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# Leaf anatomical plasticity of congeneric rain forest tree species in relation to light acclimation

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## Abstract

Leaf structure of a seedling is an important indicator of the level of shade-drought-tolerance and the use of carbon assimilates of a species. The more shade-tolerant and conservative species have greater anatomical plasticity compared to more shade-intolerant and exploitive species. This study examined seedling leaf structure variation of four *Syzygium* species in the family Myrtaceae to different combinations of light environments. These species are usually found in the canopy/sub canopy in lowland rain forests. Six combinations of irradiance and spectral quality that found within the rain forest understorey to full open were used for this investigation. Two treatments exposed seedlings to different duration of direct sunlight (2h and 6h sunflecks). One treatment had the forest understorey light quality and quantity (50 $\mu$mol m$^{-2}$ S$^{-1}$) and a control treatment exposed seedlings to full sunlight (1600 $\mu$mol m$^{-2}$ S$^{-1}$).

Seedlings of *S. firmum, S. makul, S. operculatum, S. rubicundum* were grown for two years within 24 environmental shelters. At the end of two years, leaves were sampled from each species in each light treatment. Subsequently, leaf blade thickness, stomatal frequency, and thickness of upper and lower epidermal and palisade mesophyll cell layers were measured. Analysis tested for differences and interactions among species and light treatments. In general, leaf dimensions and cell layers of all species increased with increase in amount of light and were greatest in the full sunlight treatment (1600 $\mu$mol $m^{-2}S^{-1}$) *S. firmum* had the thickest leaf anatomical attributes while it was lowest for *S. rubicundum*. Greatest densities of stomata were measured for *S. operculatum* followed in declining order by *S. makul, S. firmum and S. rubicundum. S. makul* exhibited double rows of cells within the palisade mesophyll and lower epidermal layers while *S. firmum* exhibiited double rows of cells only within the Palisade mesophyll.

*S. firmum*, with the thickest cuticle and leaf blade in full sun seems that it has a conservative carbon economy. The thinner leaf anatomical attributes in *S. rubicundum* when growing in the sun suggest that it is more exploitive compared to others but less water wasteful due to its lower stomatal frequency. *S. operculatum* is the most drought susceptible species due to its highest stomatal frequency. *S. makul* is moderate in terms of drought- tolerance and the use of carbon assimilates. The results demonstrate a close relationship between species leaf anatomy and their efficiency in use of water and carbon assimilates that had allowed them to occupy different sites within the rain forest environment.

**Key words. Leaf anatomy, light, seedlings, Syzygium.**

## 1. Introduction

Light intensity varies in tropical rainforest understories due to the process of gap formation, which are created by disturbances. The frequency and scale of disturbances promotes differentiation of regeneration survival of tree species and affect the composition of mature forest (Ashton *et al.*, 1995; Thompson *et al.*, 1992; Turner; 1989; Whitmore, 1978). Hence the role of gaps of various sizes and the change in light quantity, quality and duration for the regeneration of tropical rainforests have received considerable discussion. The ability of seedlings to acclimatize to those sudden changes in light is an important factor for their survival (Fetcher *et al.*, 1983).

Seedling leaves are subjected to affect above changes in light since those are the mostly exposed morphological features of a seedling. Light is one of the most important environmental factor that affect seedling leaf anatomy and there by for the rate of photosynthesis. Therefore the changing leaf anatomical attributes of seedling leaves, with the change of light intensity gives an enormous ecological understanding of their shade-drought tolerance and carbon economy, since leaves are the principal organs in which photosynthesis is carried out.

Several investigators have reported that the intensity and different duration of direct sunlight affect the development of a leaf and modify its anatomy including the number and dimensions of mesophyll cells (Thompson *et al.,* 1992; Ashton and Berlyn, 1992, 1994; Lee *et al.,* 1990; Simon *et al.,* 1990; Carpenter and Smith, 1980; Cutler, 1978; Wilson and cooper, (1969). These anatomical modifications of leaves differ dramatically between species. Anatomical differences in stomatal density, leaf thickness, epidermal thickness and palisade mesophyll thickness of tree species have been related to their successional status, their age and crown position in the canopy (Ashton and Berlyn, 1994; Carpenter and Smith, 1975; Jackson, 1967; Loach, 1967; Wylie, 1951).

