leaf establishments related to hydraulic and growth parameters of *E. agallocha* or not, was studied. Further, hydraulic and assimilate resistances were increased at the above ground levels by long term manipulations of xylem - phloem notching and phloem girdling in December, 2005 and it responses were investigated in March, 2006.

Leaf establishments and xylem – phloem manipulations of notching and girdling has shown impact on the growth parameters of *E. agallocha* in terms of stem over bark diameter, total leaf area, total leaf dry mass, total stem dry mass and specific leaf area (SLA). Hydraulic parameters of maximum absolute hydraulic conductivity (k_{max}), leaf specific hydraulic conductivity (k_l, k_{max} / leaf area distal) and specific hydraulic conductivity (k_s, k_{max} / sapwood area) were significantly increased concurrently with leaf establishments and xylem - phloem notched *E. agallocha.* Surprisingly, xylem - phloem notched branches shown higher k_s compared with branches measured at fully unfolded stage and this could be suggested as a survival strategy (drought avoider) of *E. agallocha*, while alleviating k_s under stem level stress condition. k_l increased with leaf establishments and in manipulated xylem – phloem notched stems. In accordance, a positive relationship was established between k_l and vessel diameters. Vessel diameters measured at distal ends of stems were progressively and relatively widened and shown significant differences in early unfolded, fully unfolded and notched *E. agallocha*. This suggests that progressive increased hydraulic characteristics were an impact of vessel diameter of stem. Further, *E. agallocha* need not necessarily depend on the leaves for conservative water use like other forms, as this species falls under semi-deciduous with a phenomenon of shedding leaves.

Keywords: *Excoecaria* spp., hydraulic characteristics, manipulations, drought resistant, survival.

Introduction

Water is essential for plant growth and cells contain water as the solvent in which biochemical reactions takes place and cell structures are maintained (Lack and Evans, 2001). Water stress is a major factor limiting terrestrial plant productivity (Pyrke and Kirkpatrick, 1994). As soil water depleted, water flow through the soil and plant decreases to one - tenth the maximum rate but both the soil resistance and plant resistance increased (Blizzard, 1980). Mangrove species exhibit a range of water use behaviors related to salinity and their zonation patterns parallel to shore (Sobrado, 1999).

In this present study, *Excoecaria agallocha*, a true and dominance mangrove in Batticaloa district, above ground stem growth characteristics and hydraulic architecture was assessed during the leaf establishment stages. *E. agallocha* is a semidecidious mangrove and sheds their leaves 2 to 3 times annually in Batticaloa, Sri Lanka. A main shedding of leaves occurs immediately after the first break of North – East monsoon in the middle of October and this prolongs up to December. Gradual changes of green into orange / red of leaves and then shedding and sprouting of new leaves often attract the travelers passing the A_{15} highway through the proposed study site of Sathurukkondan, Batticaloa.

To our knowledge, no literatures data on hydraulic architecture, particularly during the periods of leaf establishments is available in *E. agallocha*, as well as in other semi deciduous mangroves. However, a little work was carried out in *Fraxinus excelsior* concurrently with leaf shedding and establishments, which is a temperate tree (Cochard *et al*., 1997). Hence, it was decided to assess the hydraulic architecture of above grounds in *E. agallocha* (tropical). In this study objectives were three folds, (a) To assess the above ground stem hydraulic architecture during the early stage of leaf establishment (early unfolded stage) and of later stage of leaf establishment (fully unfolded stage), to investigate is there any relationship associated with leaf establishment or not, with measured growth and hydraulic characteristics (b) Hydraulic resistances and assimilate resistances were increased by xylem – phloem notching and phloem girdling manipulations in the stems and morphological responses were observed (c) Further such manipulations (here only notching) whether had an impact on the hydraulic characteristics and it's growth was assessed while compared the data of (a) keeping as a control.

Materials and Methodology

Study site: The plant of semi deciduous true mangrove of *Excoecaria agallocha* growing naturally along the shoreline and inland in Sathurukkondan, Batticaloa were used in this study to investigate the proposed objectives from December 2005 to March 2006. The numbers of *E. agallocha* patches in the sampling area were counted and randomly selected eight plant patches used as replicates for this study.

Fig. 1 An overview of sampling patches of *Excoecaria agalloha* at the time of leaf shedding site at Sathurukkondan, Batticaloa, Sri Lanka (November, 2005).

