International Journal of Scientific Research and Management (IJSRM)
||Volume||11||Issue||02||Pages||B-2023-203-213||2023||
Website: www.ijsrm.in ISSN (e): 2321-3418
DOI: 10.18535/ijsrm/v11i02.b01

Thickness of Leaf Anatomical Tissue of Dicot Ornamental Plants at Different Light Intensities

[1] Nadia Fransiska, [2] Entin Daningsih, [3]Asriah Nurdini Mardiyyaningsih

[123] Faculty of Teacher Training and Education, Tanjungpura University, West Kalimantan/78124, Indonesia.

Abstract
Different light intensities affect the leaf anatomy. This study aimed to measure the thickness of the anatomical tissue of the leaves of six dicot ornamental plants at different light intensities. This study used a quantitative experimental method with a factorial completely randomized (CRD-Factorial) design. Two factors in this study were variants of plants and types of light intensities. The six plant variants were *Codiaeum variegatum* (L.) A. Juss., *Bougainvillea glabra* Choisy, *Tabernaemontana divaricata* (L.) R, Br. Ex Roem. &Schult., *Syzygium paniculatum* Gaertn, *Excoecaria cochinchinensis* Lour., and *Aerva sanguinolenta* BI. Meanwhile, the light intensities were under two conditions: open and shading areas with three replications. Parameters were leaf anatomical tissue thickness: total, upper and lower epidermis, palisade, and spongy using a modified paraffin method from Johansen. Data were analyzed using the CRD-Factorial ANOVA followed by the LSD test and continued to correlate between palisade, spongy, and lower epidermis thickness with total tissue thickness. The results showed that the leaf anatomy of six dicot ornamental plants varied. All plants have one layer of upper and lower epidermis tissue and one layer of the spongy part. However, only *Codiaeum variegatum* (L.) A. Juss., *Syzygium paniculatum* Gaertn, and *Excoecaria cochinchinensis* Lour have two layers of palisade tissue. The thickness of the leaf tissue decreased in the shading area along with the depletion of the palisade, spongy, and lower epidermis tissue, as indicated by a correlation from moderately strong to very strong. Shrinkage of palisade tissue, spongys, and lower epidermis is a plant's attempt to get optimal light in shading areas. The lower epidermis tissue shrank, the thickness of leaf become thin while the thickness of palisade and spongy varied so the thickness of total leaf from each species also varied.

Keyword: Light intensity, thickness of leaf tissue, shading, ornamental plant, shading and open areas

Introduction

Ornamental plants function to create beauty and attractiveness in an object because they have beautiful shapes and colors, soothe the soul and preserve the environment (Widyastuti, 2018). Ornamental plants can be placed indoors and or outdoors. Leaves are one of the important organs of plants that function as a place of photosynthesis (Campbell et al., 2008). Leaves comprise of upper and lower epidermal tissues, mesophyll (palisade and spongy) tissues, and transport bundles (Wulansari et al.,2020). Each tissue gives a different appearance and provide special features so that the anatomical features can be used as a supporting tool in the identification, grouping, and kinship of plant species (Stuessy, 1990). In general, leaves have varying thicknesses, sizes, and number of leaf layers (Aini et al., 2014).

One of the environmental factors that affect leaf thickness is sunlight. According to Karyati et al., (2017), the length, width, and thickness of leaves on plants are affected by the amount of exposure to sunlight received. Nelza et al., (2018) said that the sword bean plant's shade and fertilization factors could affect both leaf and palisade thickness. The higher the shade percentage given, the leaves of the sword bean plant become thinner. The reduced leaf thickness under shading conditions is thought to be caused by the thinning of the epidermal layer and parenchyma cells that make up the leaf structure. In addition, giving low doses of fertilization can cause the thickness of the palisade tissue to decrease. Changes in the morpho-anatomical structure of leaves in low light conditions are adaptation mechanisms in capturing sunlight (Levitt, 1980).

Changes in leaf anatomy usually relate to plant efforts to carry out photosynthetic efficiency (Tholen, 2012).

Palisade tissue in leaves contains lots of chlorophyll, and changes in light cause the size and shape of cells in the palisade tissue to change to get enough light.

Costa and Daningsih (2022) show that there is leaf shrinkage in six dicot ornamental plants related to transpiration. However, Costa and Daningsih (2022) have not been able to show which tissue causes leaf shrinkage. Daningsih et al., (2022) observed leaf tissue in six dicot plants and showed that there were tissue variations between different species. This variation was investigated on the six ornamental plants placed in open areas. According to Tholen (2012), light can affect changes in leaf anatomy. An insight into changes in leaf anatomy affected by different light intensities is essential to determine the ability of plants adaptation to outdoor and indoor environments. For this reason, this study aimed to investigate the anatomical tissue thickness of those six dicot ornamental plants with different light intensities.

Materials and Method

1. Tools and Material

The tools for this study were the 30x35 cm polybags, soil shovel, scissors, ruler, slide, cover glass, test tube clamp, tweezers, 50 ml beaker, Olympus-CX 21 microscope, vials, film bottles, euromex rotary microtome, brush, plastic petri dish, alcohol thermometer S-006, lux-meter AS803, thermo-hygrometer max-min AZ-HTC-2, anemometer GM816, measuring cup, O'hauss analytical balance, Nikon D5200 DLSR camera, C-1 objective micrometre, Memert oven, Optilab advance microscope camera, staining glass, 70% paranet, and gunkol brand hot plate.

