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Changes in Leaf Morphological and Anatomical Characteristics in Vertical Canopy Light Profile in *Heriteria fomes* Buch.-Ham

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DECLARATION

I, Selina Khatun, declare that this thesis is the result of my own work and it has not been submitted or accepted for any degree to other university or institution.

I hereby, give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans, and for title and summary to be made available to outside organizations with my approval.

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08.03.18

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Selina Khatun

APPROVAL

This is to certify that the present project entitled **"Change in Leaf Morphological and Anatomical Characteristics in Vertical Canopy Light Profile in *Heriteria fomes* Buch.-Ham"** has been carried out by Selina Khatun (Student ID:130505) under my direct supervision and guidance. Project thesis submitted to the Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh in partial fulfilment of the requirements for the four years professional B.Sc. (Hons.) degree in Forestry. I have approved the style and format of the project thesis.

Supervisor

9-3-18

Dr. Md. Nabiul Islam Khan

Professor

DEDICATED

TO

MY BELOVED PARENTS

Md. Abdul Gafur and Mrs. Rebeka Khatun,

Who are always with me and without them I don't exist in this

Beautiful world.

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Selina Khatun

ABSTRACT

Heritiera fomes (Sundri) is an evergreen moderate size mangrove tree growing abundantly in Sundarbans. *H. fomes* grows well in top canopy having full sunlight and even in full shade under the canopy. This study presents the variation of leaf morphological and anatomical characteristics in (*H.fomes*) due to variation of light intensity at different canopy depth. Leaf of *H. fomes* responses to different light environments at the different canopy height. Smaller leaves are found in the top of the canopy, which allows light penetration to the lower layers, while lower leaves have a higher area so as to ensure maximum light absorption. Shade-growing leaves are thinner, have lower mass per unit area than do sun-growing leaves. Sun leaves become thicker than shade leaves because they develop longer palisade cell or an additional layer of palisade cell. In the morphological feature of sundri leaf the leaf area was increased in the bottom canopy and decreased in the top canopy. The specific leaf area also decreased in the top canopy and increased in the bottom canopy. Vertical distribution of photosynthetic active radiation at various levels within canopies of *Heritiera fomes* showed strong relationship with various leaf anatomical and morphological characteristics. ANOVA and Tukey tests showed significant difference ($p < 0.01$) in those leaf characteristics among different canopy height. However, in most cases the differences within the shade leaves were not significantly different ($P > 0.05$). For this reason multiple comparison test was performed. A principal component analysis also performed for showing the ordination of different canopy height with respect to different leaf (anatomical and morphological) characteristics.

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CHAPTER ONE

1.0 Introduction

1.1 Background and Justification of the Study

Sundarban, the largest evergreen mangrove ecosystems of the world located in India and Bangladesh has the widest range of mangrove species in its tidal influenced highly saline soil (Anderson and Briske 1990).

The name *Sundarban* was derived from the *Sundari* trees (the mangrove species *Heritiera fomes*) that are found in Sundarbans in large numbers. Alternatively, it has been proposed that the name is a corruption of *Samudraban*, *Shomudrobôn* or *Chandra-bandhe* (name of a primitive tribe). However, the generally accepted view is the one associated with *Sundari* or *Sundri* trees (Islam 2003).

Heritiera fomes is an evergreen moderate size tree growing abundantly in Sundarbans (Spalding 1997).This species is found in the upstream estuarine zone in the high intertidal region. It prefers freshwater, and is fast-growing in low saline environment (Kathiresan et al. 2010). The leaves of *Heritiera fomes* are dark green and occur 10 to 23 cm × 4 to 10 cm broadly elliptic with acute apex having cuneate base. Base may also be rounded or entirely covered beneath with minute silvery hairs (Spalding 1997).

The leaves of *Heritiera fomes* were ± horizontally oriented (41°), absorbing a relevant percentage of incident irradiance. (Anderson 1986)The vertical radiation profile showed an evident reduction of the red-far red ratio (R/FR) (Mukherjee et al. 2003).Radiation quality and quantity influenced leaf physiology and morphology. Clear differences in leaf size and specific leaf area (SLA) on the vertical profile of the forest were observed (Anderson 1986). Leaves that develop under different light levels also show different anatomical features.

Leaf orientation may be coordinated with external leaf morphology and internal anatomy for efficient light capture, according to different sunlight environments and stress levels (Gitelson et al. 2003). Typically, sun leaves of laminar-leaved plants are smaller and/or more deeply lobed, thicker, and lighter in color compared to shade leaves. Also, sun leaves are commonly more amphistomatous with well-developed palisade layers, while shade leaves are typically thinner, primarily hypostomatous, and without palisade layers. Sun leaves also tend to be more inclined in their orientation, away from horizontal (Gitelson et al. 2003).

In terms of canopy structure, the canopy height and depth, and the spatial organization of overstory stems, branches, and foliage affect the pattern of light transmission (GLI) through the canopy (Baldocchi and Collineau 1994).

In addition to the light in the mangroves be intense throughout the day, other environmental factors such as high levels of soil salinity and water available to plants, low levels of oxygen available and periodically flooded soils, make the plant species established there to develop functional and morphological adaptations that allow survival and remain in these environments (Soares 1997).

All these environmental factors may be responsible for low diversity of plant species found in mangroves (Tomlinson 1986).

It is common to find roots developing in the horizontal direction, or stems that grow toward the surface, known as pneumatophores, which act in the capture of oxygen to the tissues that are immersed in the soil, and excretory glands, which eliminate the excess of salt absorbed by the roots. The adjustments related to the anatomical structure of the leaf, as thick cuticle, epidermis and chlorenchyma, alter the scattering of radiation inside the leaf and, consequently, its spectral response.

However, it is still little known how the leaves of *Heritiera fomes* react with availability of sunlight at different canopy height in a vertical profile. Therefore, this study aims to describe and to evaluate the changes in leaf morphological and anatomical characteristics in vertical canopy light profile of the mangrove species *Heritiera fomes*.

1.2 Objectives of the Study

To study the variation of leaf morphological and anatomical characteristics in Sundri (*Heritiera fomes* Buch.-Ham) due to variation of light intensity at different canopy depth in a vertical profile.

CHAPTER TWO

2.0 Literature Review

This chapter includes two section each with several subsections. The first section gives the detailed information about leaf anatomy and leaf morphology and effect of light intensity on sun leaf and shade leaf. The second section represent the species description about *Heritiera fomes.*

2.1 Morphology of a Leaf

The main light-collecting structure on a leaf is a large, broad, flat surface called the leaf blade. The blade has many layers that not only help the plant move but also help it store materials and byproducts of photosynthesis (Larcher 2003). The blade is held away from the stem and supported by the petiole. The petiole is not exactly like a stem, but it does have xylem and phloem that transport water and sugar. The blade is supported by a system of veins that also contain both xylem and phloem. These veins prevent the blade from collapsing under its own weight (Lowman 1992). A leaf is often organized with one main vein running down the middle of the blade. This vein is called the midrib. All of the veins, the petiole, and the midrib help position the blade so that it is facing the light source (Larcher 2003).

