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Effect of Transpiration on the Monocot Ornamental Plants Leave Anatomy

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KEYWORDS

Transpiration

Leaf anatomy thickness

Palisade

Spongy

Shrinkage

ABSTRACT

Transpiration refers to the loss of water from leaves, and increased levels can lead to changes in leaf morphology and anatomy, affecting the total thickness. This study aims to determine the effect of transpiration on leaf anatomy, particularly thickness, in six types of monocots ornamental plants, namely *Rhoeo discolor* (L'Her.) Hance ex Walp., *Hymenocallis littoralis* (Jacq.) Salisb., *Cordyline fruticosa* (L.) A. Chev., *Chlorophytum laxum* R. Br, *Dracaena reflexa* Lam, and *Aglaonema commutatum* Schott. The study procedures were conducted using a Factorial Completely Randomized Design (Factorial CRD) with an experimental approach. The first factor was the type of plant, while the second was the condition before and after transpiration. The data obtained were analyzed using ANOVA, followed by LSD and Pearson correlation tests. The results showed that the plant type factor significantly affected the thickness of leaf tissues. The conditions before and after transpiration also significantly impacted all leaf tissues except for the lower epidermis. Furthermore, this finding was supported by the positive correlation between the thickness shrinkage of the upper epidermis-mesophyll and transpiration. The results also revealed that the mesophyll of *R. discolor*, *C. laxum*, *D. reflex*a, and *A. commutatum* differentiated into palisade and spongy layers, but there was no differentiation in the other two species. The transpiration rate was observed to change along with the specific anatomical structure of the leaf tissues. The lowest rate was found in *R. discolor* with thicker hypodermis tissue, while the highest was in *C. laxum* with thinner mesophyll.

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1 Introduction

Urban vegetation plays a crucial role in reducing the adverse effects of heat on the environment (Hsieh 2017; Rahman et al. 2020). Among the various vegetation types, ornamental plants are often found in urban areas due to their aesthetic appeal, which can positively impact people's minds. Furthermore, these plants produce oxygen-rich air and absorb CO_2, thereby improving air quality (Widyastuti 2018). These processes are greatly influenced by various physiological activities, such as transpiration (Williams et al. 2021; Thom et al. 2022), which is essential for growth, as it helps to regulate leaf and environmental temperatures (Salisbury and Ross 1995; Sundberg 1985; Gao et al. 2020). However, excessive transpiration can lead to issues such as wilting, drought, and death (Beckman, 1964), which diminishes the beauty of ornamental plants (Toscano et al. 2019) and change leaf anatomy (Boughalleb et al. 2014). This indicates that leaf anatomy can serve as a crucial indicator for assessing the water requirement of plants (Carr 2013) and determining the level of resistance to drought conditions.

Water loss from leaf tissue has been identified as the primary factor leading to changes in its thickness, as reported by Bachman (Burquez 1987). In a more recent study, Ningsih and Daningsih (2022) investigated the effect of transpiration on six monocot ornamental plants. The results showed alterations in the total leaf thickness of various species, including *Rhoeo discolor* (L'Her.) Hance ex Walp., *Hymenocallis littoralis H. littoralis* (Jacq.) Salisb., *Cordyline fruticosa* (L.) A. Chev., *Chlorophytum laxum* R. Br, *Dracaena reflexa* Lam, and *Aglaonema commutatum* Schott. Kutlu et al. (2009) also reported a similar finding in *Ctenanthe setos*, where the mesophyll thickness decreased under drought conditions. However, contradictory results have been reported by several studies. Such as Burnett et al. (2005) found that the leaf tissue of *Salvia splendens* remained unaffected by water shortage conditions. This suggests that different plants often develop distinct leaf anatomical adaptations to water shortages (Boughalleb et al. 2014). Meidner (1975) reported that the water from the leaves was sourced from the epidermis around the stomata, considering the proximity of the epidermal cells to the substomata cavity. However, Fahn (1995) and Onoda et al. (2015) reported that the epidermis consistently exhibited higher density and rigidity to form a compact layer without intercellular spaces. Epidermal cell walls tend to be thick, accounting for 10-20% of the total volume of leaf cells (Crang et al., 2018). The cuticle, which exists in the outer and inner layers of the epidermis, plays a vital role in preventing water loss (Wullschlenger and Oosterhius 1989). It primarily consists of cutin, an insoluble substance that acts as a barrier to water passage (Beck 2010). As an alternative adaptive mechanism, the epidermis can also thicken its walls to prevent water loss (Nawazish et al. 2006; Hameed et al. 2012; Boughalleb et al. 2014).

According to Taiz et al. (2018), most vapour leaving the leaves originates from the mesophyll tissue. This finding is consistent with Kutlu et al. (2009) and Kapchina-Toteva et al. (2014), who reported that water shortage could be affected by the mesophyll cell shrinking and reducing the number of layers. The flexibility of this tissue and the absence of cuticles can also increase the rate of water loss (Onoda et al. 2015; Crang et al. 2018). The flexibility of the mesophyll is attributed to its elastic properties, which allow it to adjust its thickness. This property is also closely related to the plant cell wall, which regulates thickness. In drought conditions, a decrease in cell size often leads to an increase in elasticity, as Martinez et al. (2007) and Xing et al. (2022) reported.

Several studies have shown that the mesophyll tissue in some plants is differentiated into two layers: palisade and spongy (Hidayat and Niksololihin 1995). The palisades contain a higher number of chloroplasts compared to the spongy cells. Therefore, previous studies have inferred that they have a higher water content due to their association with photosynthesis (Outlaw et al. 1976; Cutler et al. 2007). Further, Boughalleb et al. (2014) and Han et al. (2016) also suggested that mesophyll often experiences shrinkage under drought conditions. According to Canny et al. (2012) and Xing et al. (2022), water loss is more pronounced in the spongy part compared to the palisade.

Ningsih and Daningsih (2022) conducted a study on six types of monocot ornamental plants, and the results showed an association between leaf thickness and transpiration, but the role of shrinking tissues was unknown. The anatomical arrangement and thickness of the six types of plants provided by Daningsih et al. (2022) served as a foundation for further studies. Therefore, this study aims to examine the effect of transpiration on the leaves' anatomy.

2 Materials and Method

2.1 Time and Place

This study was conducted in a simple plant house at the Biology Education Laboratory, Faculty of Teacher Training and Education, Tanjungpura University, Indonesia, from February to September 2022.

2.2 Tools and Materials

The tools used in this study included Olympus-CX 21 microscope, C-1 objective micrometer, Optilab Advance SN: MTN004210791, vials, Euromax rotary microtome, thermometer, lux meter AS803, GM816 anemometer, HTC-2 hygrometer, measuring cup, Ohaus analytical balance, Nikon D5200 DLSR camera, *ImageJ* software, and *Image Raster 3* software. Meanwhile, the materials were vaseline, FAA solution, alcohol, safranin 2%, tert-Butyl alcohol, paraffin oil, fast green 0.5%, xylol, paraffin, Haupt's adhesive, formalin 3%, and distilled water. A total of six selected monocot ornamental plants samples were utilized for slide preparation, including Boat Lily (*Rhoeo discolor*), Spider Lily (*Hymenocallis littoralis*), Cabbage Palm (*Cordyline fruticosa*), Bichetti Grass (*Chlorophytum laxum*), Song of India (*Dracaena reflexa*), and Philippine Evergreen (*Aglaonema commutatum*).

2.3 Method and Research Design

This study used a quantitative experimental method with a Completely Randomized Design Factorial (CRD-Factorial) approach. Furthermore, it determined the effect of various treatments on the thickness of leaf tissue. The first factor was the types of ornamental plants, while the second was the condition before and after transpiration. The combination factor was the interaction of species type and conditions with three replications. In this study, the sample population consisted of 36 plant samples.

2.4 Plant Preparation

The plants used in this study had the same size and almost the same number of leaves. The samples were transferred to a homogeneous planting medium consisting of burnt soil and poor sand in a ratio of 2:1, which were placed in polybags measuring 30 x 35 cm. The plants were watered daily, and the NPK fertilizer was given once every two weeks. After two weeks of adaptation, samples were taken from the planting medium for further analysis.

