Changes of stomatal distribution and leaf thickness in response to transpiration rate in six dicot plant species

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Abstract. Changes in leaf anatomy can be influenced by the surrounding environment and several other factors. Shade plants or ornamental plants themselves also have their characteristics in responding to surrounding conditions through transpiration. This study aimed to measure the rate of transpiration related to changes in the distribution of stomata and leaf thickness of six types of dicot ornamental plants and to describe the leaf anatomy of each type. The experiment used Completely Randomized Design (CRD) with six types of plants as treatment with three replications. Transpiration rate was measured by modified lysimeter method, stomatal distribution was calculated from the ratio of the number of stomata to epidermal cells and leaf anatomy was observed using preserved slide made from paraffin method. Data were analyzed by SAS with CRD model and correlation was performed between transpiration rate and leaf thickness. The results showed that the transpiration rate, leaf thickness, and stomatal distribution were significantly different at $\alpha = 0.05$ amongst plants. The transpiration rate was inversely proportional to leaf thickness with various correlation coefficients. The leaf anatomy of six plant species showed various thicknesses between tissues. Further research needs to be done to explain the rate of transpiration with changes in leaf anatomy.

Keywords: Stomatal distribution, transpiration rate, leaf thickness, leaf anatomy, dicot.

1. Introduction

Recently, ornamental plants are increasingly attractive and in demand by various groups, not only as decoration but also as greenery. It is easily found ornamental plants in the surrounding, one of which is red shoots (*Syzygium paniculatum* Gaertn.). Ornamental plants not only serve to add the beauty of the environment but also to comfort [32]. It is related to the function of plants and transpiration. Transpiration is the process of losing water in the form of vapor from plant tissues through the stomata [35]. Stomata play an important role in the use of water. In addition, stomata are functional plant parts so that they can adapt to their environment to improve their performance [25]. This functional change can be shown by changes in the distribution of stomata of a species. According to Judge, Dorly, & Rahayu [16], the distribution of stomata which is the ratio of the number of stomata or epidermal cells in a certain area unit will change if plants are planted in different environments. Rochman & Hamida [34] showed that the distribution of stomata decreased or increased when plants were planted under certain conditions. Stomata are holes or gaps in the leaf epidermis that are limited by 2 guard cells that function to enter and exit gases, including water vapor [35]. The moisture content in plants depends on the shape of the leaf structure, including leaf thickness. According to Dwidjoseputro [8], leaf thickness can affect the rate of plant transpiration.
Leaves are parts of plants that are thin, wide, rich in a green dye called chlorophyll. Leaves are one of the plant organs originating from the stem which functions as a catcher of solar energy through photosynthesis [27]. Different leaf thickness in each plant is influenced by the amount of exposure to sunlight received [21]. Leaf thickness decreases when transpiration begins and then increases when it absorbs water from stems and soil [12].

The flexibility of the thickness of the leaf that contains water indicates the influence of leaf anatomy. According to Fahn [10], anatomical differences in leaf tissue can show kinship or differences in each species. According to Alponsin, Maideliza & Noli [2] the structure of leaf tissue has different thicknesses, especially in palisade and sponge tissues. This difference occurs because plants are exposed to different light intensities. Da Costa [7] has not yet know what the physical condition of each leaf tissue is when it transpires. The relationship between thickness shrinkage of leaf tissue and transpiration can be done by measuring the thickness of the epidermal tissue, leaf mesophyll tissue, and overall leaf tissue thickness.

This study aimed to determine changes in stomatal distribution and leaf thickness associated with transpiration rate and to describe the leaf anatomy of six dicot ornamental plants that are often planted in Pontianak City.

2. Materials and Methods
2.1 Materials
The plants study were six types of dicot plants which are commonly used as ornamental plants. The plants were planted in pots containing planting media with a 2:1 ratio of burnt soil and sand. The six types of plants were Croton or Puring (Codiaeum variegatum (L.) A. Juss.), Firestorm (Excoecaria cochinchinensis Lour), Erpah or Ki Sambang (Aerva sanguinolenta Bl.), Rombusa Putih or Crape Jasmine (Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult.,), Red Shoots or Pucuk Merah (Syzygium paniculatum Gaertn.) and Bougenville or Bunga Kertas (Bougainvillea glabra paniculatum Gaertn.). The tools used were preserved slide, microscope, ocular and objective micrometers, handcounter, 50 ml erlenmeyer, ruler, thermometer, luxmeter, wind speed meter, vial bottle, film bottle, rotary microtome, brush, plastic petridish, water bath, thermometer, hygrometer, measuring cup, analytical balance, cutter, camera phone, laptop, stationery, logbook, and printer. The materials used are clear nail polish, clear tape, scissors, vaseline, water, FAA solution, alcohol (30, 50, 70, 80, 90, 96, and 100%), 1% of safranin, xylol, paraffin, albumin, clean cotton, and label.