Studies have also shown that leaves in full sun often have similar anatomical adaptations as drought avoiding plants while leaves in shade often resemble those of drought-intolerant species (Ashton and Berlyn, 1992; Lee *et al.,* 1990) The light generalist seedlings that can grow in a range of light conditions within a forest have a larger anatomical plasticity than specialist species (Ashton and Berlyn, 1992). Little work has been done that examines these relationships between selected tree species within the same successional status (Gamage *et al.,* 1996; Ashton and Berlyn, 1992, 1994).

This study examined the variation in leaf structure among congeneric seedlings of four canopy treee species that have been characterized as late - successional. The study tested the hypothesis that *Syzygium* species would differ in leaf anatomy with more shade -intolerant and exploitive species exhibiting thin leaf blades, cuticles and palisade cells, lower number of stomata per unit area than species that are more shade-tolerant and conservative. Also the shade-tolerant and conservative species have a larger anatomical plasticity than the more shade-intolerant and expolitive species.

## 2. Materials and methods

### Study site and species.

This study was done at the field station of the Sinharaja World Heritage Site, a forest located in the southwest of Sri Lanka. This forest is an everwet mixed-dipterocarp type comprising a canopy dominated by the genera *Shorea* section Doona (Dipterocarpaceae) and *Mesua* (Clusiaceae) (De Rosayro, 1942; Gunatilleke and Ashton, 1987).

The region receives 4,000-6,000 mm of rainfall per year. Most rain falls during the southwest monsoons (May-July) and the northeast monsoons (October-January). Seasonal temperatures range between 25 and 27°C (Ashton, 1992) . The topography is undulating between valley and ridge with a mean elevation above sea level of about 600m. The elevation differences between valley and ridge are generally less than 100m. Soils are classified as ultisols following the USDA (1995) terminology, or red yellow podzols using the classification system of the Food and Agriculture Organization (Moorman and Panabokke, 1961). They vary from weakly defined humults with a thick organic pad (2cm) on the ridges to udults with little to no organic accumulation but high in weathered clays and low in coarser sands (Ashton *et al.,* 1995) The soils are derived in situ from underlying metamorphic charnokitic and knondalitic gneiss (Corray, 1967).

The four species in this study *Syzygium firmum* Thw., *S. makul* Gaerth., *S. rubicundum* Wight and Arn. and *S. operculatum* (Roxb.) Niedz., belong to the family Myrtaceae and are common in the Wet and Intermediate zones of Sri Lanka. *Syzygium firmum,* and *S. makul,* are endemic species to Southwest Sri Lanka. All four species are often dominant in the canopy *(S.firmum and S. rubicundum)* and sub canopy *(S. makul and S. operculatum)* strata of late - successional forest. They are important timber species in Sri Lanka.

Each of the four species appears to occupy a different part of the forest topography. *S. operculatum* is found to occur on the lower slopes and valleys of the topography, where small rivers and perennial streams are found. *S. firmum and S. makul* occupy the deep soils of valleys to mid slopes and *S. rubicundum* occurs on hill midslopes (Ashton, 1981).

### Design of the controlled environmental shelters

Twenty-four well ventilated environmental shelters were constructed in the full open at the Sinharaja field station in January 1996 to examine the seedling leaf physiology, morphology and anatomy. They were designed to create light treatments that represented a range of photosyntheitic photon flux density (PFD) and Red: Far-red ratios (R:FR) found within the Sinharaja forest (Ashton, 1992). Six combinations of irradiance and spectral quality were created across the twenty-four shelters as shown in Table 1.

**Table 1.** Irradiance quality and quantity that seedlings were exposed

| Light Treatment (Assigned Abbreviation) | Maximum Measured Photosynthetic Photon Flux Density (PFD) ($\mu$mol m$^{-2}$ s$^{-1}$) | Maximum Daily PFD ( mol m$^{-2}$) | Duration of Direct Light (h) | Red:Far Red Ratio |
|---|---|---|---|---|
| i. Full sun (FS) | 1600 | 38.1 | - | 1.27 |
| ii. Direct light at center of a large (300-400 m$^2$) canopy opening (LO) | 1600 | 13.3 | 6 | 1.27 |
| iii. Same as ii above, but a small (150-200 m$^2$) canopy opening (SO) | 1600 | 7.4 | 2 | 1.27 |
| iv. 60% shade with light quality similar to that at the sunny edge of a large canopy opening (OE) | 800 | 16.3 | - | 1.05 |
| v. 82% shade with light quality similar to that at a the shaded edge of a large canopy opening (FE) | 350 | 6 | - | 0.97 |
| vi. 99% shade with light quality similar to the forest understorey (FU) | 50 | 1.2 | - | 0.23 |