2.2 Anatomy of a Leaf

A leaf is organized to collect sunlight. The leaf blade is constructed of many layers that make this happen. On top of the leaf is a waxy, non cellular layer called the cuticle. The cuticle is on the leaf to prevent water from escaping. Generally speaking, plants that live in bright, arid conditions have very thick cuticle layers. The next layer on the leaf is also there for protection. The epidermis is the skin like layer of cells found on both the top and bottom surfaces of the leaf. The epidermis may be one or many layers thick. Within the leaf, there is a layer of cell called the mesophyll. Mesophyll can then be divide into two layers, the palisade layer and spongy layer. Palisade cells are more column-like, and lie just under the epidermis, the spongy cells are more loosely packed and lie between the palisade layer and the lower epidermis. The air spaces between the spongy cells allow for gas exchange. Mesophyll cells (both palisade and spongy) are packed with chloroplasts, and this is where photosynthesis actually occurs (Farquhar and Von Caemmerer 1982).

2.3 Effect of Light Intensity during Growth of a Leaf

In tropical forests, canopy opening, both by anthropic action and natural tree falling, or even canopy closing, produces a condition of light heterogeneity which is morphologically and physiologically reflected in the leaves (Chazdon and Fetcher 1984). Tree species are submitted to light variations as evidenced in the higher and lower portion of the same tree (Holbrook and Lund 1995). Leaf responses to different light environments vary widely within and among species. In general, within species, shade-growing leaves are thinner, have lower mass per unit area and have higher mass-based chlorophyll content than do sun-growing leaves (Bongers & Popma 1988). In addition, shade leaves usually have low area-based rates of photosynthesis and dark respiration and low light saturation points (Boardman 1977). As a result, leaf adjustments to low light increase the capacity of light absorption at the expense of photosynthetic capacity and minimize carbon losses through respiration (Evans 1989). In contrast, sun leaves can make more efficient use of the prevailing high light intensities for carbon gain while avoiding a possible reduction in photosynthetic performance as a result of photoinhibition (Boardman 1977).

2.4 Sun Leaf Vs Shade Leaf

Shade leaves are typically larger in area, but thinner than sun leaves. Sun leaves become thicker than shade leaves because they develop longer palisade cell or an additional layer of palisade cell (Boardman 1977) On a weight basis, shade leaves generally have more chlorophyll. In shade leaves, the chloroplasts move within the cells to take up a position where they will absorb the maximum light without shading other chloroplasts below them (Kappel 1983). In sun leaves, the chloroplasts take turns in the bright light and then shelter in the shade of others whilst they make use of the light they have absorbed - too much bright light would destroy the chloroplasts. Sun and shade leaves can differ in the amount of photosynthesis by a factor of up to 5 for the same amount of light (Chazdon and Fetcher 1984).

Sundri (*Heritiera fomes*)

2.5 Taxonomic information

Scientific classification

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Malvales

Family: Sterculiaceae

Genus: Heritiera

Species: fomes

Botanical Name: *Heritiera fomes* Buch.-Ham.

2.5.1 Description:

Heritiera fames is a medium-sized evergreen tree growing to a height of 15 to 25 metres (49 to 82 ft). The roots are shallow and spreading and send up pneumatophores(Islam 2017). The trunk develops buttresses and is grey with vertically fissured bark. Trees with girths of 2 metres (6 ft 7 in) used to be found but these large trees have mostly been harvested for their timber. The trunk has few large branches and the canopy is open. The leathery leaves are elliptical and tend to be clustered at the ends of the twigs. The pink or orange bell-shaped flowers are each about 5 mm (0.2 in) across. They form in panicles, each flower being either male or female (Islam 2017).

2.5.2 Distribution and Habitat

Heritiera fomes is native to coastal regions of the Indo-Pacific, its range extending from the east coast of India through Bangladesh and Malaysia to Myanmar and Thailand. Compared to other species of mangrove, it grows in less saline environments and on drier ground that gets inundated by the tide only infrequently. It thrives on clayey soils and is the dominant species in these habitats, typically growing on the low banks that form around the edges of saucershaped, newly-emerged island. It is the dominant mangrove species in the area and its local name, sundri, which gives the Sundarbans region its name (Polgar and Jaafar 2018).

2.5.3 Traditional Uses

Timber produced from *Heritiera fomes* is hard, fine-grained, tough and elastic. The heartwood is dark red or reddish-brown and the sapwood is a paler reddish-brown. The timber has many uses; in bridge building, house construction, boat building and joinery, as utility poles and tool handles, making hardboard and as firewood. The tree is grown commercially in plantations (Polgar and Jaafar 2018).

The bark of *H.fomes* is rich in procyanidins.The ethanol extract has been shown to have antioxidant properties. It also shows antimicrobial activities against *Kocuria rhizophila*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* and is non-toxic in brine shrimp toxicity tests (Islam 2017).

2.5.4 Medicinal Value

The chemicals produced from the species can be used for gastro-intestinal disorders (including dysentery, diarrhea, indigestion, colic, acidity, constipation, bloating, lack of appetite, stomachache (Agastian et al. 2000). Besides this, it can be used to treat hepatic disorders (including jaundice and hepatitis), insect repellent and skin diseases (including eczema, abscess, acne, boils, scabies, itch, infections, dermatitis, rash, sores, scar, warts, etc (Iftekhar and Islam 2004). Sundri has been the main timber species and is the primary resource base for 221 small saw mills and 350 pitsaw units in the region (Bangladesh Bureau of Statistics, 1983).

2.5.5 Management Practies

H. fomes is the single most important species of the Sundarbans, but the dominance of *Heritiera* forest is decreasing. As a pure crop and in mixture with *Excoecaria agallocha*, the species occupies ca. 18.2% and 62.4% of the forest area respectively (Anon 2001).

The species necessitates mass vegetative propagation, an alternative to seed propagation, for perpetuation of the species and their re-establishment in the area (Hartmann and Kester 1989). Few studies dealt with the effect of auxins (IAA, IBA & NAA) on rooting of the pregirdledstem cuttings and air-layers and the biochemical changes during initiation and development of roots in *H. fomes*. Extensive physical and biological changes in last 50 years have led to artificial assemblage of *H. fomes*, *Sonneratia apetala* etc., species owing to economic needs and environmental change (Snedaker 1982).

There are reports of development of management plans for coastal plantations targeting to achieve many objectives *viz.*, (a) to continue the establishment of coastal forest plantations and initiate management of existing ones for their timber value, (b) to protect and preserve areas of environmental value relating to conservation of biodiversity resources, (c) to integrate people's participation and development, (d) to enhance and promote recreational and tourism potential etc (Canonizado 1999).

2.5.6 Biodiversity conservation and enhancement:

Mangrove plantations are offering a new habitat to the wildlife of the Sundarbans. In addition, some parts of the mangrove forest and plantations are declared protected areas under a different status *e.g.*, wildlife sanctuaries, national park and ecologically critical areas (Iftekhar and Islam 2004).

CHAPTER THREE

3.0 Materials and Methods

3.1 Description of the Study Area

The leaf samples were collected from a mangrove plantation in Khulna university campus area which is located at latitude 22°48′4′′ N and longitude 89°32′2′′E along the lake. The annual average temperature is 26.3 °C (79.3 °F) and monthly means varying between 12.4 °C (54.3 °F) in January and 34.3 °C (93.7 °F) in May. Annual average rainfall of the study area is 1,809.4 millimeters (71.24 in). Approximately 87% of the annual average rainfall occurs between May and October (Cavendish 2007).

The area received fresh water supply through the lake and nearby adjacent area. The mangrove plantation was established in (2003-2004) and the species are Sundri (*Heritiera fomes*), Amoor (*Amoora cucullata* Roxb.), Kankra (*Bruguira sexangula*).The mangrove *Heritiera fomes* is the most dominant species in the study site. This species has wide distribution in Sundarban mangrove forest and southwestern part of Bangladesh and India.