2.2 Method
This study used an experiment with completely randomized design (CRD) and treatment of six types of dicot plants with three replications to observe the distribution of stomata, leaf thickness and transpiration rate and to describe the leaf anatomy of six types of dicot plants using preserved slides with paraffin method. These measurements were carried out from 09.00-11.00 Western Indonesia Time. Fatonah [10] stated that the stomata opened more and the diameter of the stomata opening was larger at 09.00-11.00 WIB and at this time the highest transpiration rate also occurred. Observation of stomatal distribution, leaf thickness and leaf anatomy require standardization of micrometers using an ocular and an objective lenses [17].

2.3 Stomatal distribution calculation
Stomatal distribution or also called stomata index is calculated from the ratio of the number of stomata to epidermal cells in a certain unit. The measurement method of stomatal distribution refers to Meidner & Mainsfield in Avci & Aygün [4] with the formula as follows:

\[
SI = \frac{\text{stomatal number per unit area}}{\text{stomatal number per unit area} + \text{epidermal cell number per unit area}} \times 100
\]

Explanation:
SI = stomata index per unit area
The number of stomatal and epidermal cells were counted from slide prepared using replica method. The number of stomata was counted under a microscope with 10x10 magnifications using a hand counter. The distribution of stomata is calculated from the stomata before and after the transpiration.

2.4 Transpiration Rate Measurement

Transpiration rate was measured using a modified lysimeter method refers to Gulo [14]. The first step was to make leaf patterns for each plant using paper from the same source (HVS paper) to determine leaf area. The calculation was as follows:

\[
\text{leaf area} = \frac{\text{weight of leaf pattern}}{\text{weight of paper size (2 cm}^2\text{)}} \times \text{area of paper (2 cm}^2\text{)}
\]

The measurement of the transpiration rate was calculated by the following formula:

\[
\text{Transpiration rate} = \frac{\text{total water lost (g)}}{\text{time (s) x leaf area (cm}^2\text{)}}
\]

2.5 Leaf thickness measurement

Leaf thickness was measured before and after transpiration using fresh slides of each species. Leaf thickness was measured from the upper to the lower epidermis. Measurements were observed under a microscope with a magnification of 10x10 using a calibrated micrometer [2]. Leaf measurement using the formula:

Leaf thickness (µm) = Thickness measurement result x Calibrated Micrometer (µm)

2.6 Observation of leaf anatomy

Description of the variation of leaf tissue thickness in each type of dicot plants using preserved slides with the paraffin method [15]. The thickness of each leaf tissue was measured with a calibrated micrometer under magnification of 40 x 10.

2.7 Data analysis

Data were analyzed using SAS with CRD model for stomatal distribution, transpiration rate, and leaf thickness. If the treatment was significant, followed by LSD test. Furthermore, correlation was carried out to determine the relationship between stomata distribution and transpiration rate and the relationship between stomatal distribution and leaf thickness using the Pearson correlation. The strength of the relationship was categorized according to Schober et.al [36] and Mukaka [29]. Leaf tissue thickness was described based on the average size of each tissue.

3. Results

3.1. The ratio of stomatal distribution

The results showed that stomatal distributions (Figure 1) were affected by the types of plant at \(\alpha = 0.05\) (Table 1). Based on the calculation results of the stomatal distribution on six types of dicot plants (Table 1), it was shown that stomatal distribution of red shoots (Syzygium paniculatum Gaertn.) had the highest average stomatal distribution (50.84%) and significantly different to other plants, followed by stomatal distribution of Croton (Codiaeum variegatum (L.) A. Juss.) (33.09%) and Firestorm (Excoecaria cochinchinensis Lour) (28.74%). Stomatal distribution of Bougenville (Bougainvillea glabra Choisy) (11.69%) was the lowest and significantly different to other plants (Table 1) except Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult (Table 1 & Figure 1).
Table 1. Analysis of variance of stomatal distribution in six types of dicot plants.

| Plant Type                                      | Mean of Stomatal Distribution (%) | Sign |
|------------------------------------------------|-----------------------------------|------|
| *Syzygium paniculatum* Gaertn.                 | 50.84a                            | ***  |
| *Codiaeum variegatum* (L.) A. Juss.            | 33.09b                            |      |
| *Excoecaria cochinchinensis* Lour               | 28.74b                            |      |
| *Aerva sanguinolenta* Bl.                      | 16.66c                            |      |
| *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult. | 12.78cde                          |      |
| *Bougainvillea glabra* Choisy                   | 11.69d                            |      |

Note: *** significant at α = 0.001. The difference in letters behind the mean in the same column showed significance when tested with LSD at α = 0.05.