2.5 Transpiration Measurement

Transpiration rate were measured using modified gravimetric method refered to Ningsih and Daningsih (2020). Meanwhile, the leaf area was measured using the *ImageJ* program proposed by Reinking (2007). The transpiration rate was calculated using the formula below:

$$\text{Transpiration Rate} = \frac{\text{initial weight (gr)} - \text{final weight (gr)}}{\text{time (hours)x leaf area(cm}^2\text{)}}$$

2.6 Leaf Transverse Incision Preparations

Transverse leaf incisions were made using a modified Johansen (1940) paraffin method. The modification was made in several steps, including the thickness of the incision (Sass 1951), the treatment of the paraffin tape after cutting, the adhesive preparations (Berlyn and Miksche 1976), and the use of fabric softener (containing *Diethylester Dimethyl Ammonium Chloride*) on hard paraffin blocks (Orchard et al. 2008). Samples were taken in the basal position under two conditions: before and after transpiration. Although the leaves selected for the pre and post-transpiration measurements differed, they were close to each other. It was assumed the leaves were of the same age and had received comparable amounts of nutrients, light, and water. During the investigation, the leaf samples were sliced into 1 x 1 cm sections using a razor, following the direction of the main veins, without including them. The monocot samples were taken at 9 AM (Jakarta time) before transpiration, followed by sampling after measurements. The leaves obtained before and after transpiration were soaked in FAA fixative solution for 24 hours. The process was continued with preparation, which consisted of washing, clearing, infiltration, and embedding. Subsequently, the samples were cut into a thickness of 12 µm using a microtome. The incisions were glued to the slide using Haupt's adhesive, placed in an oven at 38^{o}C for 12 hours, and stained using safranin-fast green double stain.

2.7 Measurement of leaf anatomical thickness

Leaf anatomy was observed under an Olympus C-X 21 microscope with 100x magnification, and the images observed were captured with Optilab Advance. Furthermore, the leaf anatomy thickness was measured using Image Raster 3 Software, focusing on the upper epidermis and hypodermis, mesophyll (palisade & spongy), lower epidermis, and total thickness.

2.8 Measurement of External Factor

The external factors of this study were measured using various tools. For example, a thermometer measured the temperature, while air humidity was assessed using the HTC-2 hygrometer. Furthermore, the wind speed was calculated using the GM816 anemometer, and light intensity was measured with the AS803 lux meter.

2.9 Statistical Analysis

Data on the thickness of leaf tissue anatomy were analyzed using an ANOVA test, which determined the effect of the treatment. Subsequently, further analysis was carried out using the Least Significance Different (LSD) test at the 5% level to determine the difference among all treatments. The process was then continued with the Pearson correlation test to determine the relationship between the transpiration rate and the difference in leaf tissue anatomy before and after transpiration. The classification of the correlation coefficient was carried out based on the method proposed by Amruddin et al. (2022).

3 Result

3.1 Transpiration Rate of Six Monocot Ornamental Plants

The transpiration rate data obtained in this study were subjected to normality and homogeneity tests. The results showed that the data had normal distribution ($p>0.05$) and homogeneity ($p>0.05$). Based on the ANOVA results, the transpiration rate differed significantly from one plant to another ($p<0.05$). The highest rate was obtained

Table 1 The average difference in the transpiration rates of six types of monocot ornamental plants

Plant Types	Average of Transpiration Rate ($gr.cm^{-2}hour^{-1}$)
Bichetii Grass (*C. laxum*)	0.00963^{a}
Philippine Evergreen (*A. commutatum*)	0.00484^{b}
Cabbage Palm (*C. fruticosa*)	0.00374^{bc}
Spider Lily (*H. littoralis*)	0.00262^{cd}
Song of India (*D. reflexa*)	0.00259^{d}
Boat Lily (*R. discolor*)	0.00187^{d}

Note: The difference in letters behind the numbers shows a significant difference in the LSD test (α=0.05).

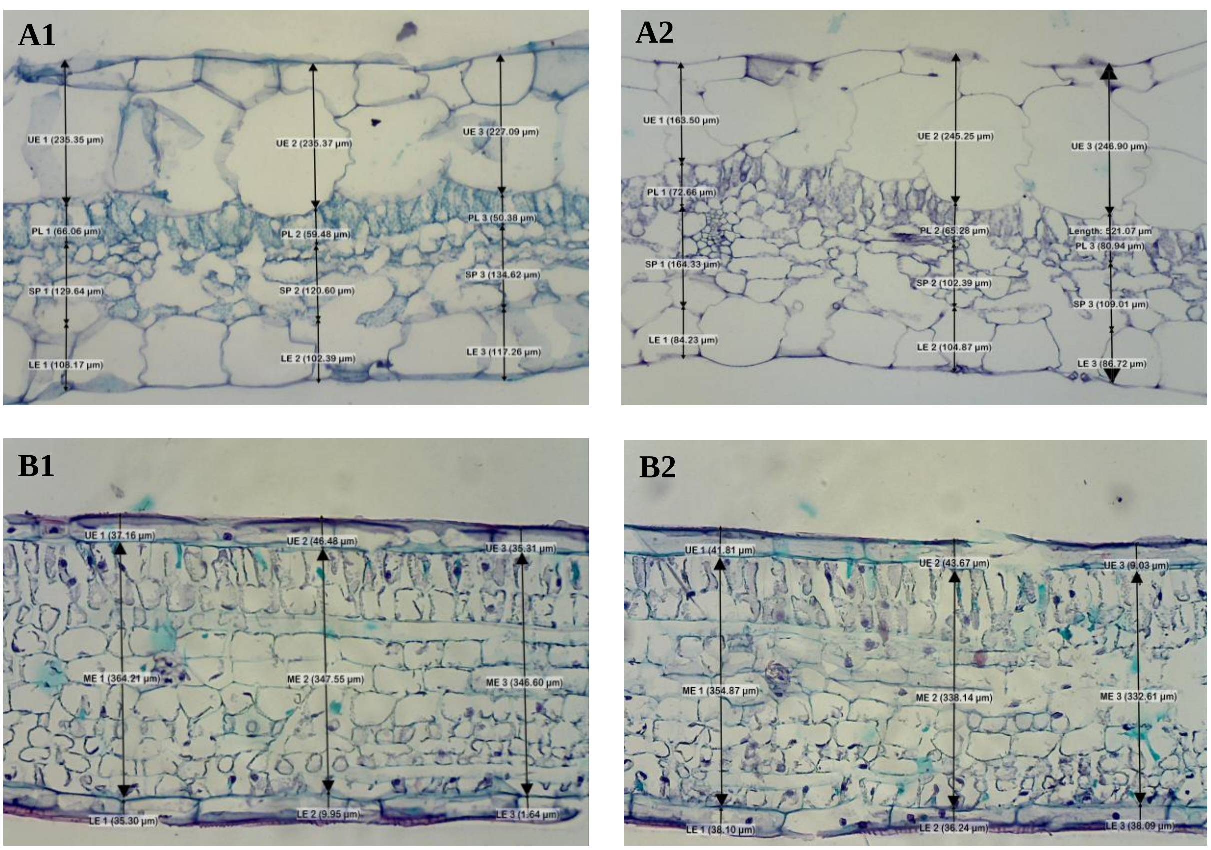
in the Bichetii Grass (*C. laxum*), while the lowest was found in Boat Lily (*R. discolor*). The results of various plants' transpiration rates recorded in this study are presented in Table 1.

Based on Table 1, the transpiration rates of Bichetii Grass were significantly different from other plants. Meanwhile, the Philippine Evergreen and Cabbage Palm had no significant difference. Similarly, Cabbage Palm and Spider Lily do not show any significant difference. The results showed that Spider Lily exhibited high similarity with the song of India and Boat Lily. These measurements were carried out at an average temperature of 35^{o}C, with air humidity level, wind velocity, and light intensity of 49%, 0.367 $m.s^{-1}$, and 103.5901 $watts.m^{-2}$, respectively.

3.2 Leaf Anatomy of Six Monocot Ornamental Plants

The leaf anatomy of six types of monocot ornamental plants consisted of the upper epidermis and hypodermis, mesophyll (palisade and spongy), and lower epidermis, as shown in Figure 1.