3.2 PPFD Measurement:

3.2.1 Measurement Instruments:

- Quantum light sensor (Li-190SA, Li-COR, USA)
- Data logger(Li-1400, Li-COR, USA)
- Height measurement pole.

3.3 Measurement technique:

Photosynthetic photon flux density (PPFD) was measured at 10 am on overcast days during 24 September 2017. PPFD measurement was performed at the bottom canopy to top canopy of a tree in vertical stratum. In each canopy height, 100 observations were taken using a data logger (LI-1400, LI-COR, USA) and a pair of quantum sensors (LI-190SA, LI-COR, USA), one of which was measured at direct sunlight and the other was measured on a height measurement pole, which can be extended to a desired height (Khan et al. 2007).The total canopy height was 8.6 m. After measuring the PPFD at top canopy (8.6m) to bottom canopy

(2m) we collected the reading which were taken on the data logger for converting the photosynthetically active radiation (PAR) into relative photosynthetically active radiation (RPAR) dividing each light data by direct sun light. One plant species was selected and analyzed for the anatomical and morphological features related to the light condition and the species is *Heritiera fomes*. Twenty leaf samples were collected in each canopy height from 2m, 3m, 4m, 5m, 6m, to top canopy. Ten leaf samples were used for measuring the leaf anatomical features and other ten leaf samples were used for measuring leaf morphological features in the laboratory.

3.4 Laboratory observation:

3.4.1 Anatomical observation:

From each canopy height ten adult leaves were selected and these samples were taken to the Bio nanomaterial lab in Forestry and Wood Technology discipline in Khulna University.

The historical slides were hand made from cross section of midrib and leaflet with the aid of common steel blade. For smooth cross section and clear observation of each cell organ we used potato and the cross sections were stained with safranin and mounted in aqueous glycerin and observed under electronic microscope.

3.4.2 Microscopic observation:

After making the slide of cross sectional view of midrib and leaflet, the slide was fixed in an electronic microscope (BOECO, Germany) and using 10x magnification for observation. Digital image were taken under the microscope coupled with digital camera. One micrometer scale was fixed for measuring the thickness of upper and lower epidermis, thickness of palisade mesophyll and thickness of spongy mesophyll. By using image j software all the portion was measured separately.

3.4.3 Morphological observation:

For morphological observation ten adult leaves from each canopy height were collected and kept on the micro oven for 24 hours for drying. After 24 hours the dry leaves were used for measuring oven dry weight by using digital oven dry weight measuring machine. After taking the reading those leaves was taken for photo scan by using an image scanner (cano scan LiDE 120).Then by using image j software the morphological traits were determined. The traits evaluated were: Leaf length, Leaf width, Leaf area, Tip angle and Base angle. The specific leaf area (SLA) was estimated as leaf area on a dry weight basis for each canopy height.

3.5 Statistical analysis:

For statistical analysis at first i showed a relationship in average RPAR (Relative photosynthetically active radiation) with different canopy height. Then we showed correlation between different leaf parameter and the canopy height in both anatomical and morphological features. A one-way Anova was used to evaluate the significance level of $p<0.01$ for morphological parameter. Because here only one factor canopy height are present. A two-way Anova was also performed to determine the significance level of $p<0.01$ for anatomical features. Because here two factor i.e canopy height and leaf position (midrib and leaflet) are present. When significant differences among treatment means were found, these were compared with Tukey's test ($P\leq0.01$). To explore the association of leaf anatomy (midrib and leaflet) and leaf morphology with canopy light condition and their behaviors in different canopy height a principal components analysis was performed. All the statistical and graphical analysis were performed by using a statistical software freely available (https://cran.r-project.org) programming language R (R Core Team 2017).

CHAPTER FOUR

4.0 Results

4.1 Vertical distribution of canopy light.

Canopy light showed an exponential decrease with increasing canopy depth (Fig.1).

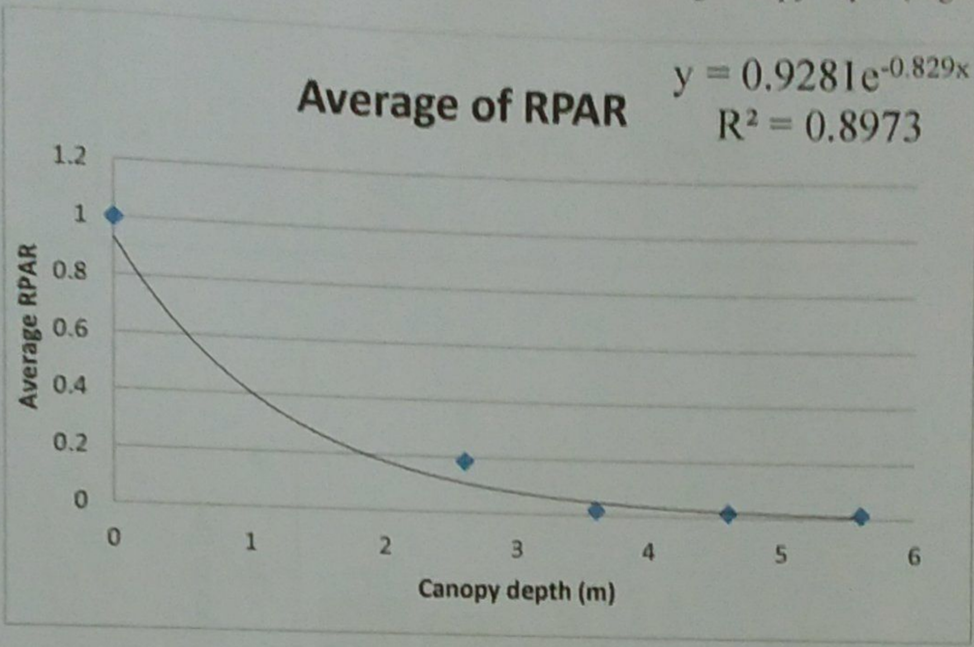

Fig. 1: Relationship between average RPAR (relative photosynthetically active radiation) and canopy height.

4.2 ANOVA Table for Anatomical Feature

4.2.1 ANOVA for thickness of upper epidermis

ANOVA showed a significant difference in thickness of upper epidermis among different canopy height ($P<0.01$). The upper epidermis was found significantly thinner (Tukey test) in the top canopy and thicker in the bottom canopy (Fig. 2).

Source of variation	Sum of Squares	df	Mean Squares	F Vaule	P Value	F Critical
Canopy Height	3539	5	707.9	11.10	P<0.01	13e-10 ***
Leaf Position	1857	1	1857.4	29.13		9.86e-08 ***

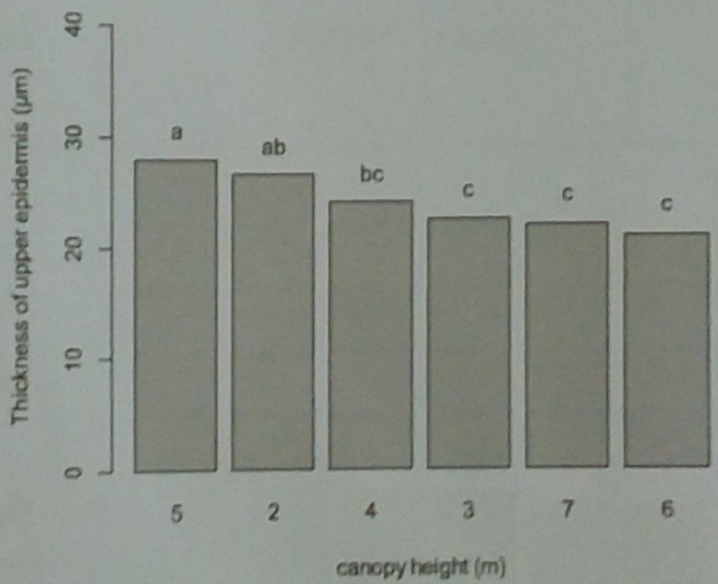

Fig. 2: Relationship between upper epidermis and canopy height. Same superscript indicates there was no significant difference among this canopy height (Tukey test).