The materials utilized in this study were Vaseline, distilled water, FAA solution (5 ml 40% formalin-90ml 70% alcohol-5 ml glacial acetic acid), various concentrations of alcohol (30%, 50%, 70%, 80%, 90%, 96% and 100%), 1% safranin (aqueous solvent), xylol, paraffin oil, 0.5% (90% alcohol solvent) Fast green, paraffin, fabric softener (Diethylester Dimethyl Ammonium Chloride), granulated alcohol (TBA), Haupt's adhesive (gelatin, sodium bicarbonate and distilled water), 3% formalin, burnt soil-poor sand-16:16:16 NPK fertilizer (Mutiara brand) and six dicot ornamental plants used as objects in this study namely: Croton (*Codiaeum variegatum* (L.) A. Juss.), Bougenville (*Bougainvillea glabra* Choisy), Crape Jasmine (*Tabernaemontana divaricata* (L.) R, Br. Ex Roem. & Schult.), Red Shoots (*Syzygium paniculatum* Gaertn), Firestorm (*Excoecaria cochinchinensis* Lour.), and Erpah (*Aerva sanguinolenta* BI).

2. Method

The research was a quantitative experiment with a factorial completely randomized design (CRD-Factorial). The two factors were the six dicot ornamental plants and the different light conditions (open and shading). The plants were Croton (*Codiaeum variegatum* (L.) A. Juss.), Bougenville (*Bougainvillea glabra* Choisy), Crape Jasmine (*Tabernaemontana divaricata* (L.) R, Br. Ex Roem. & Schult.), Red Shoots (*Syzygium paniculatum* Gaertn), Firestorm (*Excoecaria cochinchinensis* Lour.), and Erpah (*Aerva sanguinolenta* BI). The open area referred to putting plants under direct sunlight, while the shading was produced using 70% paranet. The combination factor was the interaction of plant species and each light intensity condition (open and shading areas). Each treatment was replicated three times with a total collection of 36 research samples.

3. Procedures

3.1 Plant Preparation

The plant had the same height and approximately similar number of leaves for each plant types. The plants were transferred into 30x35 cm polybags prepared with the planting medium of burnt soil and poor sand with a ratio of 2: 1. The 0.5 grams NPK fertilizer with a ratio of 16:16:16 diluted in water was given to each plant once every two weeks. Watering the plants was done once every day at the same time. All samples were placed in a simple green house. The green house was divided into two areas: the open area (direct sunlight) and the shading area (covered by the 70% paranet).

3.2 Making Transverse Slide Leaf Preparations

Leaf samples were taken from plants in the two areas (the open and shading areas). All samples were mature leaves from the first branch position. The lamina was taken by avoiding the leaf veins. The leaves' anatomical tissue section was prepared using the modified paraffin method from Johansen (1940). The modified steps were the use of adhesive (Berlyn and Miksche, 1976), and the fabric softener (Diethylester Dimethyl Ammonium Chloride) on the hard paraffin block (Orchard et al, 2008). The fabric softener was used for soaking the paraffin block which was difficult to cut for 10 minutes before putting the block in the cooler for 15 minutes.The block was cut using a microtome with 12-14 µm thickness. Samples then double stained using safranin-fast green.

3.3 Leaf Tissue Thickness Measurement

The leaf anatomy tissue was observed under a microscope with a magnification of 10x10 which was assisted by Optilab advance microscope camera. The tissue thickness was measured using Image Raster3 Software. The Thickness measurement includes the upper epidermis, lower epidermis, palisade, and spongy.

3.4 External Factors

External factors acted as supporting data for the research. The environmental factors measured consisted of light intensity (using lux-meter AS803), humidity (using thermo-hygrometer max-min AZ-HTC-2), and wind speed (anemometer GM816), which were carried out once a week for four weeks prior to sampling.

Data analysis

Leaf tissue thickness was analyzed using the CRD-Factorial model of variance using 1997 SAS version 6.12. First, data were checked for normality and homogeneity using SPSS version 24. If the data shows normal and homogeny, the analysis proceeds to test of least significant difference test (LSD) with an $\alpha=0.05$. The Pearson correlation test was carried out to determine the relationship between the total thickness of leaf tissue and each tissue part of leaf anatomy: palisade, spongys, and upper-lower epidermis. The correlation number is categorized based on Riduwan and Sunarto (2007).

Results and Discussion

1. Results

1.1 Leaf Anatomy of Six Dicot Ornamental Plants in Open and Shading Areas

Observations of leaf anatomy in this study showed variations within leaf tissue thickness in open and shading areas (Figure 1 to Figure 6).

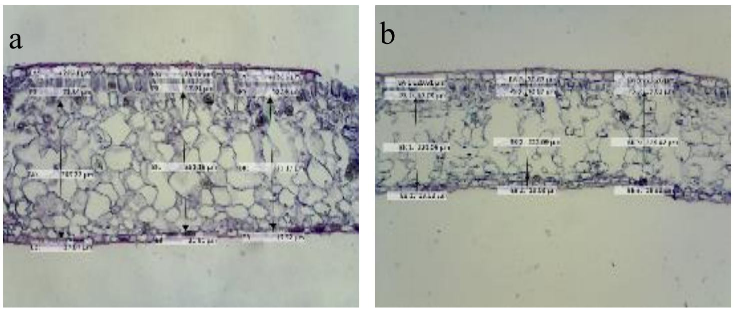

Figure 1. Croton (*Codiaeum variegatum* (L.) A. Juss.) leaf anatomy (a) open area (b) shading area. Mesophyll in Croton (*Codiaeum variegatum* (L.) A. Juss.) differentiated into palisade and spongy tissue. Croton plant has two layers of palisade tissues and consists of cells that stand vertically, while the spongy tissue was located between the palisade and the lower epidermal tissue. The anatomical thickness of the leaves in open and shading conditions was as follows: upper epidermis 25.04µm (open) and 23.86µm (shading); palisade grid 47.13µm (open) and 34.58µm (shading); spongy tissue 308.71µm (open) and 230.68µm(shading); lower epidermis 21.15µm (open) and 18.21µm (shading). Total leaf anatomy thickness were 399.55µm (open) and 307.32µm (shading).