The current study documented that the upper and lower epidermis of the six monocot ornamental plants consisted of one-layer tissue with a rectangular and tight arrangement. However, the Boat Lily had an additional tissue under the upper epidermis, known as the hypodermis. The observation showed that the upper epidermis tended to be thicker than the lower epidermis. In some plants, such as Spider Lily and Cabbage Palm, these tissues were almost the same



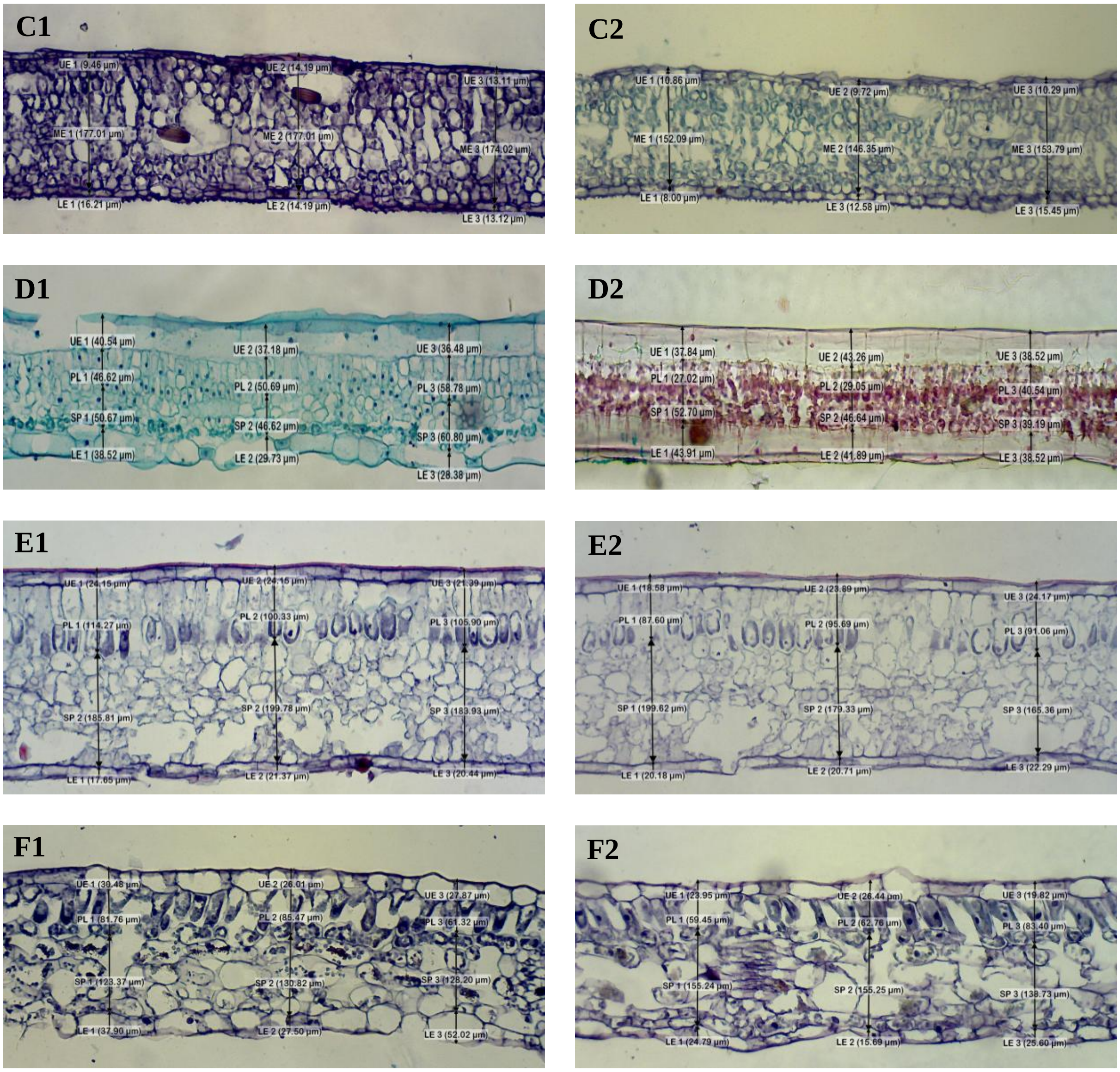


Figure 1 Leaf anatomy of six types of monocot ornamental plants, before transpiration (1), after transpiration (2), Boat Lily (*R. discolor*) (A), Spider Lily (*H. littoralis*) (B), Cabbage Palm (*C. fruticosa*) (C), Bichetii Grass (*C. fruticosa*) (D), Song of India (*D. reflexa*) (E), Philippine Evergreen (*A. commutatum*) (F).

size. This study also found that the mesophyll in Spider Lily and Cabbage Palm leaves were similar (homogeneous) in terms of the palisade and spongy segment. The undifferentiated mesophyll was due to the chlorenchymatous cells containing several chloroplasts. Meanwhile, the four other plants' mesophyll showed significant structure, shape, and arrangement differences. The palisade leaves of Boat Lily and Bichetii Grass were composed of one dense layer with a tubular shape. In Philippine Evergreen, one loosely arranged layer was observed. The palisade of Song of India was composed of two layers, which tended to be dense. The spongy tissue of Song of India, Boat Lily, and Philippine Evergreen showed larger and denser intercellular spaces compared to Bichetii Grass.

3.3 ANOVA Test Results for Leaf Tissue Anatomical Thickness

The normality test showed that all data were normally distributed ($p>0.05$). However, data on the total, mesophyll, and palisade thickness ($p<0.05$) were not homogeneous. The Factorial-CRD results using the ANOVA test showed that the plant type factor had a significant effect ($p<0.05$) on all leaf tissue. Furthermore, the condition factor had a significant effect ($p<0.05$), except in the lower epidermis. The results showed that the interaction between plant species and conditions has a significant impact ($p<0.05$) on the upper epidermis. All the leaf tissue responded differently after transpiration except the lower epidermis. Before and after

Table 2 ANOVA results of leaf thickness of six monocot ornamental plants

Factor	Average of leaf thickness (µm)					
	TT	UE	LE	ME	PL	SP
Plant Types	*	*	*	*	*	*
Boat Lily (*R. discolor*)	535.171[a]	234.087[a]	118.800[a]	182.204[d]	58.762[b]	123.425[c]
Spider Lily (*H. littoralis*)	431.266[b]	39.154[c]	38.594[b]	343.518[a]	-	-
Cabbage Palm (*C. fruticosa*)	182.152[e]	14.254[e]	12.527[c]	155.371[e]	-	-
Bichetii Grass (*C. laxum*)	174.900[e]	47.276[b]	39.274[b]	88.455[f]	39.337[c]	47.835[d]
Song of India (*D. reflexa*)	321.342[c]	24.892[d]	17.841[c]	278.609[b]	97.694[a]	180.916[a]
Philippine Evergreen (*A. commutatum*)	255.211[d]	28.662[d]	27.114[bc]	199.435[c]	61.279[b]	138.156[b]
Condition	*	*	ns	*	*	*
BeforeTanspirasi (BT)	327.727[a]	66.658[a]	42.810	218.290[a]	68.097[a]	129.341[a]
After Transpirasi (AT)	305.620[b]	62.784[b]	41.933	200.903[b]	60.439[b]	116.469[b]
Combination of plant types and condition	ns	*	ns	ns	ns	ns
BT- Boat Lily (*R. discolor*)	552.259	243.047[a]	119.360	189.887	61.159	128.692
AT- Boat Lily (*R. discolor*)	518.047	225.127[b]	118.399	174.521	56.364	118.157
BT- Spider Lily (*H. littoralis*)	437.076	38.811[e]	39.965	358.299	-	-
AT- Spider Lily (*H. littoralis*)	425.457	39.497[d]	37.223	348.737	-	-
BT- Cabbage Palm (*C. fruticosa*)	189.564	14.360[g]	13.516	161.688	-	-
AT- Cabbage Palm (*C.fruticosa*)	174.739	14.148[g]	11.537	149.053	-	-
BT- Bichetii Grass (*C. laxum*)	181.514	48.114[c]	38.855	94.754	42.679	52.087
AT- Bichetii Grass (*C. laxum*)	165.286	46.437[c]	39.693	82.155	35.996	43.583
BT- Song of India (*D. reflexa*)	342.839	26.778[ef]	26.692	297.589	105.762	191.826
AT- Song of India (*D. reflexa*)	299.846	23.006[f]	27.536	259.630	89.626	170.004
BT- Philippine Evergreen (*A. commutatum*)	263.078	28.837[e]	18.472	207.546	62.788	144.758
AT- Philippine Evergreen (*A. commutatum*)	247.348	28.488[e]	17.209	191.323	59.770	131.553

Note: *= Significant on (α=0.05), ns= Non-significant, TT= Total Thickness, UE= Upper Epidermis, LE= Lower Epidermis, ME= Mesophyll, PL= Palisade, SP= Spongy, BT= Before Transpiration, and AT= After Transpiration. The difference in letters behind the numbers indicates a significant difference in the LSD test (α=0,05).

transpiration, the interaction factor between species and condition only influenced the upper epidermis, as shown in Table 2.

3.4 Correlation Between Anatomical Shrinkage Thickness of Leave and the Transpiration Rate

This study examined the shrinkage in leaf thickness, encompassing the upper epidermis, mesophyll, palisade, spongy tissue, and total anatomical thickness of the six monocot ornamental plants. Table 3 presents a range of results, varying from no correlation to strong correlation.