Light treatments altering the duration of direct PFD (small and, large openings), were obtained by constructing a series of parallel vertical slats, aligned north-south, placed horizontally 2 m above the ground and across the complete interior of the respective shelter. As the sun rose and set, the duration and number of sunlight periods of direct PFD was controlled by slat orientation (N-S), slat height, and the proximity of slats to one another. For shelters altering te quality and intensity of PFD (open edge, forest edge, forest understorey) a mix of pigments was sprayed onto UV durable plastic film in a concentration of 10% with clear varnish. The amount sprayed regulated the R: FR ratio and the intensity of PFD. A photo spectro-radiometer (LI 1800) verified the treatment. All shelters allowed for adequate ventilation without the use of electrical power.

## Leaf anatomy measurements

Sample leaves were taken from approximately 2 years old seedings of each species in each light treatment for anatomical observations. Only undamaged, fully expanded leaves with no signs of herbivory were selected. Six seedlings were sampled for each species in each light treatment. From each seedling one mature leaf was selected and a strip$\pi$ (0.5x1.0cm) was taken from the middle portion of the lamina across the mid-rib. Leaf strips were immediately fixed in FAA (Formalin: Acetic acid: Ethanol) and transported to the laboratory for anatomical measurements.

Fixed leaf strips were thoroughly washed with running water, dehydrated in an ethanol series, immersed in xylene and embedded in wax. Cross sections of 10 $\mu$m thickness were taken using a rotary microtome, mounted on slides, stained with safranin and fast green following a modified procedure of Berlyn and Miksche (1976) and mounted in Canada balsam. One slide from each leaf strip was prepared and the thickness of the leaf, and those of the cuticle, palisade mesophyll, upper epidermis and lower epidermis at 10 separate points, avoiding the region around the midrib. These structural attributes were selected since their responsiveness has been proven to be indicative of micro-environmental surroundings.

Stomatal frequencies were observed from cellulose acetate positives of silicone-rubber impressions (Heichel,1971). Each impression was taken midway between the base and tip on both adaxial and abaxial surfaces of each leaf. Five stomatal impressions were taken from each leaf and from each peel two separate points were viewed.

## Data analysis

Analysis of variance (ANOVA) was performed on each anatomical measure using the Statistica Version 5. All data were log transformed prior to analyses. The analyses tested for differences and interactions between light and species. All F statistics that were significant ($P<0.001$) were evaluated for differences among species using Tukey's Studentized range at the 5% significance level.

# 3. Results

## Leaf blade thickness

There were significant differences and interactions among species and among light treatments (Table 2). All species produced thicker leaves when grown in full sun. Additionally there were differences among species within a light treatment.

Table: 2. Variance ratios following two-way analysis of variance on thicknesses ($\mu m$) of cuticle (CT), leaf blade (LB), upper epidemis (UE), palisade cell (PC), and lower epidemis (LE) and stomatal frequency (SF) $mm^2$) using data from the six light treatments (FU-forest understorey, FE-forest edge, OE_outside edge, FS-full sun, SO-small opening, LO-large opening) Degree of significaance:*** $P<0.01$; ** $P<0.001$; * $P<0.05$

**Anatomical attributes**

| | CT | LB | UE | LE | PC | SF |
|---|---|---|---|---|---|---|
| Light | 100.38*** | 566.80*** | 192.71*** | 9.02 *** | 94.05*** | 265.52*** |
| Species | 2101.4 *** | 4324.82*** | 86.92*** | 219.49*** | 1170.18*** | 316.24*** |
| Block | 0.45 lns | 0.953 ns | 1.2 lns | 0.865 ns | 3.30* | 2.28ns |
| Light xSpecies | 37.22*** | 95.12*** | 7.47*** | 37.24*** | 5.13*** | |

The shade-tolerant and more conservative *S. firmum* had the thickest leaves in the full sunlight treatment followed in order by *S. makul>S. operculatum>S. rubicundum*. However in the shade, *S. makul* had the thickest leaf blade and *S. operculatum* the thinnest. *S. firmum and S. rubicundum* were in -between (Fig.1). The greatest degree of leaf thickness plasticity exhibited between full sun and forest understorey leaves appeared to approximate the same species shade tolerance order (Table 3).