Fig. 2 The sampling patches of *Excoecaria agallocha* at fully unfolded stage site at Sathurukkondan, Batticaloa, Sri Lanka (March, 2006).

Stem Xylem - Phloem Manipulations

Long term manipulations of stem xylem - phloem notching and phloem girdling to increase hydraulic and assimilate resistances at above ground level was assessed in *E. agallocha* to see the nature of susceptibility. Overall morphological plant responses were observed frequently for 4 months from initial day of notching and girdling.

Hydraulic measurements: Hydraulic conductivity measurements were carried out at three consecutive sets. First measurement was taken from eight randomly collected stem segments at early stage of unfolded (19th December, 2005 – 24th January, 2006), where leaves were an immature stage and second beginning of fully unfolded (7th March, 2006 - 22nd March 2006) where leaves were almost at the flowering. Simultaneous measurements were carried out on the stems subjected to xylem – phloem notching.

Hydraulic conductivity of main branch stem segments of *E. agallocha* was measured using Low Pressure Meter (LPFM) (Sperry *et al*., 1988). The modified and costless LPFM was designed in the Department of Botany, Eastern University, Sri Lanka from the version of Sperry *et al*., (1988). The apparatus designed to measure the water flow rate of stem segment with pressure gradient of 0.016 MPa (head pressure) and the solution of 0.01M HCl used was distilled, degassed, filtered (0.47 μm, Millipore filter).

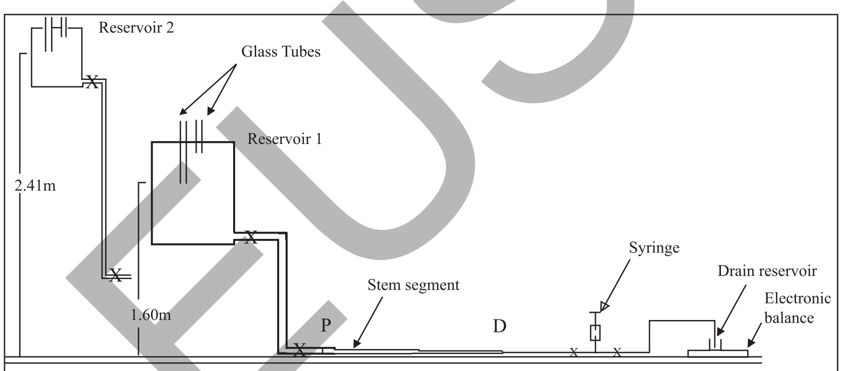


Fig 3 Schematic diagram of modified Low Pressure Flow Meter (LPFM) from the version of Sperry et al. (1988). P – Proximal end of the stem segment, D – Distal end of stem segment, X – Taps to control water supply.

The xylem vessel diameter and sap wood area of distal end of stem segment subjected to hydraulic conductivity measurement was measured using ocular meter and stage micrometer. Distal end and total leaf area of stem segments subjected to hydraulic conductivity measurements were taken by indirect method of manual calculation (Ryan *et al*., 2000, Hubbard *et al*., 2003)

Statistical Analysis

The statistical analysis of one - way ANOVA and two - way ANOVA was performed using a Statistical Package for Social Sciences (SPSS, 14.0 for windows). The relationship between maximum hydraulic conductivity (k_{max}), xylem specific conductivity (k_s) and vessel diameter were explored by using linear regression analysis of the Graph Pad Prism, Version 3.02.

Results

Morphological Changes in Stem Xylem-phloem Manipulations

The new lateral branches of [2 ± 1 (SEM) (n=8)] and [7 ± 1 (SEM) (n=8)] were established below the notched and girdled regions respectively. Identical growth responses of both notched and control (fully unfolded) canopy suggests that the notching manupulation did not caused comparable effect on canopy leaf morphology of *E. agallocha*. Newly formed lateral branches and above yellow mottled girdled canopy suggests that basipetal transport is important for *E. agallocha*.

Fig. 4 The newly established lateral branches below the (a) notched area (b) girdled area after 80 days of notching (March, 2006), (c) Regenerated phloem tissue in and around the girdled portion after 80 days of girdling (March, 2006).