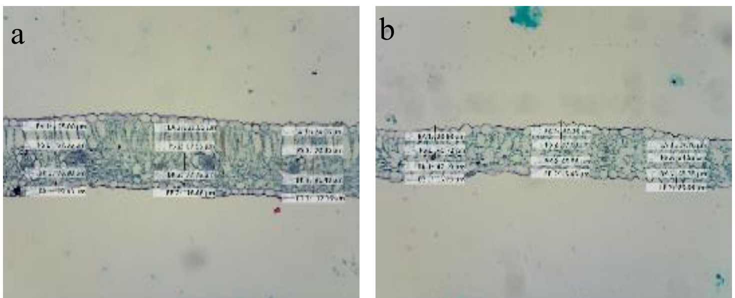

Figure 2. Anatomy of a Bougenville leaf (*Bougainvillea glabra* Choisy) in an (a) open and (b) shading area. Mesophyll in Bougenville plants (*Bougainvillea glabra* Choisy) has undergone differentiation into palisade

and spongy tissue. The palisade tissue consists of cells that stand vertically, while the spongy tissue lies between the palisade and the lower epidermal tissue. The anatomical thickness of the leavesin open and shading conditions was as follows: upper epidermis 26.89µm (open) and 23.73µm (shading); palisadegrid 74.47µm (open) and 35.74µm (shading); spongy tissue 72.39µm (open) and 58.76µm (shading); lower epidermal16.23µm (open) and 14.34µm (shading). Total leaf anatomy thickness were 189.99µm (open) and 132.58µm (shading).

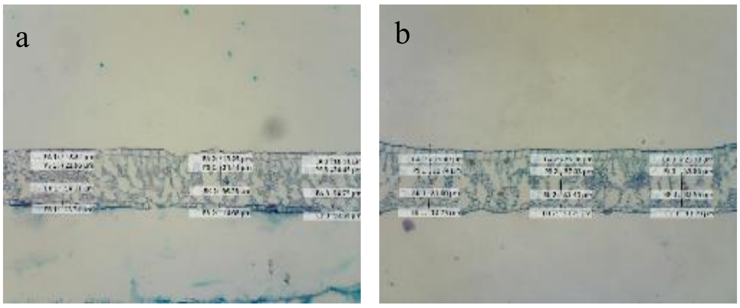

Figure 3. Anatomy of a Crape Jasmine (*Tabernaemontana divaricata* (L.) R,Br. Ex Roem. & Schult.) leaf in an (a) open area (b) shading area. Mesophyll in Crape Jasmine (*Tabernaemontana divaricata* (L.) R, Br. Ex Roem. & Schult.) differentiated into palisade and spongy tissue. The palisade tissue consists of cells that stand vertically, while the spongy tissue lies between the palisade tissue and the lower epidermal tissue. The anatomical thickness of the leaves in open and shading conditions was as follows: upper epidermis 18.52µm (open) and 24.17µm (shading); palisade grid 23.94µm (open) and 34.07µm (shading); spongy tissue 96.81µm (open) and 84.18µm (shading); lower epidermal 14.57µm (open) and 13.26µm (shading). Total leaf anatomy thickness were 153.84µm (open) and 155.78µm (shading).

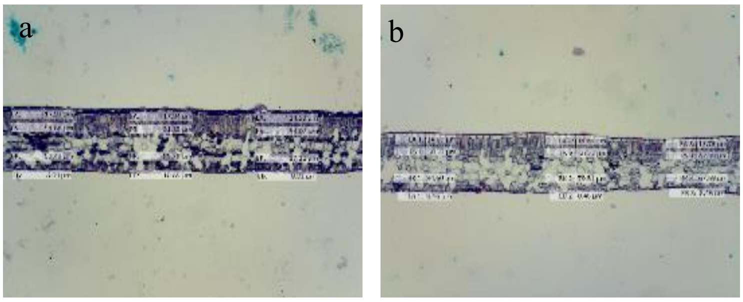

Figure 4. Red Shoots leaf anatomy (*Syzygium paniculatum* Gaertn) in an (a) open area (b) shading area. Mesophyll in Red Shoots plants (*Syzygium paniculatum* Gaertn) differentiated into palisade and spongy tissue. Red shoots plants have 2 layers of palisade tissue and consist of vertical cells, while the spongy tissue lies between the palisade tissue and the lower epidermal tissue. The tissue thickness of the leaves in open and shading conditions was as follows: upper epidermis 17.41µm (open) and14.25µm (shading); Palisade grid 48.93µm (open) and 47.45µm (shading); spongy tissue 94.86µm (open) and78.47µm (shading); lower epidermal 9.54µm (open) and 8.18µm (shading). Total leaf thickness were 173.23µm (open) and 148.34µm (shading).

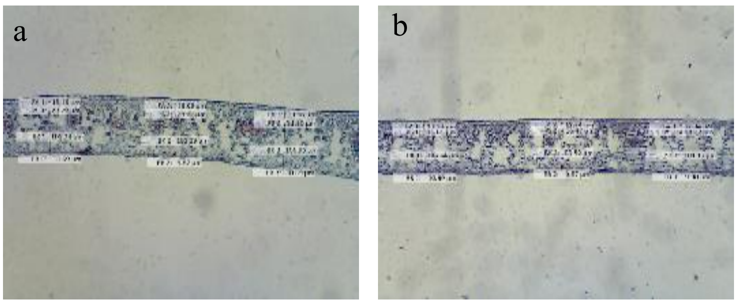

Figure 5. Firestorm (*Excoecaria cochinchinensis* Lour.) leaf anatomy in an (a) open area and (b) shading area. Mesophyll in Firestorm (*Excoecaria cochinchinensis* Lour.) plants differentiated into palisade and spongy tissue. The firestorm (*Excoecaria cochinchinensis* Lour.) has 2 layers of palisade tissue and consists of cells that stand vertically,while the spongy tissue located between the palisade tissue and the lower epidermal tissue. The anatomical thickness of the leaves in open and shading conditions was as follows: upper epidermis

11.76µm (open) and12.23µm (shading); palisade grid 27.19µm (open) and 18.13µm (shading); spongy tissue 112.9µm (open) and97.82µm (shading); lower epidermis 9.85µm (open) and 10.25µm (shading). Total anatomical thickness of the leaf was 161.79µm (open) and 138.43µm (shading).