The result showed that each plant had different anatomical thicknesses due to the varying response to transpiration. This served as a criterion for selecting plants more resistant to drought. Furthermore, the correlation coefficient showed a mixed relationship between the shrinkage of leaf anatomical thickness and the transpiration rate. This indicated the tendency that the response was caused by plant genetic characteristics rather than transpiration.

3.5 The Potential Relationship of Transpiration Rate and Thickness Shrinkage of Leaf Tissue

The data explained in the previous section were used for mapping the relationship between the shrinkage of anatomical tissue thickness and the transpiration rate. Table 4 shows the pattern of specific anatomical shrinkage with the transpiration rate (from high to low).

Table 3 Correlation between the shrinkage of leaf tissue anatomical thickness and the transpiration rate on six types of monocot ornamental plants

Plant Types	Transpiration	Leaf Anatomical Thickness Correlation Coefficient Value				
		TT	UE	ME	PL	SP
Boat Lily (*R. discolor*)	2.00929×10^{-3}	0.711	0.935	0.596	0.005	0.680
		strong	very strong	moderate	uncorrelated	strong
Spider Lily (*H. littoralis*)	2.62796×10^{-3}	0.166	0.613	0.993	-	-
		very weak	strong	very strong		
Cabbage Palm (*C. fruticosa*)	3.74401×10^{-3}	0.924	0.672	0.936	-	-
		very strong	strong	very strong		
Bichetti Grass (*C. laxum*)	9.63219×10^{-3}	0.999	0.695	0.998	0.705	0.932
		very strong	strong	very strong	strong	very strong
Song of India (*D. reflexa*)	2.59619×10^{-3}	0.999	0.256	0.806	0.875	0.245
		very strong	very strong	strong	strong	Very weak
Philippine Evergreen (*A. commutatum*)	4.84362×10^{-3}	0.949	0.458	0.942	0.057	
		very strong	weak	very strong	uncorrelated	very strong

Note: TT= Total Thickness, UE= Upper Epidermis, ME= Mesophyll, PL= Palisade, and SP= Spongy.

Table 4 Potential relationship between transpiration rate and shrinkage of leaf tissue thickness on six types of monocot ornamental plants.

Transpiration Rate	High →			Low
Leaf Tissue Shrinkage	SP (I)	SP (I)	SP (II)	SP (I)
	PL (I)	PL (II)	PL (I)	PL (II)
The thickness of UE&LE	Medium	Thin	Thin	Thick
Species	*Chlorophytum laxum*	*Aglaonema commutatum*	*Dracaena reflexa*	*Rhoeo discolor*

Note: PL=Palisade, SP= Spongy, UE= Upper Epidermis, LE=Lower Epidermis. I= very strong-strong, II= uncorrelation-moderate.

Based on Table 4, the palisade's and spongy tissue's thickness impacted leaf shrinkage due to transpiration. The results showed that the transpiration rate increased following a decrease in the thickness of the spongy and palisade tissue, as observed in Bichetti Grass. In this study, Bichetti Grass strongly correlated with the shrinkage of the upper epidermis, palisade, and spongy. This characteristic indicated that its tissue could not withstand the transpiration rate, leading to massive water loss. Based on these findings, it was concluded that the species cannot live in water shortage conditions. However, lower transpiration occurred when only one of the tissues experienced high shrinkage, such as in the Philippine Evergreen and Song of India. The transpiration rate was also lower when the upper and lower epidermis was thick, such as in Boat Lily.

Discussion

Transpiration involves the process of releasing water from leaves into the air in the form of vapour. In plants, this process was often controlled by stomata cells and was aimed at achieving energy (Hopkins and Huner 2009; Kocchar and Gujral 2020). Furthermore, the plants with the highest and lowest transpiration rates among the six types of monocot ornamental plants had the same sequence pattern as the findings of Ningsih and Daningsih (2022). The highest rate was found in Bichetti Grass, while the Boat Lily had the lowest (Table 1). The four other plants showed varying veining patterns compared to Ningsih and Daningsih (2022). This difference was influenced by internal and external factors (Salisbury and Rose 1995). The internal factors included plant variations (Wang et al. 2022) and leaf thickness (Giulani et al. 2013), while the external were temperature (Yang et al. 2012; Sugiarto et al. 2020), wind speed (Kuiper 1961; Schymanski and Or 2015), humidity (Tullus et al. 2012), and light intensity (Park et al. 2020).

Ningsih and Daningsih (2022) measured the transpiration rate at a temperature of 32°C, with 20.30 watts.m^{-2} light intensity, 42% humidity, and 1 m.s^{-1} wind speed. Furthermore, the external factors' value was lower than the current study, except for the wind speeds. The transpiration rate obtained in this study was lower than that of Ningsih and Daningsih (2022). This difference was caused by the high temperature, which led to the closure of the stomata (Schulze et al. 1973). This finding was consistent with Slot and Winter (2017) that the stomata often closed at an average temperature of 30^{o}C. Lugassi et al. (2015) also reported that high light intensity could lead to the closure of the organ, thereby causing a decrease in transpiration. This phenomenon occurs in citrus plants which are expressed with *Arabidopsis* hexokinase. The closing of the stomata has long been known as a plant's adaptation strategy to reduce excessive transpiration (Agurla et al. 2018). Therefore, it could be assumed that the transpiration rate depended on the environmental conditions. In this study, Bhicetti Grass had the highest rate, followed by Philippine Evergreen, Cabbage Palm, Spider Lily, Song of India, and Boat Lily.

Several studies reported transpiration's association with leaf anatomy (Giulani et al. 2013; Da Costa and Daningsih 2022; Daningsih et al. 2022; Ningsih and Daningsih 2022). According to Cutler et al. (2007), the anatomical structure of leaves was generally the same, consisting of the epidermis, mesophyll, and vascular tissue. However, each species had distinctive features despite belonging to the same group (Budel et al. 2017). These variations were dominantly influenced by genetics (Olsen et al. 2013), leaf position, and leaf age (Xie and Luo 2003). Environmental factors (Cassola et al. 2019), including light intensity (Coble and Cavelari 2017), temperature (Von Caemmerer and Evans 2014), humidity, and water content affecting stomata (Fanourakis et al. 2013) have been reported to also contribute to these variations. The differences in leaf anatomical structure could be used as a basis for species identification (Mabel et al. 2014; Budel et al. 2017; Ozcan et al. 2015; Bahadur et al. 2018; Chatri et al. 2020; Da Costa Santos et al. 2020), as well as an essential indicator for determining plant adaptation to different habitats (Carr 2013; Bertel et al. 2016; Oguchi et al. 2018).

The six types of monocot ornamental plants showed varying leaf characteristics, consistent with Wang et al. (2022), that each species had distinctive features. According to Wang et al. (2022) the 6 test plants were unique, as exemplified by Boat Lily with hypodermis tissue. The observation results showed that the mesophyll of Boat Lily, Bichtti Grass, Song of India, and Philippine Evergreen differentiated into palisades and spongy layers. Meanwhile, mesophyll was not differentiated in the Spider Lily and Cabbage Palm, as shown in Figure 1. The variation in leaf features was likely influenced by the genetics of each species (Coneva and Chitwood 2018).

Six monocot ornamental plants' upper and lower epidermis comprised a single layer of dense tissue. They were typically rectangular, except for the upper epidermis of Boat Lily, which was equipped with a hypodermis (Figure 1). The result presented in Table 2 indicated that the samples' upper epidermis was thicker than the lower epidermis. According to Tihurua et al. (2020), a thick epidermis could reduce the transpiration rate, thereby preventing changes in thickness. Boughalleb et al. (2014) also found that the thickness in the A*stragalus gombiformis* Pomel plant did not change due to water outflow (Fahn 1995; Becraft 1999; Cutler et al. 2007; Kutschera 2008; Crang et al. 2018).

This study showed a significant difference in the epidermal thickness of six monocot ornamental plants between the pre and post-transpiration conditions (Table 2). Based on the results, there were no changes in the thickness of the upper epidermis of Cabbage Palm, Bichetti Grass, Song of India, and Philippine Evergreen due to the rigidity of the cells. However, the differences in this parameter and the effects of transpiration were more visible in Boat Lily and Cabbage Palm. These variations were indicated by the combined LSD shown in Table 2. The difference in the thickness of the upper epidermis was probably caused by the different adaptations in some plants, specifically Cabbage Palms, which experienced thickening due to transpiration. The observation results showed that the Boat Lily experienced shrinkage due to the reduction in the thickness of the hypodermis.