Further, tissue regeneration was observed in and around the notched (7 out of 8 stems) and girdled (5 out of 8 stems) portions and this suggests the recovery ability of xylem-phloem tissues in *E. agallocha.*

Hydraulic Parameters

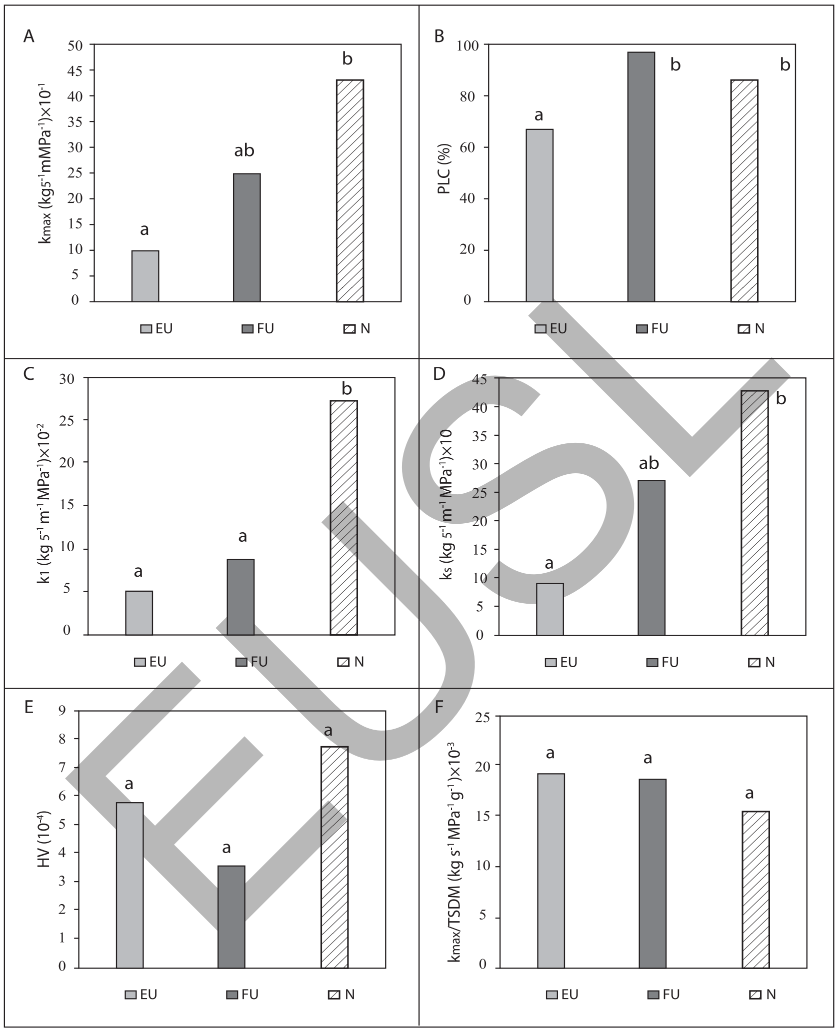


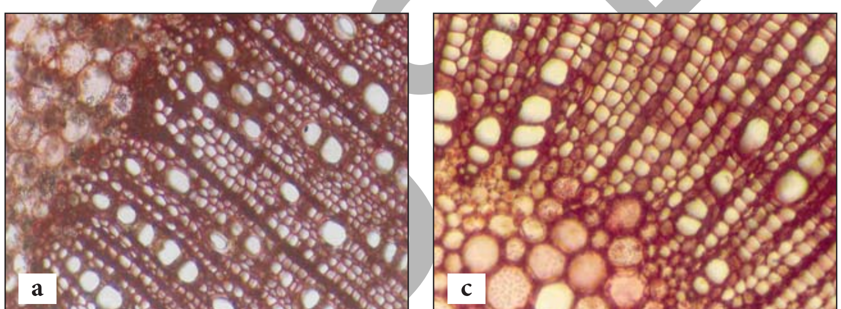
Fig 5 Hydraulic characteristics of *E. agallocha* at early unfolded (EU), fully unfolded (FU) and notched (N) stages. (A) Maximum absolute hydraulic conductivity of stem segments (k_{max}), (B) Percentage loss in hydraulic conductivity after flushing of embolism, (C) Leaf specific hydraulic conductivity (k_l); k_{max} normalized by leaf area at the distal end, (D) Specific hydraulic conductivity (k_s); k_{max} normalized by sap wood area at the distal end, (E) k_{max} normalized by total shoot dry mass ($k_{max}/_{TSDM}$), (F) Huber value (HV). Mean ± SEM (n=7-8). Different letters indicate significant differences at P<0.05 (one - way ANOVA.