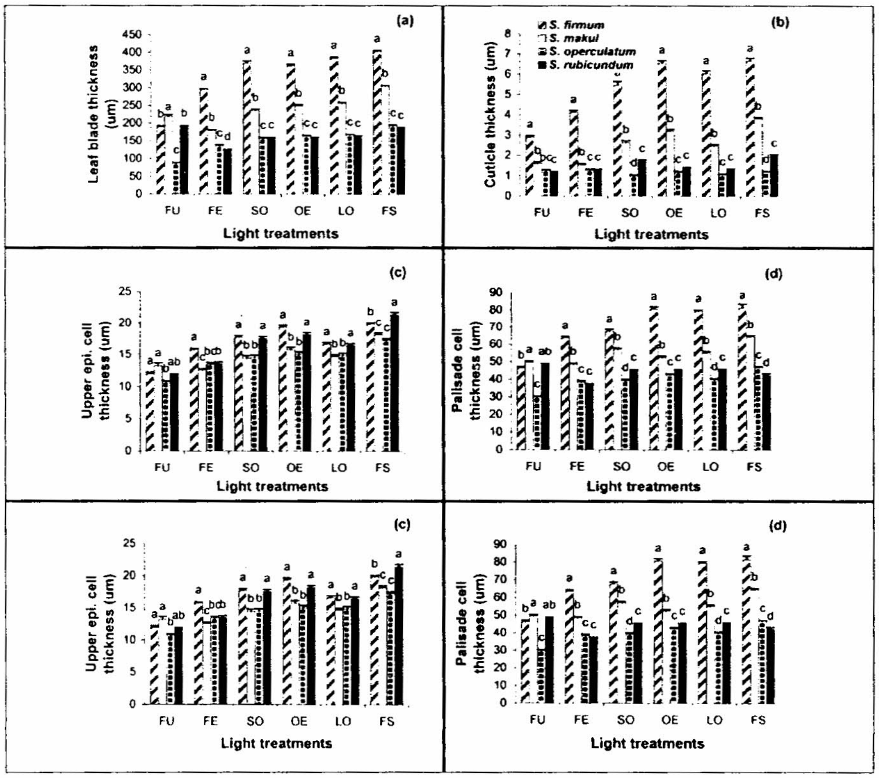
**Table 3.** Summary of anatomical variables for the four *Syzygium* species in different light treatments (FU-forest understorey, FE-forest edge, OE-outside edge, FS-full sun, SO-small opening, LO-large opening). Values are given for anatomical plasticity P (FS/FU). Data are means from six different leaves with standard errors in parentheses. Species followed by the same letter within a treatment are not significantly different at the 5% level.