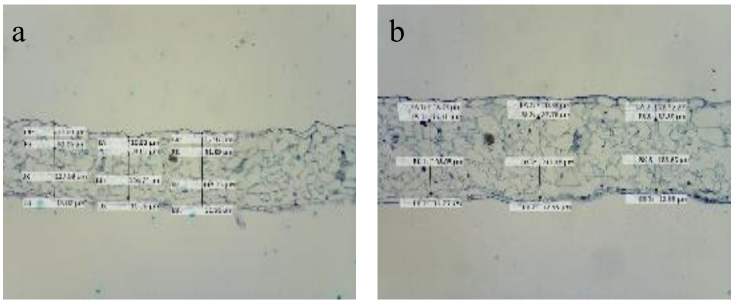

Figure 6. Erpah leaf anatomy (*Aerva sanguinolenta* BI). (a) Open area. (b) Shading area. Mesophyll in Erpah (*Aerva sanguinolenta* BI) differentiated into palisade and spongy tissue. The anatomical thickness of the leaves in open and shading conditions was as follows: upper epidermis 16.14µm (open) and 16.98µm (shading); Palisade grid 41.41µm (open) and 39.66µm (shading); Spongy tissue 128.05µm (open) and 175.09µm (shading); lower epidermal tissue 12.82µm (open) and 13.46µm (shading). The total thickness of the leaf was 197.84µm (open) and 243.96µm (shading).

1.2 Leaf Anatomical Tissue Thickness Variation

All data were distributed normally (p=0.05). However, only the data for upper and lower epidermal tissues and spongy had homogeneity distribution while the palisade and total leaf tissue thickness were not (p=0.02 and p=0.01, respectively).

The results from factorial ANOVA (Table 1) showed that plant species affected significantly all parameters of leaf thickness total (KT), upper epidermis (EA), lower epidermis (EB), palisade (PS), and spongy (BK). However, the light intensity (condition) affected all leaf thickness parameters, except for upper epidermis. Furthermore, the combination between plant species and conditions had a significant effect on all leaf anatomical tissue thicknesses.

Table 1. Results of variance with the CRD-Factorial model of six dicot ornamental plants

Factor	**Average leaf thickness in open and shading conditions (µm)**				
	KT	**EA**	**EB**	**PS**	**BK**
Plant Species	*	*	*	*	*
Croton (*Codiaeum variegatum* (L.) A. Juss.)	353.44^{a}	24.45^{a}	19.68^{a}	40.85^{c}	269.69^{a}
Erpah (*Aerva sanguinolenta* BI)	220.89^{b}	16.56^{a}	13.14^{c}	40.54^{c}	151.57^{b}
Bougenville (*Bougainvillea glabra* Choisy)	161.28^{c}	25.31^{b}	15.28^{b}	55.11^{a}	65.58^{e}
Red Shoots (*Syzygium paniculatum* Gaertn)	160.78^{c}	15.83^{c}	8.86^{e}	48.19^{b}	86.66^{d}
Crape Jasmine (*Tabernaemontana divaricata* (L.) R,Br. Ex Roem. & Schult.)	154.81^{cd}	21.39^{c}	13.91^{c}	29.01^{d}	90.49^{d}
Firestorm (*Excoecaria cochinchinensis* Lour.)	150.11^{d}	11.99^{d}	10.05^{d}	22.66^{e}	105.40^{c}
Condition	*	ns	*	*	*
Open (O)	212.71^{a}	19.29	14.03^{a}	43.85^{a}	135.64^{a}
Shading (S)	187.74^{b}	19.22	12.95^{b}	34.94^{b}	120.83^{b}
Combination of Plant Species and Conditions	*	*	*	*	*
O x Croton (Codiaeum variegatum (L.) A. Juss.)	399.55^{a}	25.05^{b}	21.15^{a}	47.13^{b}	308.71^{a}
S x Croton (Codiaeum variegatum (L.) A. Juss.)	307.32^{b}	23.86^{bc}	18.21^{b}	34.57^{d}	230.68^{b}
O x Erpah (Aerva sanguinolenta BI)	197.34^{d}	16.14^{e}	12.82^{f}	41.41^{c}	128.05^{d}
S x Erpah (Aerva sanguinolenta BI)	243.96^{c}	16.97^{e}	13.46^{e}	39.66^{c}	175.09^{c}
O x Bougenville (Bougainvillea glabra Choisy)	189.98^{e}	26.89^{a}	16.23^{c}	74.47^{a}	72.39^{i}
S x Bougenville (Bougainvillea glabra Choisy)	132.57^{k}	23.73^{c}	14.34^{d}	35.74^{d}	58.76^{j}