Figure 1. Stomatal distribution of Crape Jasmine (*Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult.).

3.2. Transpiration rate
The results showed that the rate of transpiration amongst species ranged from 0.004811 gr.cm$^{-2}$.hour$^{-1}$ to 0.011033 gr.cm$^{-2}$.hour$^{-1}$ (Table 2). *Aerva sanguinolenta* Mrs. had the highest transpiration rate and significantly different to other plants. *Syzygium paniculatum* Gaertn having highest stomatal distribution (Table 1) had the second highest transpiration rate but significantly different to that of *Aerva sanguinolenta* Mrs.

Table 2. Analysis of Transpiration Rate in Six Types of Dicot Plants

| Plant Type                                      | Mean of Transpiration Rate (gr.cm$^{-2}$.hour$^{-1}$) | Sign |
|------------------------------------------------|-----------------------------------------------------|------|
| *Aerva sanguinolenta* Mrs.                     | 0.011033a                                           | ***  |
| *Syzygium paniculatum* Gaertn.                 | 0.008133b                                           |      |
| *Tabernaemontana divaricata* (L.) R. Br. ex Rome. & Schult. | 0.007356bc                                         |      |
| *Excoecaria cochinchinensis* Lour.              | 0.006511bcd                                         |      |
| *Bougainvillea glabra* Choisy                   | 0.005122cd                                         |      |
| *Codiaeum variegatum* (L.) A. Juss.            | 0.004811d                                           |      |

Note: *** significant at α = 0.001. The difference in letters behind the mean in one column shows significance when tested with LSD at α = 0.05.

3.3. Leaf thickness
The highest leaf thickness before and after transpiration was *Codiaeum variegatum* (L.) A. Juss. (croton) (Figure 2 & 3) and significantly different from the others (Table 3). The decrease in leaf thickness also occurred in *Aerva sanguinolenta* Mrs. In contrast, *Syzygium paniculatum* Gaertn (Figures 4 & 5), followed by *Excoecaria cochinchinensis* Lour had the thinnest thickness (Table 3). However, the shrinkage occurred more in *Syzygium paniculatum* Gaertn.
### Table 3. Analysis of leaf thickness on six types of dicot plants.

| Plant Type | Mean of Leaf Thickness (µm) Before Transpiration | Sign | Mean of Leaf Thickness (µm) After Transpiration | Sign | Difference |
|------------|-----------------------------------------------|------|-----------------------------------------------|------|------------|
| *Codiaeum variegatum* (L.) A. Juss. | 119,489a | *** | 89,467a | *** | 30,022 |
| *Aerva sanguinolenta* Mrs. | 95,089b | | 81,400ab | | 13,689 |
| *Tabernaemontana divaricata* (L.) R. Br. ex Rome. & Schult. | 94,600b | | 72,844b | | 21,756 |
| *Bougainvillea glabra* Choisy | 92,644b | | 71,867b | | 20,777 |
| *Excoecaria cochinchinensis* Lour. | 65,511c | | 49,867c | | 15,644 |
| *Syzygium paniculatum* Gaertn. | 64,289c | | 43,267c | | 21,022 |

Note: *** significant at $\alpha = 0.001$. The difference in letters behind the mean in one column shows significance when tested with LSD at $\alpha = 0.05$.

### 3.4. Correlation between stomatal distribution ratio and transpiration rate and between leaf thickness and transpiration rate.

The correlation between the stomatal distribution ratio and the transpiration rate showed the best proportional relationship with the correlation coefficient that varied between each species.

The correlation between stomatal distribution ratio and transpiration rate showed an inverse relationship (Figure 6) with various correlation coefficients amongst species. In the species *Excoecaria cochinchinensis* showed a low correlation between stomatal distribution and transpiration rate but in *Aerva sanguinolenta* Bl. (Erpah or ki sambang) this correlation was very strong (Figure 6a & 6b). This
result showed that there was a relationship between stomatal distribution and transpiration rate that had negative values from low to high.