| | **Light treatments** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **FU** | **FE** | **OE** | **FS** | **SO** | **LO** | **P** |
| **Leaf blade thickness (μm)** | | | | | | | |
| *S. firmum* | 189.17 (3.53)b | 295.17 (2.13)a | 363.17 (4.87)a | 404.50 (5.98)a | 371.00 (3.81)a | 384.67 (2.64)a | 2.14 |
| *S. makul* | 218.50 (5.15)a | 179.83 (1.89)b | 248.83 (4.61)b | 305.67 (4.33)b | 236.17 (3.04)b | 257.00 (2.05)b | 1.40 |
| *S. operculatum* | 87.67 (1.10)c | 136.00 (3.47)c | 164.33 (2.08)c | 193.67 (2.69)c | 157.50 (2.12)c | 167.50 (2.47)c | 2.21 |
| *S. rubicundum* | 191.50 (1.67)b | 125.67 (1.39)d | 159.50 (1.95)c | 186.83 (1.75)c | 158.83 (1.75)c | 164.17 (2.89)c | 0.98 |
| **Cuticle thickness (μm)** | | | | | | | |
| *S. firmum* | 2.89 (0.084)a | 4.13 (0.086)a | 6.58 (0.123)a | 6.69 (0.175)a | 5.48 (0.138)a | 6.06 (0.139)a | 2.31 |
| *S. makul* | 1.60 (0.087)b | 1.54 (0.054)b | 3.19 (0.096)b | 3.82 (0.104)b | 2.64 (0.091)b | 2.47 (0.073)b | 2.39 |
| *S. operculatum* | 1.26 (0.043)bc | 1.29 (0.048)c | 1.18 (0.049)c | 1.20 (0.048)d | 1.01 (0.043)d | 1.07 (0.038)c | 0.95 |
| *S. rubicundum* | 1.16 (0.054)c | 1.28 (0.054)c | 1.39 (0.051)c | 2.00 (0.077)c | 1.71 (0.057)c | 1.31 (0.065)c | 1.72 |
| **Upper epidermal cell thickness (μm)** | | | | | | | |
| *S. firmum* | 11.98 (0.275)a | 15.76 (0.212)a | 19.36 (0.332)a | 19.88 (0.307)b | 17.72 (0.302)a | 16.64 (0.241)a | 1.66 |
| *S. makul* | 13.20 (0.508)a | 12.56 (0.209)c | 15.76 (0.412)b | 18.20 (0.424)c | 14.44 (0.299)b | 14.68 (0.280)b | 1.38 |
| *S. operculatum* | 10.78 (0.172)b | 13.48 (0.311)bc | 15.00 (0.451)b | 17.32 (0.411)c | 14.52 (0.388)b | 14.88 (0.266)b | 1.61 |
| *S. rubicundum* | 11.82 (0.176)ab | 13.52 (0.286)b | 18.20 (0.324)a | 21.44 (0.284)a | 17.60 (0.321)a | 16.44 (0.367)a | 1.81 |
| **Lower epidermal cell thickness (μm)** | | | | | | | |
| *S. firmum* | 8.64 (0.184)a | 10.42 (0.181)a | 12.00 (0.278)a | 10.84 (0.166)a | 10.00 (0.182)a | 10.14 (0.232)a | 1.25 |
| *S. makul* | 7.44 (0.344)b | 8.28 (0.218)c | 8.18 (0.207)c | 9.04 (0.223)b | 8.08 (0.180)b | 8.38 (0.170)b | 1.22 |
| *S. operculatum* | 8.02 (0.157)ab | 7.40 (0.189)d | 6.94 (0.203)d | 7.04 (0.203)c | 8.04 (0.204)b | 7.48 (0.202)c | 0.88 |
| *S. rubicundum* | 8.34 (0.282)ab | 9.42 (0.197)b | 9.28 (0.224)b | 10.32 (0.200)a | 10.40 (0.218)a | 8.92 (0.214)b | 1.24 |
| **Palisade cell depth (μm)** | | | | | | | |
| *S. firmum* | 46.30 (1.051)b | 63.72 (0.913)a | 80.80 (1.302)a | 81.64 (1.193)a | 67.72 (0.861)a | 79.64 (2.291)a | 1.76 |
| *S. makul* | 49.64 (0.926)a | 48.52 (0.894)b | 52.44 (0.971)b | 64.56 (0.897)b | 57.04 (0.965)b | 55.16 (0.908)b | 1.30 |
| *S. operculatum* | 30.04 (0.530)c | 38.44 (0.950)c | 42.68 (0.484)c | 46.92 (0.762)c | 39.48 (0.866)d | 40.04 (0.654)d | 1.56 |
| *S. rubicundum* | 48.48 (0.540)ab | 37.32 (0.344)c | 45.16 (0.577)c | 42.56 (0.642)d | 45.24 (0.528)c | 45.56 (0.987)c | 0.88 |
| **Stomatal frequency ($mm^{-2}$)** | | | | | | | |
| *S. firmum* | 279.20 ( 8.55)bc | 349.87 (4.79)b | 473.60 (10.40)c | 482.93 ( 9.23)a | 472.27 (10.36)a | 455.73 (15.39)c | 1.73 |
| *S. makul* | 287.20 ( 6.10)b | 363.73 (5.69)b | 529.87 (7.29)a | 541.07 ( 7.72)a | 491.73 ( 8.78)a | 526.13 (11.66)b | 1.88 |
| *S. operculatum* | 342.13 ( 7.79)a | 393.33 (8.22)a | 567.47 (7.66)b | 597.60 ( 9.34)a | 499.20 (14.09)a | 626.40 (23.51)a | 1.75 |
| *S. rubicundum* | 248.80 (17.20)c | 281.87 (4.13)c | 353.07 (5.52)d | 386.93 (11.71)b | 347.73 ( 6.98)b | 342.40 (10.04)d | 1.56 |