The hypodermis was typically a colourless tissue with the primary function of storing water and was located in the upper (adaxial) or lower (abaxial) epidermis (Das 1999; Nurida et al. 2012). It was often found in plants inhabiting aqueous or halophyte habitats (Cutler 2007; Rashid et al. 2020; Tihurua et al. 2020), as well as minimal water areas (xerophytes) (Cutler 2007; Griffiths and Male 2017; Hafiz et al. 2013). Although the hypodermis served as a storage area, its water content could still diffuse out, leading to a decrease in thickness. In this current study, the thickness of the hypodermis decreased along with a reduction in that of the upper epidermis caused by transpiration (Table 2). Kutlu et al. (2009) found that the hypodermis, located under the upper epidermis of *Ctenanthe setosa*, had the flexibility to reduce its size under minimal water conditions, leading to the curling of the leaves in some cases.

The measurement of the upper epidermis thickness of the Boat Lily was not carried out separately in this study. Future studies should perform separate measurements of the upper epidermis and hypodermis tissue to determine the reduction level. The upper epidermis of the Boat Lily did not change significantly after transpiration, except for the hypodermis layer. Furthermore, the hypodermis experienced a reduction in size due to transpiration. Under these conditions, the shrinkage could lead to a decrease in the upper epidermis of Bichetti Grass. The bottom epidermal tissue of the six types of monocot ornamental plants was visually thin compared to the upper epidermis. The results also showed non-significant differences between the conditions before and after transpiration, as shown in Table 2. The absence of differences revealed that the epidermal tissue was composed of rigid and stiff cells. Therefore, the lower epidermis only experienced a slight reduction and did not differ much between both conditions. Several studies have also reported that this tissue layer could protect the component above it (Beck 2010).

Table 2 shows some significant changes in the mesophyll of the six monocot ornamental plants before and after transpiration. Galmes et al. (2013) stated that changes in mesophyll were some of the strategies of plants to adapt to water availability. Based on morphology, the leaves of Bichetii Grass were curved after exposure to high temperatures and light with a lack of water. This indicated that the plant could not adapt well to water drought conditions. According to Boughalleb et al. (2014) and Han et al. (2016), mesophyll could experience shrinkage due to the occurrence of transpiration. Zhang et al. (2014) and Vastag et al. (2020), changes in the thickness of the tissue were caused mainly by the accumulation of water. Consequently, the mesophyll became thicker compared to others (Table 2). The tissue also had an elastic ability that caused changes in its size due to the cellular component and a shift in the balance between the evaporation rate and water supply (Canny and Huang 2005; Xing et al. 2022). A previous study reported that a size reduction could also occur due to decreased intercellular space (Canny and Huang 2005; Kutlu et al. 2009).

Four monocot ornamental plants, including Boat Lily, Bichetti Grass, song of India, and Philippine evergreen had mesophylls that differentiated into palisades and spongy tissues. Meanwhile, the other two plants, spider lily and Cabbage Palm, were undifferentiated. Monocots commonly had undifferentiated mesophylls, and this was consistent with the finding of El-Gawad and El-Amier (2017) on three species, including *Arundo donax*, *Pennisetum setaceum*, and *Saccharum Spontaneum*. However, some of the plants could have differentiated mesophylls palisade. According to Sumardi and Wulandari (2010), *Musa* spp., a monocot plant, had a leaf anatomy consisting of palisade and spongy tissues with numerous chloroplasts. Similar results were also obtained by Kocyigit et al. (2023) in *Allium sphaeronixum*. Cutler et al. (2007) highlighted that undifferentiated mesophyll was commonly found in the monocot species, with few exceptions.

The results showed a relationship between the size or the number of mesophylls layer and the transpiration rate (Table 3). The Boat Lily had the lowest transpiration rate among the six plants due to its unique anatomical structure. The hypodermis, located between the upper epidermis and the palisade, was only found in Boat Lily, suggesting that this extra layer was the cause of the low rate (Figure 1). The results showed that the thicker the mesophylls, the lower the level of transpiration, as observed in undifferentiated and differentiated plants. The spider lily had the thickest mesophylls (343.518 µm, Table 2) and showed the lowest rate.

Species with differentiated mesophylls consisting of two palisade layers, including Bichetti Grass and Song of India showed lower transpiration rates than those with one layer, such as Philippine evergreen, as shown in Figure 1. Palisade was usually composed of compact and dense cells and appeared as the densest part of the mesophyll tissue on visual observation (Cutler et al. 2007). Several studies have shown that this tissue's thickness could help prevent water loss (Ashton and Berlyn 1992; Oliveira et al. 2018), leading to resistance to environmental changes. In this study, the palisade tissue in the Song of India was composed of two thicker layers, leading to lower transpiration rates (Figure 1; Table 1). Meanwhile, its looseness in the Philippine evergreen caused a higher transpiration rate than India's song. These phenomena indicated that thick palisade tissue played a role in resisting evaporation, but transpiration could also occur due to the spacing between the palisade cells, as shown in Figure 1 and Table 1. This was evidenced by the significant shrinkage experienced after transpiration in four types of monocot ornamental plants. According to Kapchina-Toteva et al. (2014), environmental differences could affect the number of palisade layers. In this study, the four monocot ornamental plants did not show any change in the number of palisade layers because they grew in the same environment.

Four of the six monocot ornamental plant types showed irregular spongy tissue forms (Figure 1). Spongy tissue had a wide extracellular space, accommodating more H_2O and CO_2 (Xing et al. 2022), which played an important role in the physiological processes in leaves (Salisbury and Ross 1995). According to Canny et al. (2012), it experienced higher water loss levels than Palisades. Furthermore, Kapchina-Toteva et al. (2014) found a reduction in spongy thickness in *Lamium album* L. plants, which were grown under different conditions, but the structure did not change. In this study, the spongy thickness of the four types of monocot ornamental plants before and after transpiration showed a significant decrease, indicating the loss of water (Table 2). Canny et al. (2012) stated that the shrinkage of this tissue could occur due to a reduction in the spaces between the cells.

Bichetti Grass visually had the thinnest spongy thickness, with denser inter-cell spaces compared to Boat Lily, song of India, and Philippine evergreen (Figure 1). Although the spaces between the spongy cells in Bichetti Grass were denser, the transpiration rate was significantly higher than in other plants (Table 1). Bichetti Grass contained water in its palisade tissue and spongy cells. Consequently, the water discharge from the leaves caused shrinkage of the two tissues, as indicated by the total mesophyll thickness, which was thinner than other species. Bichetti Grass had the thinnest total thickness compared to other plants (Table 2).

According to Buckley et al. (2015), reducing the leaves' total thickness could increase the hydraulic conductivity outside the xylem. Similar results were reported by Rahman et al. (2020) in species with thin leaves and simple shapes. The shape indicated the presence of higher water loss compared to species with thicker or more compound leaves. This study also found a similar condition, specifically in Bichetti Grass, where the plants had a high transpiration rate due to thinner leaves. A total of types of monocot ornamental plants, namely spider lily and Cabbage Palm, had mesophyll that was not differentiated into palisades and spongy (Figure 1). Spider lily have thicker and denser mesophyll than Cabbage Palm, which has dense but thin tissue. Consequently, the transpiration rate was lower than Cabbage Palm, as shown in Figure 1. According to Bosabalidis and Kofidis (2002), denser mesophyll cells could reduce the transpiration rate.

The leaf anatomy thickness changes significantly impacted by reducing the total thickness (Figure 1; Table 2). According to Osakabe et al. (2013), a reduction in leaf thickness indicated the loss of water from the cells, leading to a decrease in turgor pressure, softening, and a decrease in size. The correlation test results showed a mixed relationship between shrinkage in leaf anatomical thickness and transpiration (Table 3).

Each plant had various leaf anatomical characteristics and could adapt to different environmental habitats (Bertel et al. 2016). Plants with thick leaves could adapt better in environments exposed to sunlight than compared to others (Zwieniecki and Boyce 2014). Based on previous studies, plants with narrow and thick leaves, such as Boat Lily adapted to their habitat by forming a thicker epidermis. The leaves were also equipped with a hypodermis below the upper epidermis, leading to low transpiration rates. This indicated that the Boat Lily was more drought-resistant. Furthermore, the presence of the hypodermis played a role in storing water when the Boat Lily experienced drought. Boat Lily and Song of India also had the potential to resist environmental changes, as indicated by the two layers of palisade and low transpiration rate.

Conclusion

Transpiration caused leaf anatomy shrinkage in six monocot ornamental plants, specifically in the mesophyll tissue. The upper epidermis generally did not experience changes, but shrinkage occurred in the hypodermis of Boat Lily. The results showed that transpiration increased along with shrinkage of the palisade and spongy, such as in Bichetti Grass. However, lower rates were observed when only one of the tissues between the palisades or spongy experienced reduction, as shown in the song of India, Philippine evergreen, and Boat Lily, which experienced a decrease in the hypodermis. Among the six monocotyledon ornamental plants, the leaves of Boat Lily were more adaptive and resistant to growing in sunlight and water-deficient conditions than Bichetti Grass.