4.2.2 ANOVA for thickness of lower epidermis.

I found significance difference in thickness of lower epidermis among different canopy height ($p<0.01$) in the Anova table. From the Tukey test the lower epidermis was found significantly thinner in the top canopy and thicker in the bottom canopy (Fig.3).

Source of Variation	Sum of Squares	df	Mean Squares	F Vaule	P Value	F Critical
Canopy Height	4550	5	910	6.005	P<0.01	1.98e-05 ***
Leaf Position	10419	1	10419	68.762		7.70e-16 ***

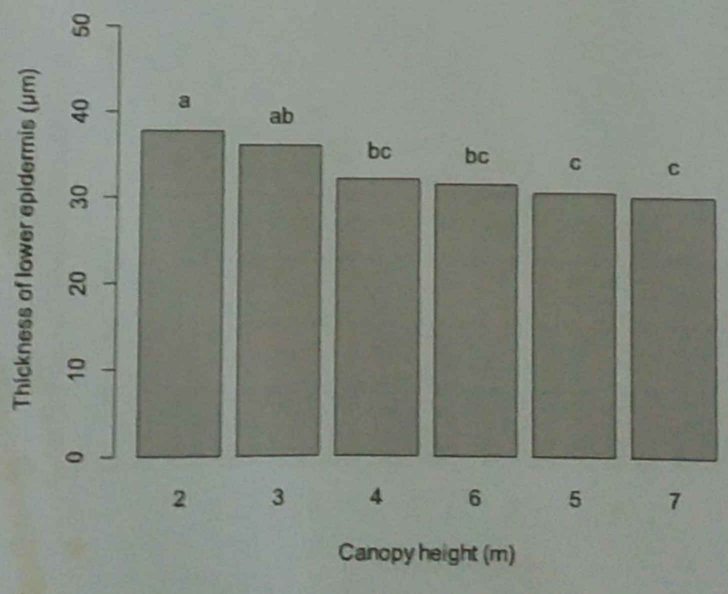

Fig.3: Relationship between lower epidermis and canopy height. Same superscript indicates there was no significant difference among this canopy height (Tukey test).

4.2.3 ANOVA for thickness of palisade mesophyll.

ANOVA showed significance difference in thickness of palisade mesophyll among different canopy height(p<0.01) The palisade mesophyll was found significantly thicker(Tukey test) in the top canopy and thinner in the bottom canopy (Fig.4).

Source of Variation	Sum of Squares	df	Mean Squares	F Value	P Value	F Critical
Canopy Height	119437	5	23887	10.64	P<0.01	8.43e-10 ***
Leaf Position	788619	1	788619	351.37		< 2e-16 ***

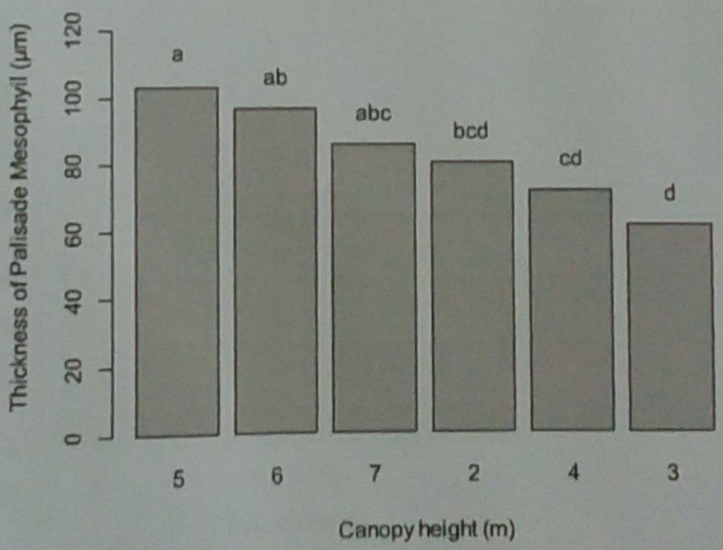

Fig.4: Relationship between thickness of palisade mesophyll and canopy height. Same superscript indicates there was no significant difference among this canopy height (Tukey test).

4.2.4 ANOVA for spongy mesophyll.

The boxplot represents the relationship between thickness of spongy mesophyll among different canopy height seperating the leaf position (midrib and leaflet) in (Fig.6). The ANOVA showed significance difference in the thickness of spongy mesophyll among different canopy height($p<0.01$).The spongy mesophyll was found significantly larger(Fig.5) in the top canopy and smaller bottom canopy.

Source of Variation	Sum of Squares	df	Mean Squares	F Value	P Value	F Critical
Canopy Height	111089	5	22218	24.30	P<0.01	<2e-16 ***
Leaf Position	42226	1	42226	46.19		2.66e-11 ***

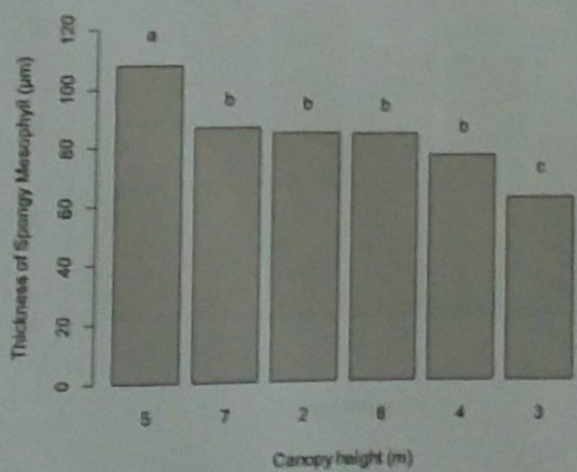

Fig.5: Relationship between thickness of spongy mesophyll and canopy height. Same superscript indicates there was no significant difference among this canopy height (Tukey test).

4.3 ANOVA for Morphological Feature

4.3.1 ANOVA for leaf area:

The ANOVA showed significant difference in the leaf area among different canopy height ($p<0.01$). The leaf area is significantly smaller in the top canopy (Fig.6: Tukey test) and larger in the bottom canopy.

Source of Variation	Sum of Squares	df	Mean Squares	F Value	P Value	F Critical
Canopy Height	7443	5	1488.7	3.745	P<0.01	0.00554 **

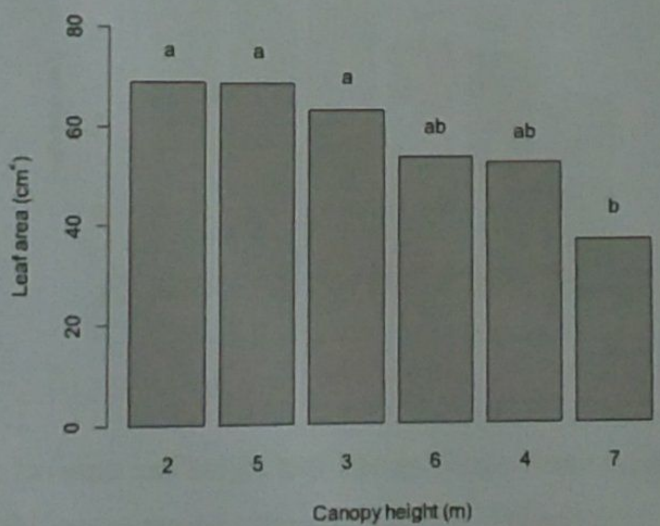

Fig.6: Relationship between leaf area and canopy height. Same superscript indicates there was no significant difference among this canopy height (Tukey test).