O x Crape Jasmine (Tabernaemontana divaricata (L.) R,Br. Ex Roem. & Schult.)	153.84^{h}	18.52^{d}	14.57^{d}	23.94^{f}	96.81^{f}
S x Crape Jasmine (Tabernaemontana divaricata (L.) R,Br. Ex Roem. & Schult.)	155.78^{h}	24.27^{bc}	13.26^{ef}	34.07^{d}	84.17^{g}
O x Red Shoots (Syzygium paniculatum Gaertn)	173.23^{f}	17.41^{de}	9.54^{h}	48.93^{b}	94.86^{f}
S x Red Shoots (Syzygium paniculatum Gaertn)	148.34^{i}	14.25^{f}	8.17^{i}	47.45^{b}	78.47^{h}
O x Firestorm (Excoecaria cochinchinensis Lour.)	161.78^{g}	11.76^{g}	9.85^{gh}	27.19^{e}	112.98^{e}
S x Firestorm (Excoecaria cochinchinensis Lour.)	138.43^{j}	12.23^{g}	10.25^{g}	18.13^{g}	97.82^{f}

Note: *: significance, ns: non-significance, O: Open, S: Shading, KT: total thickness, EA: upper epidermis, EB: lower epidermis, PS: palisade, dan BK: spongy.

The following LSD test results showed Croton (*Codiaeum variegatum* (L.) A. Juss.) had the highest number for total, upper, lower and spongy epidermis tissue thickness, while Firestorm (*Excoecaria cochinchinensis* Lour.) had the lowest thickness for total, upper, lower and palisade tissue thickness. Interestingly, the Firestorm (*Excoecaria cochinchinensis* Lour.) had a thicker spongy tissue thickness than the Red Shoots (*Syzygium paniculatum* Gaertn), Crape Jasmine (*Tabernaemontana divaricata* (L.) R, Br. Ex Roem. & Schult.) and Bougenville (*Bougainvillea glabra* Choisy). A comparison between open and shading areas showed that the total thickness of leaf, lower epidermis, palisade, and spongy tissue were higher than of the shading area, except for the upper epidermal tissue, which was similar between the open and shading areas. In general, the combinations between plant species and light intensities affected significantly the tissue thicknesses (Table 1).

1.3 Correlation between Total Leaf Tissue Thickness and Palisade, Spongy, and Lower Epidermis Thickness

The correlation between the total thickness of the leaf tissue and the thickness of the palisade, spongy, and lower epidermis is shown in Table 2.

Table 2. Pearson correlation analysis results of total leaf tissue thickness with palisade, spongy, and lower epidermis thickness.

Plant Species	Condition	Correlation Coefficient					
		PS		BK		EB	
		r	Category	r	Category	r	Category
Croton (*Codiaeum variegatum* (L.) A. Juss.)	T	1	Very strong	0.91	Very strong	-0.22	Weak
	N	0.99	Very strong	1	Very strong	0.04	Very weak
Bougenville (*Bougainvillea glabra* Choisy)	T	-0.63	strong	0.80	Very strong	0.95	Very strong
	N	-1	Very strong	0.96	Very strong	0.48	Strong enough
Crape Jasmine (*Tabernaemontana divaricata* (L.) R,Br. Ex Roem. & Schult.)	T	0.98	Very strong	0.93	Very strong	0.01	Very weak
	N	0.99	Very strong	-0.34	Weak	0.32	Weak
Red Shoots (*Syzygium paniculatum* Gaertn)	T	0.34	Weak	0.99	Very strong	0.91	Very strong
	N	0.99	Very strong	-0.92	Very strong	-0.63	Strong
Firestorm (*Excoecaria cochinchinensis* Lour.)	T	-0.12	Very weak	0.60	strong	-0.42	Strong enough
	N	-0.99	Very strong	0.97	Very strong	-0.90	Very strong

Erpah (*Aerva sanguinolenta* BI)	T	0.55	Strong enough		0.65	strong		-0.86	Very strong
	N	-0.93	Very strong		0.99	Very strong		0.97	Very strong

Note: O: open, S: shading, PS: palisade, BK: Spongy, EB: Lower epidermis.

The correlation between the total leaf thickness and the other tissues parameters (palisade, spongy, and lower epidermis) varied among the six dicots ornament plants. For example, the total leaf thickness of Croton (*Codiaeum variegatum* (L.) A. Juss.) showed the very strong correlation to the palisade and spongy thickness both in open and shading areas; however, it did not correlate with the thickness of the lower epidermis. On the other hand, Bougenville (*Bougainvillea glabra* Choisy) showed a strong correlation coefficient between total and palisade thickness in open areas and a very strong one for shading areas. These were different from the very strong correlation of thickness between the total and the spongy, the total and the lower epidermis both in open and shading areas.

1.4 External Factors

The average of measurements for light intensity, humidity, windspeed in open and shading area were as followed 282 $W.m^{-2}$ and 78 $W.m^{-2}$; 74% and 76%; 0 $m.s^{-1}$ respectively.

2. Discussion

Based on observations of leaf anatomy (Figure 1 to Figure 6) the mesophyll of all six dicot ornamental plants had differentiated into palisade and spongy tissue which was linear with the statements of Sumardi and Pudjoarinto (1992) who cited that mesophyll in all dicot ornamental plants had been differentiated into palisade and spongy tissue. The complete dicots leaves anatomical tissue comprised of upper and lower epidermal tissue, and the mesophyll which was composed of palisade and spongy tissue (Tihurua et al.,2020). The results showed that all plants had one layer of upper and lower epidermis tissue (Figure 1 to Figure 6). Croton (*Codiaeum variegatum* (L.) A. Juss.), Red Shoots (*Syzygium paniculatum* Gaertn), and Firestorm (*Excoecaria cochinchinensis* Lour.) had two layers of palisade tissue (Figure 1, Figure 4 and Figure 5 respectively), whereas Bougenville plants (*Bougainvillea glabra* Choisy), Crape Jasmine (*Tabernaemontana divaricata* (L.) R, Br. Ex Roem. & Schult.) and Erpah (*Aerva sanguinolenta* BI) had only one layer of palisade.