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Conflict of Interest

The authors declare that there is no conflict of interest in this study.

References

Agurla, S., Gahir, S., Munemasa, S., Murata., & Y., Raghavendra, A.S. (2018). Mechanism of Stomatal Closure in Plants Exposed to Drought and Cold Stress. *Advances in Experimental Medicine and Biology*, *1081*, 215-232. DOI: 10.1007/978-981-13-1244-1_12.

Amruddin, Priyanda, R., Agustina, T.S., Ariantini, N.S., et al. (2022). *Metode Penelitian Kuantitatif*. Pradina Pustaka.

Asthon, P.M.S., & Berlyn, G.P. (1992). Leaf Adaptation of Some *Shorea* Species to Sun and Shade. *New Phytologist Foundation*, *121*(4), 587-596. DOI: https://doi.org/10.1111/j.1469-8137.1992.tb01130.x.

Bahadur, S., Ahmad, M., Zafar, M., Sultana, S., et al. (2018). Palyno-Anatomical Studies of Monocot Taxa and its Taxonomic Implications Using Light and Scanning Electron Microscopy. *Microscopy Research Technique*, *82*(4), 1-21. DOI: 10.1002/jemt.23179.

Beck, C. B. (2010). *An Introduction to Plant Structure and Development Plant Anatomy for the Twenty-First* (2nd ed). Cambridge University Press.

Beckman, C. H. (1964). Host Responses to Vascular Infection. *Annual Review of Pgytopathology*, *2*, 231-252. DOI: https://doi.org/10.1146/annurev.py.02.090164.001311.

Becraft, P.W. (1999). Development of the Leaf Epidermis. *Developmental Biology*, *45*, 1-40. DOI: https://doi.org/10.1016/S0070-2153(08)60313-0.

Berlyn, J. P., & Miksche, J.P. (1976). *Microtechnique Cytochemishtry*. Lowa State University Press.

Bertel, C., Schönswetter, P., Frajman, B., & Holzinger, A. (2016). Leaf Anatomy of Two Reciprocally non-monophyletic Mountain Plant (*Heliosperma* spp.): does heritable adaptaions to divergent growing sites accompany the onset of Speciation?. *Protoplasma*, *254*(3), 1411–1420. DOI: https://doi.org/10.1007/s00709-016-1032-5.

Bosabalidis, A. M., & Kofidis, G. (2002). Comparative Effects of Drought Stress on Leaf Anatomy of Two Olive Cultivars. *Plant Science*, *163*(2), 375-379. DOI: https://doi.org/10.1016/S0168-9452(02)00135-8.

Boughalleb, F., Abdellaoui, R., Ben-Brahim, N., & Neffati, M. (2014). Anatomical adaptations of *Astragalus gombiformis* Pomel. under drought stress. *Central European Journal of Biology*, *9*(12), 1215-1225. DOI: 10.2478/s11535-014-0353-7.

Buckley, T. N., John, G.P., Scoffoni, C., Lawren, T. (2015). How Does Leaf Anatomy Influence Water Transport Outside The Xylem?. *Plant Physiology*, *168*(4), 1616-1635. DOI: https://doi.org/10.1104/pp.15.00731.

Budel, J. M., Raman, J., Monteiro, L. M., Almeida,V. P., et al. (2017). Foliar anatomy and microscopy of six Brazilian species of *Baccharis* (Asteraceae). *Miscrocopy Research and Techenique*, *81*(8), 832-842. DOI:https://doi.org/10.1002/jemt.23045.

Burnett , S., Pennisi, S., Thomas, P.A., & Iersel, M.W. (2005). Controlled Drought Affects Morphology and Anatomy of *Salvia splendens*. *Journal of the American Society for Horticultural Science*, *130*(5), 775-781. DOI: https://doi.org/10.21273/JASHS.130.5.775.

Burquez, A. (1987). Leaf Thickness and Water Deficit in Plants: A Tool for Field Studies. *Journal of Experimental Botany*, *38*(186), 109-114, DOI: https://doi.org/10.1093/jxb/38.1.109.

Canny, M., Wong, S.C., Huang, C., & Miller, C. (2012). Differential Shrinkage of Mesophyll Cells in Transpiring Cooton Leaves: Implications for Static and Dynamic Polls of Water, and for Water Transport Pathways. *Functional Plant Biology*, *39*(2), 91-102. DOI: http://dx.doi.org/10.1071/FP11172.

Canny, M.J., & Huang, C. X. (2005). Leaf Water Content and Palisade Cell Size. *New Phytologist Foundation*, *170*(1), 75-85. DOI: https://doi.org/10.1111/j.1469-8137.2005.01633.x.

Carr, M.K.V. (2013). The Water Relations and Irrigation Requirements of Olive (*Olea europaea* L.): a Review. *Experimental Agriculture*, *49*(04), 1-43. DOI: 10.1017/S0014479713000276.

Cassola, F., Silva, M.H.R., Borghi, A.A., Lusa, M.G., Sawaya, A.C.H.F., Garcia, V.L., & Mayer, J.L.S. (2019). Morphoanatomical Characteristics, Chemical Profiles, and Antioxidant Activity of Three Species of *Justicia* L. (Acanthaceae) Under Different Growth Conditions. *Industrial Crops & Products*, *131*, 257-265. DOI: https://doi.org/10.1016/j.indcrop.2019.01.053.

Chatri, M., Mella, C. E., & Des, M. (2020). Characteristics of Leaves Anatomy of Some Syzigium (*Myrtaceae*). *International Conference on Biology, Sciences and Education* (ICoBioSE 2019). DOI: 10.2991/absr.k.200807.005.

Coble, A.P., & 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*(10), 1337-1551. DOI: https://doi.org/10.1093/treephys/tpx016.

Coneva, V., & Chitwood, D. H. (2018). Genetic and Developmental Basis for Increased Leaf Thickness in the Arabidopsis Cvi Ecotype. *Frontiers in plant science*, *9*, 322. https://doi.org/10.3389/fpls.2018.00322.

Crang, R., Lyons-Sobaski, S., & Wise, R. (2018). *Plant Anatomy: A Concept-Based Approach to the Structure of Seed Plants*. Springer, Cham. DOI: https://doi.org/10.1007/978-3-319-77315-5.

Cutler, D. F., Botha, T., & Stevenson, D. W. (2007). *Plant Anatomy an Applied Approach*. Blackwell Publishing.

Da Costa Santos, J. V., De Oliveira, M. F. V., Santos Filho, F. S., Dos Santos Silva, L. N.N., & Silva Araujo, J. S. (2020). The Taxonomic Value of Leaf Anatomy for Species *Byrsonima:* a Difficult *genus* of *Malpighiaceae* Juss. *Acta Botanica Brasilica*, *34*(3), 570-579. DOI: 10.1590/0102-33062020abb0144 .

Da Costa, Y.O., & Daningsih, E. (2022). Ketebalan Daun dan Laju Transpirasi pada Tanaman Hias Dikotil. *Jurnal Ilmu Pertanian Indonesia*, *27*(1). 40-47. DOI: 0.18343/jipi.27.1.40.

Daningsih E., Mardiyyaningsih, A. N., Da Costa, Y. O., Primawati, R., & Karlina, S. (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*. DOI: 10.1088/1755-1315/976/1/012060

Das, S., (1999). An adaptive feature of some mangroves of Sundarbans, West Bengal. *Journal of Plant Biology*. *42*(2), 109-116. DOI: https://doi.org/10.1007/BF03031018.

EL-Gawad, A. M. A., & El-Amier, Y. (2017). Anatomical features of three perennial swampy plants of Poaceae, grown on the water stream banks in Nile Delta, Egypt. *Journal of Medicional Botanny*, *1*, 58-64. DOI: https://doi.org/10.25081/jmb.2017.v1.863.

Fahn, A. (1995). *Anatomi Tumbuhan*. *Edisi Ketiga*. Terjemahan: Ahmad Soediarto, Trenggono Koesoemaningrat, Machmud Natasaputra dan Hilda Akmal. Gadjah Mada University Press.

Fanourakis, D., Heuveluink. EP., & Carvalho, S.M.P. (2013). A Comprehensive Analysis of The Physiological and Anatomical Components Involved in Higher Water Loss Rates After Leaf

Development at High Humidity. *Journal of Plant Physiology*. *170*(10), 890–898. DOI: https://doi.org/10.1016/j.jplph.2013.01.013.