4.3.2 ANOVA for leaf length:

The ANOVA showed the significance difference between leaf length and canopy height ($p<0.01$). The leaf length was found significantly smaller (Tukey test) in the top canopy and larger in the bottom canopy (Fig.7).

Source of Variation	Sum of Squares	df	Mean Squares	F Value	P Value	F Critical
Canopy Height	263.9	5	52.77	7.208	P<0.01	3.13e-05 ***

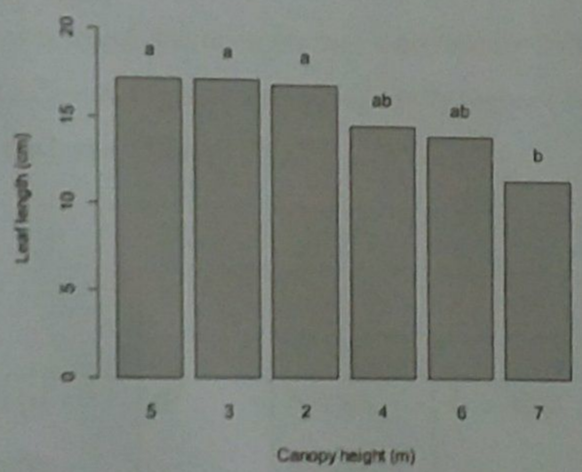

Fig.7: Relationship between leaf length and canopy height. Same superscript indicates there was no significant difference among this canopy height (Tukey test).

4.3.3: Leaf Width Variation

In the bottom canopy leaf width is higher and in the top canopy leaf width is lower (Fig.8)

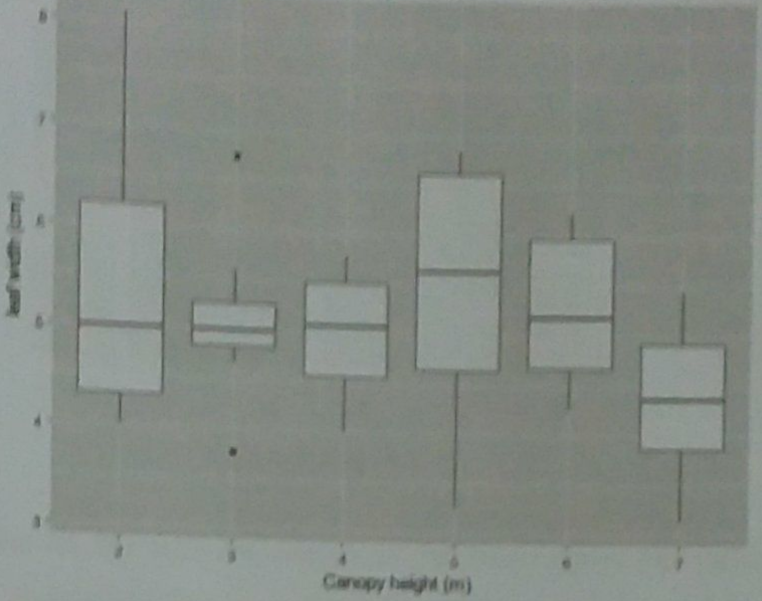

Fig.8: Relationship between leaf width and canopy height.

4.3.4 ANOVA for tip angle:

From the ANOVA it was found that tip angle was significantly different among different canopy height ($p<.01$). Tip angle was found significantly higher (Tukey test) in the top canopy and lower in the bottom canopy (Fig.9)

Source of Variation	Sum of Squares	df	Mean Squares	F Value	P Value	F Critical
Canopy Height	1890	5	378.1	4.297	P<0.01	0.00231 **

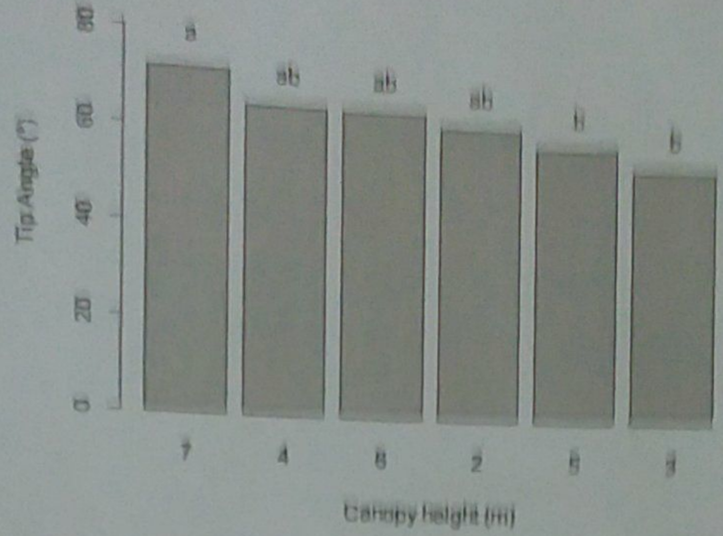

Fig.9: Relationship between tip angle and canopy height. Same superscript indicates there was no significant difference among this canopy height (Tukey test).

4.3.5 ANOVA for base angle:

The base angle was found significantly difference in different canopy height ($p<0.01$) in the ANOVA. From Tukey test it was found that the base angle is significantly higher in the top canopy and lower in the bottom canopy. (Fig 13)

Source of Variation	Sum of Squares	df	Mean Squares	F Value	P Value	F Critical
Canopy Height	1652	5	330.5	2.969	P<0.01	0.0194 *

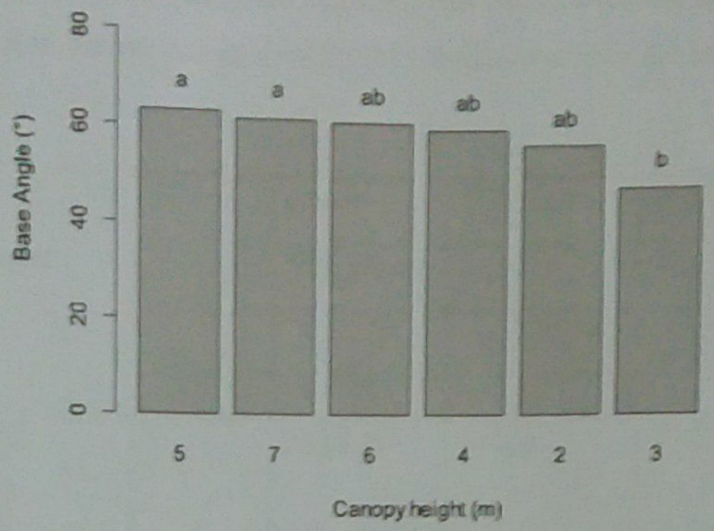

Fig.10: Relationship between base angle and canopy height. Same superscript indicates there was no significant difference among this canopy height (Tukey test).