The leaf in shading condition produced thinner palisade tissue than those in the open area (Table 1). According to Ekawati and Aziz (2016) the light intensity affected both the shape and anatomy of the leaves, including the epidermal and mesophyll cells. The changes act as a mechanism for controlling the quality and amount of light used by leaf chloroplasts. Furthermore, the lower epidermis was shortening which was shown to reduce the surface area of the leaf in contact with the outside environment so that transpiration could be controlled (Samsuri, 2013). This was supported by the opinion of Wu et al., (2017); Sundari et al., (2008) whose said that shading on soybeans causes a decrease in cell size, especially in palisade cells which results in thin leaves. Meanwhile, spongy tissue in shading conditions was thinner than spongy tissue in open conditions. Plant adaptation to the low light intensity occurred in two ways, namely increasing leaf area to reduce the use of metabolites and reducing the amount of light transmitted and reflected (Hale and Orcutt, 1987).

Another interesting finding is the differences of palisade layers formation of the sample plant. The palisade of Croton (*Codiaeum variegatum* (L.) A. Juss.) and Firestorm (*Excoecaria cochinchinensis* Lour.) found in the open areas consists of two layers; in contrast the counterpart in the shading area only consisted of one layer. This fact was consistent with the Weaver and Clements (1986) and Heyneke et al., (2013) stated those different light intensities affected the palisade layers. In a low light intensity, plants tend to develop one layer of palisade tissue, whereas at high light intensity plants develop two layers of palisade tissue. This condition did not occur for the leaves of Red Shoots (*Syzygium paniculatum* Gaertn). The palisade was on two layers both in open and shading conditions; however, the palisade tissue in shading conditions was smaller and thinner than the palisade tissue in open conditions (Figure 4).

Based on the results of the CRD-Factorial analysis of variance (Table 1), there were that shading had a significant effect on thinner anatomical tissue thickness for total anatomical leaf, lower epidermis, palisade, and spongy but had no significant effect on the upper epidermal tissue. In this study, there were a correlations between the thickness of the palisade, spongy, and lower epidermis to the total thickness of the leaf tissue from moderately strong to very strong (Table 2). This condition was also found in Coble and Cavaleri (2017) research with *Acer saccharum* Marshall plant which has a different anatomical response to two light intensity, namely open and shading condition. The leaf anatomy in the open conditionwas thicker while in the shading condition the leaf anatomy was thinner. This was consistent with the observation that plants placed openly had thicker leaf thickness than those in the shade (Table 1, Figure 1 to Figure 6).

In this study, Croton (*Codiaeum variegatum* (L.) A. Juss.), Bougenville (*Bougainvillea glabra* Choisy), Red Shoots (*Syzygium paniculatum* Gaertn), and Firestorm (*Excoecaria cochinchinensis* Lour.) had a thicker but a smaller leaves in open conditions (Figure 1, Figure 2, Figure 4, and Figure 5). Paluvi et al., (2015) also said that plants exposed with high intensity sunlight causing stems to grow faster, the perfection of vessel arrangement, the thicker leaves but smaller size compared to plants at low light intensity. High light intensity affected the anatomical tissue of the leaves of six dicot ornamental plants to had a thicker tissue (Table 1). According to Muhuria et al., (2006) high light intensity causes plants to adapt so that plants had thicker leaves. Sulistyaningsih et al., (1994) and Ramadhan and Hariyono (2019) said that the use of shade could reduce air temperature and increase humidity so that it could reduce the rate of transpiration. The shading reduces the intensityof light that will be received by plants. Hamdani et al., (2009) said that shading could result in the differences in the microclimate environment including light intensity, air temperature, soil temperature, and air humidity. The higher the level of the shading, the air temperature, soil temperature, and light intensity becomes lower, but the air humidity increases. Environmental factors indicated that the light intensity was higher in open areas. Meanwhile air humidity increased in shading areas for 2%. Observations showed that leaf thickness decreased in shading areas and thickened at high light intensity (Table 1).

Sirait (2008) said that the canopy of plants growing in shading conditions would receive a small amount of solar radiation as a result of which shade would affect the processes of photosynthesis, respiration, protein synthesis,hormone production, and translocation. Pantilu et al., (2012) said that differences in shade levels affected light intensity, temperature, and air humidity of the plant environment so that the intensity of light received by plants was different and affected the availability of light energy which will be converted into heat energy and chemical energy. Haryanti (2010) said that plant response would increase with increasing temperature and light intensity. Evans and Poorter (2001) said that if plants experienced low light stress, the plants would carry out a shade avoidance response in the form of maximizing light capture by changing the anatomy and morphology of the leaves for efficient photosynthesis, i.e. the leaves of shading plants became thinner and had a wider surface area. As a result leaves became wider. In a study conducted, leaf area and cells in leaf tissue had not been measured, but the visual appearance showed an increase in leaf area in shading areas. Therefore, further research is suggested to measure leaf area due to dilation of tissue cells that occurs in shading conditions. In addition, Chabot and Chabot (1977) said that increasing light intensity could cause leaf thickness, leaf density, and surface area of mesophyll cells as well as leaf surface area to increase.

In this study, Croton (*Codiaeum variegatum* (L.) A. Juss.), Bougenville (*Bougainvillea glabra* Choisy), Red Shoots (*Syzygium paniculatum* Gaertn), Firestorm (*Excoecaria cochinchinensis* Lour.) were able to adapt to shading areas by reducing the thickness of the leaf tissue, namely palisade tissue, spongy, and the lower epidermis so that the leaves became thinner. Meanwhile Crape Jasmine (*Tabernaemontana divaricata* (L.) R, Br. Ex Roem. & Schult.) and Erpah (*Aerva sanguinolenta* BI) plants adapt to shading areas by increasing the thickness of their leaves.