Galmes, J., Ochogavia, J. M., Gago, J., Roldan, E, J., Cifre, J., & Conesa, M.A. (2013). Leaf Responses to Drought Stress in Mediterranean Accesions of *Solanum lycopersicum*: Anatomical Adaptations in Relation to Gas Exchange Parameters. *Plant, Cell, & Environtment*, *36*, 920-935. DOI: 10.1111/pce.12022.

Gao, K., Santamaouris, M., & Feng, J. (2020) On the Efficiency of Using Transpiration Cooling to Mitigate Urban Heat. *Climate*, *8*(69), 2-16. DOI: https://doi.org/10.3390/cli8060069.

Giulani, R., Koteyeva, N., Voznesenskaya, E., Evans, M. A., Cousins, A. B., & Edwards, G. E. (2013). Coordination of Leaf Photosynthesis, Transpiration, and Structural Traits in Rice and Wild Relatives (Genus Oryza). *Plant Physiology*, *162*(2), 1632-1651. DOI: https://doi.org/10.1104/pp.113.217497.

Griffiths H., & Males J. (2017). Succulent Plants. *Current Biology*, *27*(17), R890-R896. DOI: https://doi.org/10.1016/j.cub.2017.03.021.

Hafiz, P., Dorly., & Rahayu, S. (2013). Karakteristik Anatomi Daun dari Sepuluh Spesies *Hoya* Sukulen Serta Analisis Hubungan Kekerabatannya. *Buletin Kebun Raya*, *16*(1), 58-73. DOI: 10.14203/bkr.v16i1.27.

Hameed, M., Batool, S., Nas, N., Nawaz, T., Ashraf, M. (2012). Leaf Structural Modification for drought tolerance in some differentially adapted ecotype of blue panic (*Panicumantidotale* Retz.). *Acta Physiologiae Plantarum*, *34*(4), 1479-1491. DOI: 0.1007/s11738-012-0946-6.

Han, Ji-Mei., Meng, Hao-Feng., Wang, Sai-Yu., Jiang, Chuang-Dao., Li, F., Zhang, Wang-Feng., & Zhang, Ya-Li. (2016). Variability of mesophyll conductance and its relationship with water loss use effiecency in cotton leaves under drought pretreatment. *Journal of Plant Physiology*, *194*, 61-71. DOI: https://doi.org/10.1016/j.jplph.2016.03.014.

Hidayat, E. B., & Niksololihin, S. (1995). *Anatomi Tumbuhan Berbiji*. Penerbit ITB

Hopkins, W. G., &Huner, N. P. A. (2009). *Introduction to Plant Physiology Fourth Edition.* New Jersey: John Wiley & Sons, Inc.

Hsieh, C. (2017). Effects Of Tree Shading and Transpiration on Building Cooling Energy Use. *Energy and Buildings*, *159*, 382-397. DOI: https://doi.org/10.1016/j.enbuild.2017.10.045.

Johansen, D.A. (1940). *Plant Microtechnique*. McGraw-Hill Book Company Incorporation

Kapchina-Toteva, V., Dimitrovaa, M. A., Stefanova, M., Koleva, D., Kostov, K., Yordanova, Z.P. (2014). Adaptive changes in photosynthetic performance and secondary metabolites during white dead nettle micropropagation. *Journal of Plant Physiology*, *171*, 1344-1353. DOI: http://dx.doi.org/10.1016/j.jplph.2014.05.010.

Kocchar, S.L & Gujral, S. K. (2020). *Plant Physiology Second Edition*. Cambridge University Press

Kocyigit, M., Salmeri, C., Ozhatay, N., Kaya, E., & Brullo, S. (2023). *Allium sphaeronixum* (Amaryllidaceae), *A New Species from Turkey*. *Plants*, *12*(11), 1-19 DOI: https://doi.org/10.3390/plants12112074

Kuiper, P. J. C. (1961). *The Effect of Environmental Factors on The Transpiration of Leaves, With Special Reference to Stomatal Light Response*. PhD thesis submitted to the Wageningen University, Veenman. https://edepot.wur.nl/184838.

Kutlu, N., Terzi, R., Tekeli, C., Senel, K., Battal, P., & Kadiogli, A. (2009). Changes in Anatomical Structure and Levels of Endogenous Phytohormones during Leaf Rolling in *Ctenanthe setosa* Under Drought Stress. *Turkish Journal of Biology*, *33*(2), 115-122. DOI: 10.3906/biy-0806-6.

Kutschera, U. (2008). The Growing Outer Epidermal Wall: Design and Physiological Role of a Composite Structure. *Annals of Botany*, *101*(5), 615-621. DOI: https://doi.org/10.1093/aob/mcn015.

Lugassi, N., Kelly, G., Fidel, L., Yaniv, Y., Attia, Z., et al. (2015). Expression of *Arabidopsis* Hexokinase in Citrus Guard Cells Controls Stomatal Aperture and Reduce Transpiration. *Original Research*, *6*(1114), DOI: https://doi.org/10.3389/fpls.2015.01114.

Mabel, A. F., Johnson, A. K., Olufemi, O. O., Ayomipo, A. A. T. (2014). Foliar anatomy of some species of *Asteraceae* in South Western Nigeria. *African Journal of Plant Sciences*, *8*(9), 426-440. DOI: 10.5897/AJPS2014.1196.

Martinez, J.P., Silva, H., Ladent, J.F., & Pinto, M. (2007). Effect of Drought Stress on the Osmotic Adjustment, Cell Wall Elasticity and Cell Volume of Six Cultivars of Common Beans (*Phaseolus vulgaris* L.). *European Journal of Agronomy*, *26*(1), 30-38. DOI: https://doi.org/10.1016/j.eja.2006.08.003.

Meidner, H. (1975). Water Supply, Evaporation, and Vapour Diffusion in Leaves. *Journal of Experimental Botany*, *26*(5), 666-672. DOI: https://doi.org/10.1093/jxb/26.5.666.

Nawazish, S., Hameed, M., & Naurin, S. (2006). Leaf Anatomical Adaptation of *Cenchrus ciliaris* L. from the Salt Range, Pakistan Against. *Pakistan Journal Botany*, *38*(5), 1723-1730.

Ningsih, C., & Daningsih, E. (2022). Ketebalan Daun dan Laju Transpirasi Tanaman Hias Monokotil. *Jurnal Ilmu Pertanian Indonesia*, *27*(4), 514-520. DOI: 10.18343/jipi.27.4.514

Nurida, M.K, Talip, N., & Ruzi, A.R. (2012). Nilai Taksonomi Ciri Anatomi Lamina Daun dan Adaptasi Kepada Persekitaran Spesies Hutan Paya Bakau (*Rhizophoracea*). *Jurnal of Tropical Marine Ecosystem*, *2*(2), 37-44.

Oguchi, R., Onoad, Y., Terashima, I., & Tholen, D. (2018). Leaf Anatomy and Function. In Adams III, W., Terashima, I. (eds). *The Leaf: A Platform for Performing Photosynthesis 44*, DOI: https://doi.org/10.1007/978-3-319-93594-2_5.

Oliveira, I., Meyer, A., Afonso, S., & Goncalves, B. (2018). Compared Leaf Anatomy and Water Relations of Commercial and Traditional *Prunus dulcis* (Mill.) Cultivars Under Rain-Fed Conditions. *Scientia Horticulturae, 229,* 226-232. DOI: https://doi.org/10.1016/j.scienta.2017.11.015

Olsen, J. T., Caudle, K.L., Johnson, L, C., Baer, S,G., & Maricle, B. R. (2013). Environmental an Genetic Variation in Leaf Anatomy Among Populations of *Andropogon gerardii* (Poaceae) Along a Precipitation Gradient. *American Journal of Botany, 100*(10), 1957-1968. DOI: https://doi.org/10.3732/ajb.1200628

Onoda, Y., Schieving,F., & Anten, N.P.R. (2015). A Novel Method of Measuring Leaf Epidermis and Mesophyll Stiffness Shows The Ubiquitous Nature of The Sandwich Structure of Leaf Laminas in Broad-Leaved Angiosperm Species. *Journal of Experimental Botany*, *66*(9), 2487-2499. DOI: https://doi.org/10.1093/jxb/erv024

Orchard, G. E., Torres, P. & Sounthararajah, R. (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. DOI: 10.1080/09674845.2008.11732799.

Osakabe, Y., Shinozaki-Yamaguchi, K., Shinozaki, K., & Trans, L. P. (2013). ABA Control of Plant Macroelement Membrane Transport Systems in Response to Water Deficit and High Salinity. *New Phytologist*, *2*(1), 35-49. DOI: https://doi.org/10.1111/nph.12613.