4.3.6 Dry weight variation:

In this graph it was found that there was a less variation within dry weight and canopy height. In the bottom canopy the dry weight is less higher than top canopy (Fig.11)

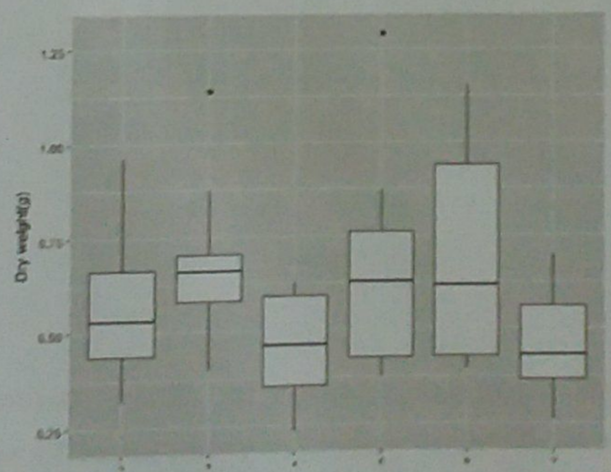

Fig.11: Relationship between dry weight and canopy height

4.3.7 ANOVA for specific leaf area (SLA):

The ANOVA showed significantly difference between specific leaf area (SLA) among different canopy height ($p<0.01$). The graph represent that the specific leaf area (SLA) is significantly decreased in the top canopy (Tukey test) and increased in the bottom canopy (Fig.12).

Source of Variation	Sum of Squares	df	Mean Squares	F Value	P Value	F Critical
Canopy Height	13142	5	2628.4	4.023	P<0.01	0.00356 **

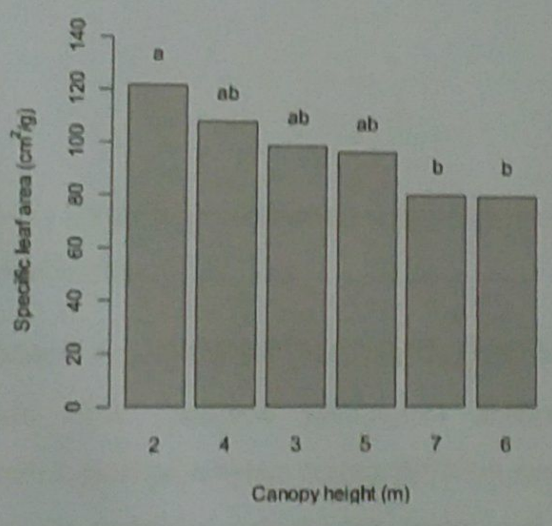

Fig.12: Relationship between specific leaf area and canopy height. Same superscript indicates there was no significant difference among this canopy height (Tukey test).

4.4 Principal Component Analysis (PCA) for Anatomocal Feature

In multiple comparison analysis PCA (principal component analysis) was showed relationship between leaf position in both midrib and leaflet and canopy height ($p<0.01$).PC1 for midrib was negatively correlated with the top canopy and positively correlated with the bottom canopy.PC2 for midrib is negatively correlated with top and bottom canopy. PC1 for leaflet was positively correlated with the top and bottom canopy.PC2 for leaflet was indicate positive correlation with the top and bottom canopy. Here different colors indicated different canopy height (Fig.13).

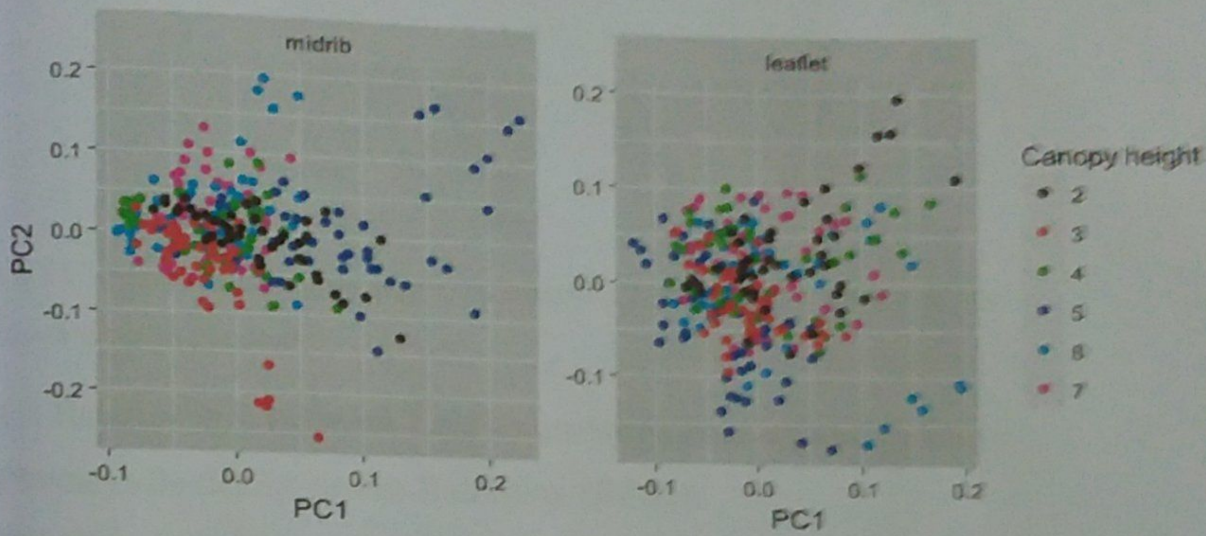

Fig.13. Ordination of canopy height with combined relationships among all leaf anatomical characteristics in midrib and leaflet.

4.5 Principal Component Analysis for Morphological Feature

In morphological feature PCA (principal component analysis) indicated combined relationship between morphological parameters among different canopy height ($p<0.01$). PC1 and PC2 both were negatively correlated with the top canopy and positively correlated with the bottom canopy. Different colors indicated different canopy height. (Fig.14).

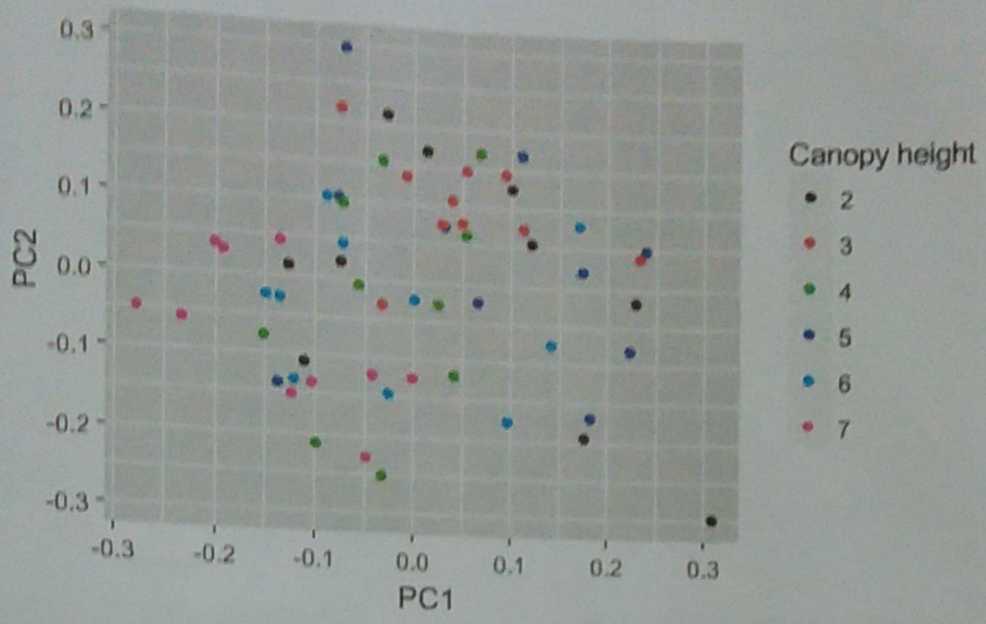

Fig.14: Ordination of canopy height with combined relationships among all leaf morphological parameter.