Table 3. Pattern of relationship between thickness total leaf tissue and palisade, spongy, and lower epidermis of six dicot ornamental plants

Species	**Croton**		**Erpah**		**Bougenville**		**Crape Jasmine**		**Red Shoots**		**Firestorm**	
Total thickness	**Thick**	→										**Thin**
Condition	**O**	**S**	**O**	**S**	**O**	**S**	**O**	**S**	**O**	**S**	**O**	**S**
Tissue												
PS	III	II	II	II	III	II	I	II	III	III	I	I
BK	III	III	III	III	I	I	II	II	II	I	II	II
EB	III	III	II	II	III	II	II	II	I	I	I	I

Note: O: open, S: shading, PS: palisade, BK: Spongy, EB: lower epidermis, I: thin, II: enough, III: thick.

The pattern of total leaf thickening was related to the lower epidermis particularly (Table 3). Shrinkage of palisade tissue, spongy, and lower epidermis was a plant's attempt to get optimal light in shading areas. The more shrinking lower epidermis the thiner the leaf thinckness became while the thickness of palisade and spongy varied so the thickness of total leaf from each species also varied (Table 3).

Conclusion

The leaf anatomy of six dicot ornamental plants varied. All plants had one layer of upper and lower epidermis tissue. In Croton (*Codiaeum variegatum* (L.) A. Juss.), Red Shoots (*Syzygium paniculatum* Gaertn), and Firestorm (*Excoecaria cochinchinensis* Lour.) have two layers of palisade tissue while in Bougenville (*Bougainvillea glabra* Choisy), Crape Jasmine (*Tabernaemontana divaricata* (L.) R, Br. Ex Roem. & Schult.) and Erpah (*Aerva sanguinolenta* BI) had one layer of palisade tissue. Meanwhile, the spongy tissue in all plants had one layer of tissue. The thickness of the leaf tissue decreased in the shading area along with the depletion of the palisade, spongy, and lower epidermis tissue as indicated by a correlation from moderately strong to very strong. Reduction of the palisade tissue could occur in a flatter shape or reduce its layers, such as Croton (*Codiaeum variegatum* (L.) A. Juss.) and Firestorm (*Excoecaria cochinchinensis* Lour.). Meanwhile, Red Shoots (*Syzygium paniculatum* Gaertn) still had two layers of palisade tissue but with a smaller size. Shrinkage of palisade tissue, spongy, and lower epidermis was a plant's attempt to get optimal light in shading areas. The shrinking lower epidermis the thinner leaf thickness became, while the thickness of palisade and spongy varied so the thickness of total leaf from each species also varied.

List of Abbreviations

The abbreviations used are defined in the text.

Data Availability

The data used are already available on the results and discussion.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Funding Statement

This research was partly funded by the DIPA of the Teaching Training and Education Faculty of Tanjungpura University in 2021 and partly using independent funds.

Author Contribution

The first author conducted the study and collected the data. The second author fasilitated funds and materials and together with other author wrote manuscript.

References

1. Aini, N., Setyati, D., dan Umiyah. 2014. Struktur Anatomi Daun Lengkeng (*Dimocarpus longan* Lour.) Kultiyar Lokal Ito, Pingpong dan Diamond River. Berkala Saintek 2(1): 31-35.
2. Berlyn, J.P., dan Mikesche, J.P. 1976. Micotechnique Cytochemishtry. Lowa State University Press, Lowa.
3. Campbell, N.A., dan Reece, J.B. 2008. Biologi Edisi 8, Jilid 1. Erlangga, Jakarta.
4. Chabot, B.F., dan Chabot, J.F. 1977. Effects of Light and Temperature on Leaf Anatomy and Photosynthesis in Fragaria Vesca. Oecologia 24(4): 363-377.
5. Coble, A.P., dan Cavaleri, M.A. 2017. Vertical Leaf Mass Per Area Gradient of Mature Sugar Maple Reflects Both Height-Driven Increases in Vascular Tissue and Light-Driven Increases in Palisade Layer Thickness. Tree Physiology 37: 1337-1351.
6. Costa, Y.O.D., dan Daningsih, E. 2022. Ketebalan Daun dan Laju Transpirasi pada Tanaman Hias Dikotil. Jurnal Ilmu Pertanian Indonesia (JIPI) 27(1): 40-47.
7. Daningsih, E., Mardiyyaningsih, A.N., Costa, Y.O.D., Primawati, R., dan Skarlina. 2022. Changes of Stomatal Distribution and Leaf Thickness in Response to Transpiration Rate in Six Dicot Plant Species. IOP Conference Series: Earth and Environmental Science 976: 1-10.