## Cuticle thickness

There were significant differences in cuticle thickness between leaves grown in full sun and those grown in the forest understorey (Table 2). Cuticles of full sun leaves were significantly thicker than shade leaves. As depicted in Fig.1, *S. firmum* had the thickest cuticle in full sun followed in order by *S.makul>S.rubicundum>S. operculatum. S. firmum* also had the thickest cuticle in each of the remaining light environment, compared to that of the three remaining species. *S. operculaatum* and *S. rubicundum* showed the thinnest cuticle in all light environments while that of *S. makul* was always moderate.

Fig . 1 Anatomical attributes of (a) leaf blade thickness, (b) cuticle thickness, (c) upper epidermal cell thickness, (d) palisade cell thickness, (e) lower epidermal cell thickness and (f) stomatal frequency for Syzygium species in different light treatments (FU-forest understorey, FE-forest edge, OE-outside edge, FS-full sun, SO- small opening, LO-large opening). Bars indicate one standard error of the mean. Species not sharing the same letter within a light treatment differ signifiicantly at P<0.05 level within a light treatment.



The cuticle thickness plasticity between full sun and forest understorey were high for *S. firmum* and *S. makul* and least for S. *operculatum* (Table 3).

## Component dimensions

As depicted in Table 2, the species exhibit significant differences in epidermal cell thickness. These differences followed similar trends among species for each light treatment for both upper and lower epidermal cells. All species had their thickest upper epidermal cell dimensions in the full sun and among them S. *rubicundum and* S. *firmum* had the thickest upper epidermis. The trends shown by the lower epidermis among the light treatments were less consistent, and varied in the different species. The lower epidermis plasticity was more or less similar for *S. firmum, S. makul* and *S. rubicundum*. Among the four species only *S. makul* exhibited multiple layers in the lower epidermis. Thus it had the greatest lower epidermal layer thickness. This phenomenon was most visible in the leaves that received greater amounts of PFD.

Changes in palisade cell thickness were significant for all species (Table 2). This may be caused either by cellular elongation or the differentiation of multiple palisade layers in response to increased light. In general full sun leaves had thicker palisade compared to that of shade leaves. This was most noticeable for *S. firmum>S. makul>S. operculatum>S. rubicundum*. In the shade *S.operculatum* had the lowest palisade cel depth compared to other species. This species was thickest in full sun treatment. *S. rubicundum* and *S. makul* also had relatively thicker palisade cell depth in the shade while *S. firmum* was in between. In the sun *S. firmum* was highest while *S.rubicundum* was the lowest. *S.makul* also had relatively thicker dimensions (Fig. 1) Both *S. makul* and *S firmum* had a double layer of palisade cells. This was most noticeable for *S. makul*. Thus double layering ratio was calculated for *S. firmum*. This phenomenon was mostly reported in shelters that simulated direct sunlight. Plasticity of palisade cells was greatest in the more shade-tolerant *S. firmum* and the lowest for *S. rubicundum* that of *S. makul* and *S. operculatum* were moderate.

## Stomatal frequency

Stomata were found only on the lower leaf surface of *Syzygium* seedlings. All species had significantly higher numbers of stomata per unit area in leaves that were exposed to full sun (Table 3). Differences among species were also apparent, and seen both under shade and full sun. *Syzygium operculatum* had significantly higher stomatal frequency compared to the other species in both sun and shade. In this species the highest stomtal frequency was recorded in seedlings grown in the shelter where the light levels was similar to that in a large opening. *Syzygium rubicundum* seedlings had fewer stomata in both sun and shade compared to that of other species. It had the highest stomatal frequency in the full sun treatment. *S. firmum* had higher stomata per unit area in several light treatments such as OE, FS, and small and large opening shelters. *S. makul* was highest in OE, FS and large opening shelters. The greatest degree of plasticity was exhibited by *S. makul* while that of *S. rubicundum* was the lowest (Table 3).