4.6 Relationship between Leaf Anatomical Feature and Relative Photosynthetically Active Radiation (RPAR)

The graph showed the relationship between photosynthetically active radiation (RPAR) and leaf anatomical feature in shade leaf and sun leaf by separating the leaf position (midrib and leaflet). In both (midrib and leaflet), the thickness of upper epidermis and lower epidermis are larger in the bottom canopy and smaller in the top canopy. The thickness of palisade mesophyll and spongy mesophyll are also higher in shade and lower in the full sun light (Fig: 15).

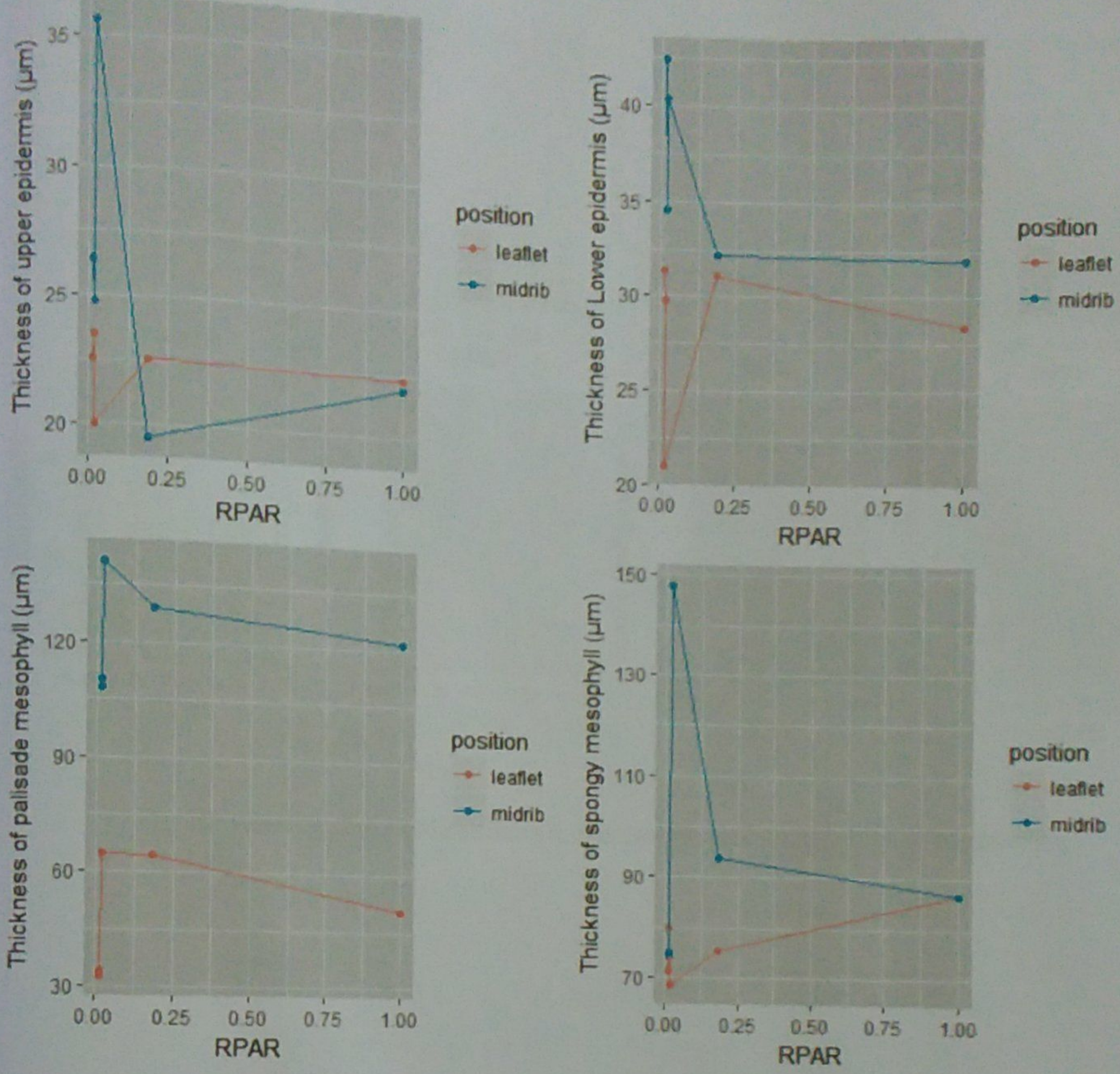

Fig 15: Relationship between RPAR and leaf anatomical parameter.

4.7 Relationship between Leaf Morphological Feature and Relative Photosynthetically Active Radiation (RPAR)

The graph showed the relationship between relative photo synthetically active radiation and different leaf morphological parameter. In shade leaf (leaf area, leaf length, leaf width and specific leaf area) are increased and decreased in the sun leaf. The tip angle and base angle those are higher in the top canopy and lower in the bottom canopy. The specific leaf mass (SLM) are also higher in the top canopy and lower in the bottom canopy (Fig.16).

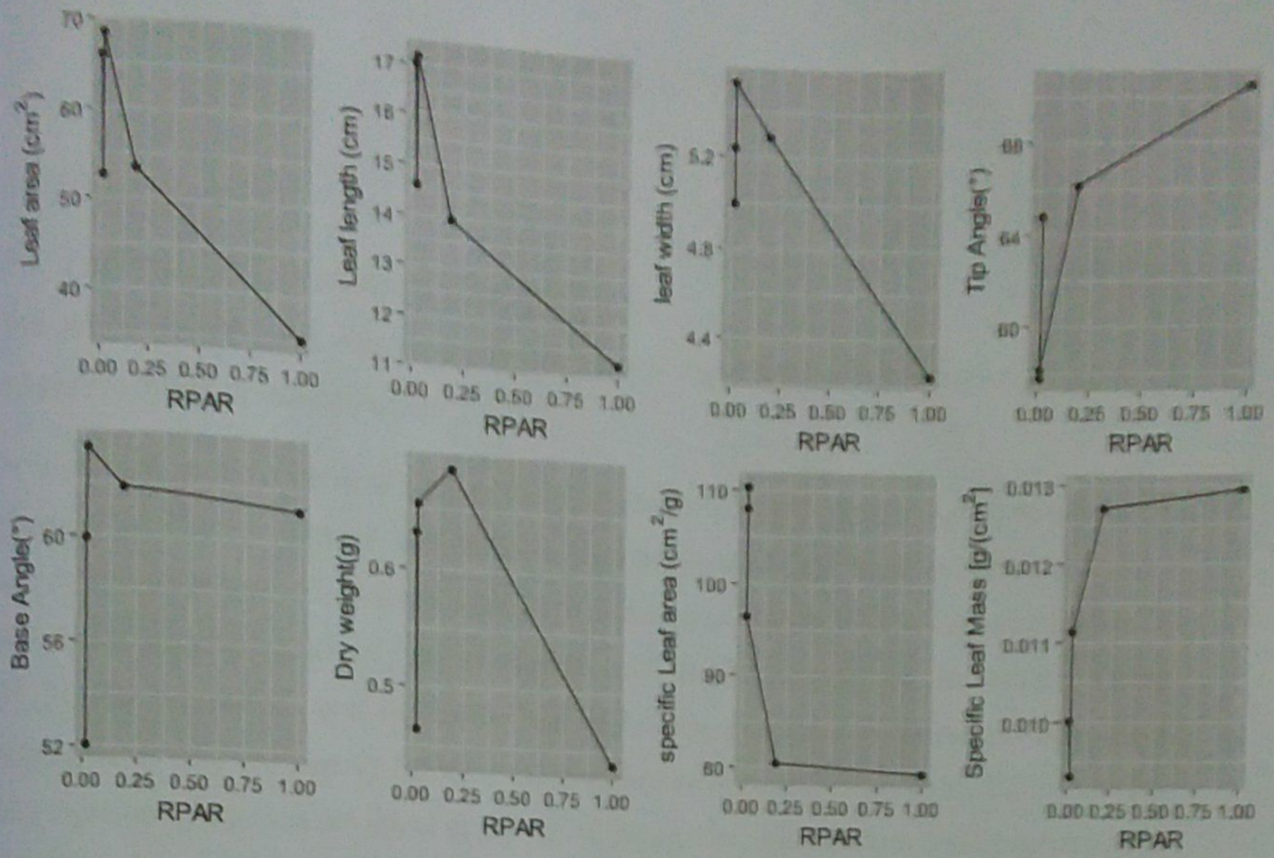

Fig.16: Relationship between RPAR and various leaf morphological parameters.