Vessel Diameter

The vessel diameter of xylem - phloem notched *E. agallocha* was progressively and relatively widened and significantly wider than early stages of unfolded and fully unfolded stages (F = 55.39, P= 0.00).

Table 1 Xylem vessel diameter at the distal end of *E. agallocha* stem segments at early unfolded, fully unfolded and notched stages. Mean ± SEM (n = 7 - 8). Within columns, different letters indicate significant differences at P<0.05 (One - way ANOVA)

Treatments / stages	Vessel diameter (mm) × 10^{-2}
EU	2.94 ± 0.09^{a}
FU	4.19 ± 0.07^{b}
N	4.95 ± 0.07^{c}



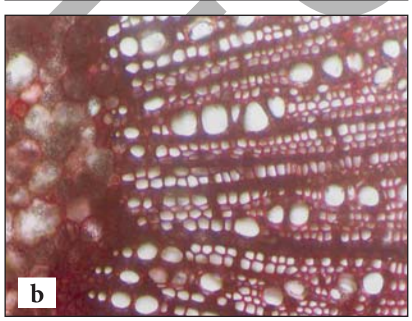


Fig 6 Transverse section of *Excoecaria agallocha* stem at distal end of early unfolded stage - January, 2006 (A), fully unfolded stage - March, 2006 (B) and notched stage - March, 2006 (C). A sector enlarged to show xylem vessels (Photographic microscope, magnification ×100).

Relationship between Vessel Diameter and Maximum Hydraulic Conductivity (k_{max}) and Specific Hydraulic Conductivity (k_s)

k_{max} tend to linearly increased (r^2 = 0.98; linear regression, SPSS 14.0) with increasing xylem vessel diameter from early unfolded to notched stages. Likewise, specific hydraulic conductivity also linearly increased (r^2 = 0.99; linear regression, SPSS 14.0) with increasing vessel diameter. This suggests that responses of specific hydraulic conductivity was an impact on hydraulic architecture (increasing vessel diameter) of stem and need not necessarily depend on the leaves for conservative water use, as this species falls under semi deciduous condition, where partial shedding leaves occurs 2 to 3 times and with a complete shedding of once at annually.

Growth Parameters

Table 2 Above ground growth parameters at early unfolded (EU), fully unfolded (FU), notched (N) and girdled (G) stages in *E. agallocha.* Mean ± SEM (n = 7 - 8). Within columns, different letters indicate significant differences at $P<0.05$ (One -way ANOVA).

Treatments / stages	Total leaf area (m^2)	Total leaf dry mass (kg)	Total stem dry mass (kg)	Specific leaf area (m^2kg^{-1})
EU	0.304 ± 0.042 ^{a}	0.010± 0.001 ^{a}	0.082 ± 0.009 ^{a}	16.7 ± 1.78 ^{a}
FU	0.308 ± 0.09 ^{a}	0.031 ± 0.004 ^{b}	0.140 ± 0.019 ^{b}	9.61 ± 1.8 ^{b}
N	0.394 ± 0.042 ^{a}	0.034 ± 0.004 ^{c}	0.280 ± 0.03 ^{c}	10.88 ± 0.72 ^{c}
G	0.580 ± 0.14 ^{a}	0.046 ± 0.007 ^{d}	0.290 ± 0.16 ^{d}	12.53 ± 0.90 ^{d}

Discussion

Plant Hydraulic Characteristics

The maximum absolute hydraulic conductivity increased with leaf establishments. Higher hydraulic conductivities are desirable for growth performances of plants (Nardini and Tyree, 1999; Nardini and Salleo, 2000; Tyree, 2002) and thus increased the conductivity with leaf establishments. Interestingly, xylem notched stems shown a highest hydraulic conductivity than control of FU instead of expected higher hydraulic resistances by this notched manipulations. But in this study, increased in k_s and k_{max} related with increased vessel diameter. Therefore it is also possible to put forward that ameliorated k_s and k_{max} as a consequent of changes in xylem diameter, possibly a mechanism to overcome a stressful condition which caused by notching in the stem.

Further newly formed xylem tissues possibly support this phenomenon and suggested that conservative water use possibly regulated by widened xylem structure in the stem.