## 4. Disscussion

### Differences in seedling leaf anatomy and plasticity

Changes in anatomical characteristics taken in combination partly determine the physiological shade-and drought-tolerance of a species (Ashton and Berlyn, 1992). Thick leaf blades, lower epidermal cells, palisade cells and cuticles suggest reasons for *S. firmum* to be the most conservative of the four species and one that can tolerate heat better than the other *Syzygium* species. Its lower stomatal frequency also allows it to conserve water better. This species had a higher plasticity for leaf blade, palisade cells, lower epidermis between leaves grown in forest understorey and those grown in full sun. *S. perculatum* with higher stomatal frequency, thin leaves, and cuticles compared to *S. firmum* reveals that it is the least drought-tolerant and less conservative of the four species. It had a thicker cuticle than the most shade-intolerant, *S. rubicundum* and also higher plasticity for leaf blade compared to *S. firmum.*

Cuticle, leaf blade, uper epidermis and palisade cells for *S. makul* was second only to the shade-tolerant, *S. firmum*. However this species had double rows of cells in palisade mesophyll and in lower epidermis. The stomatal frequency of *S. makul* was lower than the frequency of *S. operculatum*. Anatomical plasticity for component dimensions was lower for both *S. firmum and S. operculatum*. Hence *S. makul* is moderate in both shade - and drought - tolerance.

*S. rubicundum* had thinner palisade cells and leaf blades in sun. They were moderate size in the shade. The cuticle was thinner in both sun and shade compared to the other species. The lower anatomical attributes for *S. rubicundum* suggest that it is susceptible to desiccation and is more exploitive than the other species. The thinner palisade mesophyll does not enable it to maximize photosynthesis in low light conditions. Therefore the species requires higher light intensities for optimum carbon assimilation. It also had the least stomatal frequency and anatomical plasticity. Therefore it is more water efficient. Only upper epidermis plasticity was greatest in *S. rubicundum.*

## Comparison of leaf anatomy of Syzygium species with other late-successional genera

Differences in cuticle thickness between *Syzygium* and *Shorea* species show that *Syzygium* have a relatively thicker cuticle (Table 4). However, *S. firmum,* which is shade-tolerant, had the thickest cuticle compared to the more shade-tolerant *Shorea worthingtonii* in both sun and shade. Also the more shade-intolerant *S. rubicundum* was thinner than the shade-intolerant *Shorea megistophylla.* The shade-intolerant *S.operculatum* also had a thinner cuticle. The moderate light loving species *S. makul* was medium in thickness. However it was higher than *S. megistophylla* in the sun. Although differences in cuticle thickness between species is related to shade- and drought tolerance of a species the opposite trend has been reported for *Syzygium.* The relatively shade intolerant *Shorea* species, *S. megistophylla* also had a thicker cuticle compared to its relatives in the sun. The shade-tolerant *S. worthingtonii* had the thickest cuticle in the shade. This suggests that the *Syzygium* species, which are shade-intolerant, are more exploitive and water - wasteful compared to the *Shorea* species.

**Table 4**. Comparison of leaf anatomical attributes among different genera (SR-*Syzygium rubicundum*. SO-*S. operculatum*. SM-*S.makul*. SF-*S. firmum*. ST-*Shorea trapezifolia*, SW-*S. worthingtonii*. SD-*S. disticha*. SM-*S. megistophylla*, MF-*Mesua ferrea*. MN-*M. nagassarium*, DZ-*Dipterocarpus zeylanicus*. DH-*D. hispidus*).