8. Ekawati, R., dan Aziz, S.A. 2016. Respon Pertumbuhan dan Fisiologi *Plectranthus amboinicus* (Lour.) Spreng pada Cekaman Naungan. Agrovigor 9(2): 82-89.
9. Evans, J.R., dan Poorter, H. 2001. Photosynthetic Acclimation of Plants to Growth Irradiance: The Relative Importance of Specific Leaf Area And Nitrogen Partitioning in Maximizing Carbon Gain. Plant Cell Environ 24: 755- 767.
10. Fiqa, A.P., Nursafitri, T.H., Fauziah, dan Masudah, S. 2021. Pengaruh Faktor Lingkungan Terhadap Pertumbuhan Beberapa Aksesi *Dioscorea alata* L. Terpilih Koleksi Kebun Raya Purwodasdi. Jurnal Argo 8(1): 25-39.
11. Hale, M.G., dan Orcutt, D.M. 1987. The Physiology of Plants Under Stress. John Wiley and Sons, New York.
12. Hamdani, J.S., Sumadi, Suriandinata YR, Martins L. 2016. Pengaruh Naungan dan Zat Pengatur Tumbuhan Terhadap Pertumbuhan dan Hasil Tanaman Kentang Kultivar di Daratan Medium. J. Argon. Indonesia 44(1): 33-39.
13. Haryanti, S. 2010. Pengaruh Naungan yang Berbeda Terhadap Jumlah Stomata dan Ukuran Porus Stomata Daun *Zephyranthes rosea* Lindl. Buletin Anatomi dan Fisiologi 18(1): 41–48.
14. Heyneke, E., Ebengreuth, N.L., Krajcer, I., Wolkinger, V., Müller, M., dan Zechmann B. 2013. Dynamic Compartment Specific Changes in Glutathione and Ascorbate Levels in *Arabidopsis* Plants Exposed to Different Light Intensities. BMC Plant Biology 13(1): 1-19.
15. Johasen, D.A. 1940. Plant Microtechnique. McGRAW-HILL BOOK COMPANY,Inc. New York and London.
16. Karyati, Ransun, J.R., dan Syafrudin, M. 2017. Karakteristik morfologis dan antomis daun tumbuhan herba pada paparan chaya berbeda di hutan Pendidikan Fakultas Kehutanan Universitas Mulawarman. Jurnal Agrifor 16(2): 243-256.
17. Levitt, J. 1980. Responses of Plantas to Enviromental Stresses. Ater, Vol. II. Academic Press, Inc, London.
18. Muhuria, L., Tyas, K.N., Khumaida, N., Trikoesoemaningtyas, dan Sopandie, D. 2006. Adaptasi tanaman kedelai terhadap intensitas cahaya rendah: karakter daun untuk efisiensi penangkapan cahaya. Bul. Agron 34(3): 133-140.
19. Nelza, A., Suharsi, T.K., dan Surahman, M. 2018. Karakter vegetatif kacang koro pedang (*Canavalia ensiformis* L.) pada kondisi naungan dan pemupukan yang berbeda. Jurnal Agroteknologi Universitas Andalas 2(2): 27-34.
20. Orchard, G.E., Torres, P., dan Sounthararajah. 2008. Use of Softening Agents to Improve the Production of Formalin-Fixed. Paraffin-Embedded Sections of Nail Tissue: An Assessment. British Journal of Biomedia Science 65(2): 68-70.
21. Paluvi, N., Mukarlina, dan Linda, R. 2015. Struktur Anatomi Daun, Kantung dan Sulur *Nepenthes gracilis* Korth. yang Tumbuh di Area Intensitas Cahaya Berbeda. Protobiont 4(1): 103-107.
22. Pantilu, L.I., Mantiri, F.R., Nio, S.A., dan Pandiangan, D. 2012. Respon morfologi dan anatomi kecambah kacang kedelai (*Glycine Max* (L.) Merill) terhadap intensitas cahaya yang berbeda. Bioslogos 2(2): 80-87.
23. Ramadhan, A.F., dan Hariyono, D. 2019. Pengaruh pemberian naungan terhadap pertumbuhan dan hasil pada tiga varietas tanaman stroberi (*Fragaria chiloensis* L.). Jurnal Produksi Tanaman 7(1): 1-7.
24. Riduwan dan Sunarto, H. 2007. Pengantar Statistika. CV Alfabeta, Bandung.
25. Samsuri T. 2013. Pengaruh Berbagai Intensitas Cahaya Terhadap Perubahan Struktur Anatomi Daun Tanaman Gaharu (*Gyrinops versteegii* (Gilg) Domke). Jurnal Ilmiah Biologi "Bioscientist" 1(1): 5-13.
26. Sirait, J. 2008. Luas Daun, Kandungan Klorofil dan Laju Pertumbuhan Rumput pada Naungan dan Pemupukan yang Berbeda. JITV 13(2): 109-116.
27. Stuessy, T.F. 1990. Plant Taxonomy: the Systematic Evaluation of Comparative Data. Columbia University Press, New York.
28. Sumardi, I., dan Pudjoarinto, A. 1992. Struktur dan Pengembangan Tumbuhan. Universitas Gajah Mada, Yogyakarta.
29. Sundari, T., Soematono, Tohari, dan Mangoendidjojo, W. 2008. Anatomi Daun Kacang Hijau Genotipe Toleran dan Sensitif Naungan. Bul. Agron 36(3): 221-228.

30. Tholen, D. 2012. Opining: Prospects for Improving Photosynthesis by Altering Leaf Anatomy. Plant Science 197: 97-101.
31. Tihurua, E.F., Astuti, I.P., dan Rugaya. 2012. Anatomi Helaian Daun *Murraya* SPP. (*Rutaceae*) di Jawa. Berita Biologi 11(3): 411-419.
32. Weaver, J.E., dan Clements, F.E. 1986. Plant Ecology. 2nd Edition. Tata McGraw-Hill Publishing Company, Ltd. New Delhi.
33. Widyastuti, T. 2018. Teknologi Budidaya Tanaman Hias Argribisnis. CV Mine, Yogyakarta.
34. Wu, Y., Wanzuo, G., dan Wenyu, Y. 2017. Shade Inhibit Leaf Size by Controlling Cell Proliferation and Enlargment in Soybean. Scientific Report 7: 1-10.
35. Wulansari, T.Y.I., Agustiani, E.L., Sunaryo, Tihurua, E.F., dan Widoyanti. 2020. Struktur Anatomi Daun sebagai Bukti Dalam Pembatasan Takson Tumbuhan Berbunga: Studi Kasus 12 Suku Tumbuhan Berbunga Indonesia. Buletin Kebun Raya 23(2): 146-161.