HV more directly related to mechanical properties than hydraulic properties of stems (Tyree and Ewers, 1991). High HV recorded in notched stem of *E. agallocha* could be related to mechanical support of stem and for established leaves. The high recorded values of k_{max} and k_l of notched stems showed that stem notching manipulation provides efficient water transport compared with fully unfolded stems, thus suggested as drought avoider's ability in *E. agallocha*. Leaf specific conductivity (k_l) is a useful parameter, because it is the proportionality constant relating the average transpiration to the stem needed to supply water to the leaves fed by the stems (Tyree *et al*., 1991). Shoot k_l increases with leaf establishments and manipulated xylem – phloem notched stem concurrently with increased vessel diameter. High k_l appeared in notched stem, possibly to prevent excessive water stress to ameliorate the increased hydraulic resistances. Specific conductivity (k_s)is a measure of porosity of xylem conduit (Tyree *et al.*, 1991). In this study, increased vessel diameter associated with increased k_s from EU to FU and this was supported by significant differences of vessel diameter at EU and FU stages. In this study, xylem-phloem notched branches shown higher specific hydraulic conductivity compared with branches measured at fully unfolded stage and this could be a survival strategy of *E. agallocha*, while alleviating k_s under stem level stressful condition and could be classified as drought avoiders. The increased values of k_{max}, k_l and k_s in notched *E. agallocha* suggested the presence of redundancy pathway.

Plants normally have "native embolism" *i.e* they have considerable amount (10-15%) of embolism during rapid transpiration under well watered condition, causing a 5 - 20% loss of hydraulic conductance. The PLC reported here was greater than native embolism and this could have increased by xylem tension at early unfolded, fully unfolded and notched stages. In this study, vessel diameter increased with leaf establishments and notching manipulations. Increased vessel diameter was an impact of hydraulic architecture of stem and thus increases hydraulic characteristics and need not necessarily depend on the leaves for conservative water use. New xylem and phloem tissues formed around xylem phloem notched and girdled portions of stems and considered as survival strategies of *E. agallocha* under assimilate- water stress conditions.

Plant Growth Characteristics

The total leaf area, total leaf dry mass and total stem dry mass increased with increasing hydraulic characteristics of k_{max}, k_l and k_s from early unfolded to fully unfolded stages and further increased to notched *E. agallocha*. Greater leaf area intercepts higher amount of light and increases carbon assimilation (Sheriff, 1992) and hence increased growth performance in terms of total leaf dry mass and total stem dry mass. Notched *E. agallocha* showed higher growth performances compared with control of fully unfolded stage, because of high k_{max} of notched stem could have increases transpiration and this increases CO_2 assimilation and thus increases growth. This suggests that imposed stress condition by notching and girdling manipulation did not affect growth performance and this species could be considered as drought avoiders.

In conclusion, Leaf establishment stages and notching affected the above ground hydraulic characteristics of *E. agallocha* in terms of k_{max}, k_s, k_l, HV and PLC. *E. agallocha* branches shown progressive increases in k_l from EU, FU and notched stems. Leaf areas and PLC also increased concurrently with measured k_l parameter. Increased leaf areas were thus balanced against xylem hydraulic transport capacities in the branches of EU and FU. Further increased k_l in notched stem of *E. agallocha* could be a strategy to ameliorate the hydraulic efficiency at it's given stress condition. Surprisingly, notched branches shown higher k_s compared with branches measured at fully unfolded stage and this could be a survival strategy, while alleviating k_s under stem level stressful condition. Vessel diameter were progressively and relatively widened with growth trends of EU and FU and further increased to notched stems of *E. agallocha*. The increased vessel diameter was an impact of hydraulic architecture of stem and thus increases hydraulic characteristics. As such need not necessarily depend on the leaves for conservative water use. The growth performances increased with leaf establishments and notching manipulation with increased hydraulic characteristics of k_{max}, k_s and k_l. This suggests that imposed stress condition by xylem – phloem manipulation did not affect the growth performance of *E. agallocha*, thus this species considered as drought avoider. New xylem and phloem tissues considered as survival strategies. New lateral branches established below the girdled regions and above canopies exhibited yellow mottled symptoms suggests important of basipetal assimilate transport. There were positive correlation between vessel diameter and k_{max} and k_s that support the hydraulic limitation hypothesis.

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