| | SR | SO | SM | SF | ST | SW | SD | SM | MF | MN | DZ | DH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cuticle thickness (μm) | | | | | | | | | | | | |
| shade | 1.16 | 1.20 | 1.60 | 2.89 | 1.9 | 2.6 | 1.9 | 2.2 | NA | | | |
| sun | 2.00 | 1.26 | 3.82 | 6.69 | 2.8 | 1.9 | 2.9 | 3.5 | | | | |
| Leaf blade thickness (μm) | | | | | | | | | | | | |
| shade | 191.50 | 87.67 | 218.50 | 189.17 | 91.7 | 131.0 | 140.9 | 151.7 | 173.90 | 179.40 | 126.07 | 85.60 |
| sun | 186.83 | 193.67 | 305.67 | 404.5 | 97.4 | 146.0 | 153.1 | 177.2 | 224.56 | 209.94 | 161.13 | 112.07 |
| Palisade cell depth (μm) | | | | | | | | | | | | |
| shade | 48.48 | 30.04 | 49.64 | 46.30 | 18.90 | 15.60 | 15.3 | 13.1 | 34.86 | 31.80 | 31.14 | 28.63 |
| sun | 42.50 | 46.92 | 64.56 | 81.64 | 18.10 | 20.3 | 21.9 | 13.2 | 46.23 | 43.20 | 42.73 | 38.36 |
| Stomatal frequency ($mm^{-2}$) | | | | | | | | | | | | |
| shade | 249 | 342 | 287 | 279 | 478 | 262 | 437 | 337 | 272 | 226 | 140 | 247 |
| sun | 387 | 598 | 541 | 483 | 572 | 415 | 586 | 502 | 314 | 284 | 216 | 332 |

(*Shorea*, *Mesua* and *Dipterocarpus* data were taken from Ashton and Berlyn, 1992; Gamage *et al.*, 1996)

Leaf blades were thicker in *Syzygium* species than that in *Shorea* and *Dipterocarpus.* However *S. rubicundum and S. operculatum* had thinner leaf blades than *Mesua* species. *S. firmum* and *S. makul* that are considered shade-tolerant and partial shade-tolerant were thickest in their leaf blades. Hence they are more resistant to heat loading than to *Shorea, Dipterocarpus* and *Mesua.*

Anatomical differences are also apparent in palisade cell thickness. The *Syzygium* species had thicker palisade cells than that of *Shorea, Dipterocarpus,* and *Mesua* species. Therefore the *Syzygium* species are able to capture more light than other species since thicker palisade cells enable an increase in chloroplasts and greater efficiency in light capture (Lee et al., 1990). Among the *Syzygium* species the more shade-intolerant species. *(S. rubicumdum and S. operculatum)* had lower palisade cell depth compared to more shade-tolerant species *(S.makul and S.firmum)* in both sun and shade. Results for *Shorea* species in Table 4, indicate that in the sun the more shade-intolerant *Shorea megistophylla* was thicker in palisade cell depth than the more shade-tolerant *Shorea worthingtonii.* The opposite trend was reported in the shade.

Absolute differences reveal that *Syzygium* has a lower stomatal freqency than *Shorea* species but a higher than those of *Dipterocarpus* and *Mesua* (Table 4). Thus *Syzygium* are moderate in stomatal frequency. This suggest that they are moderate in drought-tolerance since species with higher stomata are less drought-tolerant, while species having lower stomatal frequency are moderate in drought-tolerance (Ashton and Berlyn, 1992).

The stomatal frequency for all genera increased with the increase of light intensity. These differences in stomatal frequencies could be a result of environmental influence on stomatal differentiation as well as due to cell expansion (Volkenburgh and Davies, 1977). Stomatal frequency varies due to a change in leaf size under differing light and water stressed conditions. Low light can cause a decrease in stomatal frequency due to an increased leaf area but has no effect on stomatal mother cell differentiation. Similarly under water stress, restricted leaf expansion can cause an increase in stomate (Chia and Brun, 1975). However these conditions do not seem to be related to *Syzygium* since these species increased leaf area as well as in stomatal frequency with increasing light. In other tropical species *(Shorea)* there was little change in leaf size under varying light environments (Ashton and Berlyn, 1992). Stomatal frequency was affected by prevailing environmental conditions (Levickaja, 1961; Cutler, 1978; Simon *et al.,* 1990) and reactions of auxins in stomatal cells (Cameron, 1969). Therefore the increase in stomatal frequency with the increasing light intensity could be related to the effect of prevailing environmental conditions on the initiation of stomata or to the reaction of auxins in *Syzygium* species.

When all anatomical measures for *Syzygium* were considered they were most significant in the full sun. This reveals that *Syzygium* are relatively shade-intolerant and behave like early-successional species since they generally have thicker leaves. *Syzygium* species grow on valleys through to midslopes. Gaps in these sites are more moist with more light available than on ridgetops. These anatomical findings support natural distribution of *Syzygium* within forest topography.

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