Outlaw, W. H., Schmuck, C. L., & Tolbert, N. E. (1976). Photosynthetic Carbon Metabolism in the Palisade Parenchyma and Spongy Parenchyma of *Vicia faba* L. *Plant Physiology*, *58*(2), 186-189. DOI: https://doi.org/10.1104/pp.58.2.186.

Ozcan, M., Demiralay, M., & Kahriman, A. (2015). Leaf Anatomical Notes on Cirsium Miller (*Asteraceae*, *Carduoideae*) from Turkey. *Plant Systematic and Evolution*, *301*, 1995-2012. DOI: https://doi.org/10.1007/s00606-015-1209-y.

Park, S.W., An, S., & Kwack, Y. (2020). Changes in Transpiration Rates and Growth of Cucumber and Tomato Scions and Rootstocks Grown Under Different Light Intensity Conditions in a Closed Transplant Production System. *Journal of Bio-Environment Control*, *29*(4), 339-405. DOI: https://doi.org/10.12791/KSBEC.2020.29.4.399.

Rahman, M. A., Stratopoulos, Laura, M.F., Moser-Reischl, A., Zolch, T., Haberle, K., Rotzer, T., Pretzcah, H, & Pauleit, S. (2019). Traits of Trees for Cooling Urban Heat Islands: A Meta-analysis. *Building and Environment, 170*, 1-31. DOI: https://doi.org/10.1016/j.buildenv.2019.106606.

Rashid, P., Shethi, K. J., & Ahmed A. (2020). Leaf Anatomical Adaptation of Eighteen Mangrove Plant Species from The Sundarbans in Bangladesh. *Bangladesh Journal of Botany, 49*(4), 903-911. DOI: https://doi.org/10.3329/bjb.v49i4.52496.

Reinking, L. (2007). *Examples of Image Analysis Using ImageJ*. Retrieved from https://imagej.nih.gov/ij/docs/pdfs/examples.pdf

Salisbury, F. B., & Ross, C. W. (1995). *Fisiologi Tumbuhan* (Eds. 1)). Penerbit ITB Bandung.

Sass, J. E. (1951). *Botanical Microtechnique Second Edition*. The Lowa State College Press.

Schulze, E.D., Lange., O.L., Kappen, L., Buschbom, U., & Evenari, M. (1973). Stomatal Responses to Changes in Temperature at Increasing Water Stress. *Planta*, *110*, 29-42.DOI: 10.1007/BF00386920.

Schymanski, S.J & Or, D. (2015). Wind Effects on Leaf Transpiration Challenge the Concept of "Potential Evaporation". *Proceeding of IAHS*, *371*, 99-107. DOI: https://doi.org/10.5194/piahs-371-99-2015, 2015.

Slot, M., & Winter, K. (2017). *In Situ* Temperature Relationships of Biochemical and Stomatal Controls of Photosynthesis in Four Lowland Tropical Tree Species. *Plant, Cell & Environment*, *40*(2), 3055-3068. DOI: https://doi.org/10.1111/pce.13071.

Sugiarto, A., Marisa, H., & Sarno, S. (2020). Pemodelan Pengaruh Peningkatan Suhu Udara Terhadap Laju Transpirasi Bibit *Lansium domesticum* Corr Menggunakan Metode Potometer yang Dimodifikasi. *Sriwijaya Bioscientia*, *1*(2), 31-34. DOI: https://doi.org/https://doi.org/10.24233/sribios.1.1.2020.165.

Sumardi, I., & Wulandari, M. (2010). Anatomy and morphology character of five Indonesian banana cultivars (*Musa* spp.) of different ploidy level. *Biodiversitas*, *11* (4), 167-175. DOI: 10.13057/biodiv/d110401.

Sundberg, M. D. (1985). Trends in Distribution and Size of Stomata in Desert Plants. *Desert Plants*, *7*(3), 154-157.

Taiz, L., Zeiger, E., Moller, I. M., & Murphy, A. (2018). *Fundamentals of Plant Physiology*. Oxford University Press.

Thom, J. K., Fltcher, T.D., Lisvesley, S. J., Grey, V., & Szota, C. (2022). Supporting Growth and Transpiration of Newly Planted Street with Passive Irrigation Systems. *Water Resources Research*, 58, e2020WR029526. DOI: https:// doi.org/10.1029/ 2020WR029526.

Tihurua, E. F., Agustiani, E. L., & Rahmawati, K. (2020). Karakter Anatomi Daun sebagai Bentuk Adaptasi Tumbuhan Penyusun Zonasi *Mangrove* di Banggai Kepulauan, Provinsip Sulawesi Tengah. *Jurnal Kelautan Tropis*, *23*(2), 255-264. DOI: https://doi.org/10.14710/jkt.v23i2.7048.

Toscano, S., Ferrante, A., & Romano, D. (2019). Respons of Mediterranean Ornamental Plants to Drought Stress. *Horticulturae*, *5*(6), 1-20. DOI: 10.3390/horticulturae5010006.

Tullus, A., Kupper, P., Sellin, A., Parts, L., Sober, J., Tullus, T., Lohmus, K., Sober, A., & Tullus, H. (2012). Climate Change at Northern Latitudes: Rising Atmospheric Humidity Decreases Transpiration, N-Uptake and Growth Rate of Hybrid Aspen. *Plos One*, *7*(8), 1-11. DOI: https://doi.org/10.1371/journal.pone.0042648.

Vastag, E., Cocozza, C., Orlovic, S., Kesic, L., Kresoja, M., & Stojnic, S. (2020). Half-Sib Lines of Pedunculate Oak (*Quercus robur* L.) Respond Differently to Drought Through Biometrical, Anatomical and Physiological Traits. *Forests*, *11*(153), 1-21. DOI: 10.3390/f11020153.

Von Caemmerer, S., & Evans, J.R. (2014). Temperature Responses of Mesophyll Conductance Differ Greatly Between Species. *Plant, Cells & Environment*, *38*(4), 629-637. DOI: https://doi.org/10.1111/pce.12449.

Wang, Z., Liu, S., Xu, Y., Zhu, W., & Du, A. (2022). Differences in Transpiration Characteristics among Eucalyptus Plantations of Three Species on the Leizhou Peninsula, Southern China. *Forest*, *13*(10), 1-20. DOI: https://doi.org/10.3390/f13101544.

Widyastuti, T. (2018). *Teknologi Budidaya Tanaman Hias Agribisnis*. CV Mine.

Williams, N. S. G., Bathgate, R. S., Farrell, C., Lee, K. F., et al. (2021). Ten years of greening a wide brown land: A synthesis of Australian green roof research and roadmap forward. *Urban Forestry & Urban Greening*, *62*, 1-10. DOI: https://doi.org/10.1016/j.ufug.2021.127179.

Wullschleger, S.D., & Oostherhuis, D. M. (1989). The Occurrence of an Internal Cuticle in Cotton (*Gossypium hirsutum* L.) Leaf Stomates. *Environmental and Experimental Botany*, *29*(2), 229-235. DOI: https://doi.org/10.1016/0098-8472(89)90054-3.

Xie, S. & Luo, X. (2003). Effect of Leaf Position and Age on Anatomical Structure, Photosynthesis, Stomatal Conductance and Transpiration of Asian Pear. *Botanical Bulletin of Academia Sinica*, *44*, 297-303. DOI: 10.7016/BBAS.200310.0297.

Xing, D., Wang, W., Wu, Y., Qin, X., Li, M., Chen, X, &Yu, R. (2022). Translocation and Utilization Mechanisms of Leaf Intracellular Water in Karst Plant *Orychophragmus violaceus* (L.) O. E. Schulz and *Brassica napus* L. *Holticulturae*, *8*(1082), 2-13. DOI: https://doi.org/10.3390/horticulturae8111082.

Yang, Z., Sinclair, T. R., Zhu, M., Messina, C. D., Cooper, M., & Hammer, G. L. (2012). Temperature Effect on Transpiration Response of Maize Plants to Vapour Pressure Deficit. *Environmental and Experimental Botany*, *78*, 157-162, DOI: https://doi.org/10.1016/j.envexpbot.2011.12.034.

Zhang, J., Jiang, X.D., & Cao, X.J. (2014). Photosynthesis and Ultrastructure of Photosynthetic Apparatus in Tomato Leaves under Elevated Temperature. *Photosynthetica*, *52*(3), 430-436. DOI: https://doi.org/10.1007/s11099-014-0051-8.

Zwieniecki, M., & Boyce, C.K. (2014). Evolution of a Unique Precision in Angiosperm Leaf Venation Lifts Constraints on Vascular Plant Ecology. *Proceedings of the Royal Society B*, *281*, 1-8. DOI: https://doi.org/10.1098/rspb.2013.2829.