CHAPTER FIVE

5.0 Discussions

5.1 Vartical Distribution of Canopy Light

The aim of this research was to analyze the vertical light distribution and photosynthesis in relation to different canopy height.

In a canopy, a more vertical leaf position results in a higher canopy light-use efficiency (Long *et al.* 2006). Vertical distribution characteristics of leaf photosynthetic potentials and *PAR* were analyzed in various levels within canopies.(Barritt 1988) Light attenuation in the forest occurred primarily in the upper and middle portions of the canopy (Aber 1979).Leaf dimensions and especially leaf angles are important in assessing these plant strategies, as they are directly linked to the acquisition of light.(Farque et al. 2001). Smaller and more upright leaves are found in the top of the canopy, which allows light penetration to the lower layers, while lower leaves have a higher area so as to ensure maximum light absorption (Pearcy *et al.* 1990).

Light interception follows a seasonal pattern with, on average, a lower fraction of light intercepted during summer than during winter. A main factor is that solar elevation changes during the year. The higher solar elevation in summer months results in an orientation of light rays more perpendicular to the plant canopy, causing higher light penetration and lower interception (Farque et al. 2001).

An increase in light in lower canopy layers resulted in a higher relative increase in photosynthesis. A probable explanation for this is the leaf acclimation to lower light intensities and the physiological age in lower layers in the canopy (Niinemets 2007).

Vertical distribution of canopy light has positive correlation with the relative photosynthetically active radiation (RPAR) and canopy height. The top canopy was receiving full light and the leaf area is smaller, for this reason the canopy light condition is higher in the top canopy (R2 =.8973). The larger leaf area reduced the light penetration in the bottom canopy .The result was shown in the (Fig.1)

5.2 Anatomical Characteristics of a Leaf

Leaf orientation may be coordinated with external leaf morphology and internal anatomy for efficient light capture, according to different sunlight environments and stress levels (Smith et al. 1997). Typically, sun leaves are smaller and/or more deeply lobed, thicker, and lighter in color compared to shade leaves. Also, sun leaves are commonly more amphistomatous with well-developed palisade layers, while shade leaves are typically thinner, primarily hypostomatous, and without palisade layers (Evans 1999).

In full sun light the upper epidermis and lower epidermis is thinner and thicker in the bottom canopy. The palisade and spongy mesophyll is much larger in sun leaf and smaller in the shade leaf. (Fig: 2). Each of the anatomical dimension was significantly different in different canopy height (Fig: 3) showing relation with the light intensity.

Sunlight entering leaves is attenuated with depth in much the same way as light entering a canopy of leaves shows a logarithmic attenuation with depth that follows Beer's Law (Section 12.4). Within individual leaves, the pattern of light absorption is a function of both cell anatomy and distribution of pigments (Smith et al. 1998). Light absorption initially increases from the upper surface, peaking near the base of the first palisade layer, then declines steadily towards the lower surface (Koizumi et al. 1998).

The influence of leaf anatomical features on the leaf internal light environment relies on the external light environment, such as incident light levels and the direction and wavelength of the incident light. Leaf absorptance is 2–3% lower when the leaf is illuminated with diffuse light compared with direct light, while leaf reflectance increases and transmittance decreases (Brodersen and Vogelmann 2007). Palisade cells can facilitate deeper penetration of light into the mesophyll. However, the magnitude of this effect decreases under diffuse light compared with direct light (Vogelmann & Martin 1993).

In addition to the direction, the wavelength of the incident light is another important factor affecting the impact of anatomy on the internal light environment. Many components of the leaf can absorb light of different wavelengths, e.g. leaf pigments mainly absorb light in the visible wavelength region (400–700nm), while water absorbs much light in the infrared region (1400–2500nm) (Jacquemoud and Baret 1990). Because leaf pigments have different absorption coefficients for light of different wavelengths, the spectrum of light inside a leaf gradually shifts with depth into a leaf (Terashima *et al.*2009).

5.3 Morphological Characteristics of a Leaf

The physiological function of leaves is strongly related to leaf morphological traits. (Bond et al. 1999). Individual leaf area generally increased from canopy top to ground level (Fig: 6). SLA had a strong relationship with relative PPFD. As shown in (Fig. 12), the bottom canopy leaves had high SLA values compared with upper canopy leaves, except at the canopy surface (Khan et al. 2007) .

Leaf mass per area (LMA, g m^{-2}) is an essential trait for modeling canopy function due to its strong association with photosynthesis, respiration and leaf nitrogen. Leaf mass per area, which is influenced by both leaf thickness and density (LMA = thickness × density), generally increases from the bottom to the top of tree canopies (Cornwell et al. 2008).

In early experiments on small plants, leaves exposed to greater light intensity were thicker as a result of a thicker palisade mesophyll cell layer (Nobel et al. 1975). In many canopy studies, vertical light gradients in canopies have been implicated as a primary driver for increasing LMA with height (Hutchison et al. 1986). Experimental studies that manipulated canopy light environments provided further evidence of adjustments in LMA in response to the light environment (Brooks et al. 1994).

5.4 Principal component analysis:

Principal component analysis (PCA) is one of the multivariate methods commonly used to investigate relationships in ecological studies (Potvin et al. 1990) PCA usually is used to reduce a data set with a relatively large number (p) of correlated variables to a data set with fewer ($m < p$), uncorrelated variables (components) that retain most of the information content of the original data (Rummel 1970).

The number of distinct principal components is equal to the smaller of the number of original variables or the number of observations minus one. This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to the preceding components. The resulting vectors are an uncorrelated orthogonal basis set. PCA is sensitive to the relative scaling of the original variables (Hotelling 1933).

CHAPTER SIX

6.0 Conclusion

Leaves of *Heritiera fomes* response to different light environments at the different canopy height. Smaller leaves are found in the top of the canopy, which allows light penetration to the lower layers, while lower leaves have a higher area so as to ensure maximum light absorption. Shade-growing leaves are thinner, have lower mass per unit area than do sun-growing leaves. Sun leaves become thicker than shade leaves because they develop longer palisade cell or an additional layer of palisade cell. The leaf area was increased in the bottom canopy and decreased in the top canopy. The specific leaf area also decreased in the top canopy and increased in the bottom canopy. Vertical distribution of photosynthetic active radiation, *PAR* at various levels within canopies of *Heritiera fomes* showed strong relationships with various leaf anatomical and morphological characteristics. The ordination of different canopy height with respect to different leaf characteristics as tested with principal component analysis suggests that after a certain amount of shade (canopy depth) the leaves show strong similarity.

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