Very low

Very strong

*a. Excoecaria cochinchinensis* Lour. (Firestorm)

*b. Aerva sanguinolent* Bl. (Erpah or ki sambang)

**Figure 6.** Correlation between stomatal distribution and transpiration rate. a. *Excoecaria cochinchinensis* Lour. b. *Aerva sanguinolent* Bl. (Erpah or ki sambang).

*a. Bougainvillea glabra* Choisy (Bougenville)

*b. Aerva sanguinolenta* Mrs. (Erpah or ki sambang)

**Figure 7.** Relationship diagram of leaf thickness and transpiration rate. a. *Bougainvillea glabra* Choisy b. *Aerva sanguinolenta* Mrs.

Dwidjoseputro [8] state that leaf thickness is one of the internal factors that affect the rate of transpiration. In this study, it was found that the correlation result was negative (-). The negative correlation shows that the relationship between leaf thickness and transpiration rate is inversely proportional where the thicker the leaves, the lower the transpiration rate.

Based on Oktarin, Rampea & Pelealua [31] leaf thickness will depend on the thickness of the leaf tissue. The results of six leaf anatomy showed that the thickness of each tissue varied amongst plant species (Table 4).
Table 4. The average of leaf tissue thickness of six dicot species.

| Plant                                      | Total (µm) | Thickness (µm) | Epidermis | Mesophyll |
|--------------------------------------------|------------|----------------|-----------|-----------|
|                                            |            |                | upper     | lower     |
| **Codiaeum variegatum** (L.) A. Juss.     | 165.625    | 23.75          | 15.625    | 26.25     | 100       |
| **Aerva sanguinolenta** Mrs.               | 165        | 26.25          | 27.5      |           |           |
| **Excoecaria cochinchinensis** Lour.       | 162.5      | 12.5           | 10.625    | 13.75     | 125.625   |
| **Syzygium paniculatum** Gaertn.           | 162.18     | 19.37          | 9.37      | 40.63     | 90.63     |
| **Tabernaemontana divaricata** (L.) R. Br. ex Rome. & Schult. | 152.5 | 20       | 23,125    |           | 109.375   |
| **Bougainvillea glabra** Choisy            | 130.625    | 18,125         | 12.1875   | 31.25     | 69.0625   |

The thickness of the leaf tissue varied (Figure 8). Leaf thickness was dominated by mesophyll (palisade and sponge) and supported by Ivanova et al. [19]. While the upper epidermis was thicker than that of the lower epidermis. In the upper epidermis the thickness ranges from 12.5 µm on Excoecaria cochinchinensis Lour. (Firestorm) to 26.25 µm Aerva sanguinolenta Mrs. (Erpah) (Table 4).
4. Discussion

4.1 The ratio of stomatal distribution

Avci & Aygün [4] stated that the spread of stomata in the epidermis was influenced by variations in species and varieties in types and growing conditions. In addition, Juairiah [20] stated that differences in the distribution of stomata in each plant could be caused by genetic and phenotypic factors and are influenced by environmental factors. A high stomatal distribution ratio indicates a greater number of stomata in a certain area and it will help to increase the rate of transpiration. According to Rehfeldt, Wykoff, & Ying [34] a high stomatal distribution ratio indicates the ability of plants to adapt to a drought-prone environment. Red Shoots (*Syzygium paniculatum* Gaertn.) is a plant that is usually used as an ornamental plant as well as shade plants.

4.2 Transpiration rate

This result was consistent that many stomata can result in high transpiration rate [9]. However, the relationship between stomatal distribution and transpiration rate was not always inline (Table 2). It is supported by Allen [1] that each species has a different transpiration. According to Wallace and Jannet [40], each vegetation has a different root and canopy structure. The canopy structure, plant physiology, leaf area index and stomata opening affect transpiration.

4.3 Leaf thickness

In this study, leaf thickness before and after transpiration was significantly affected by plant species (Table 3). According to Izza and Laily [18] differences in leaf thickness before and after transpiration are influenced by plant species. Thinner leaves (*Syzygium paniculatum* Gaertn.) had a higher transpiration rate (Table 2). According to Karyati [22] stated that thin and wide leaves tend to transpire more. It is associated with stomata with a larger stomata area [16]. Furthermore, Papuangan, Nurhasanah & Mudmainah [32] state that the number of stomata per unit area will accelerate transpiration.

4.4 Correlation between stomatal distribution ratio and transpiration rate and between leaf thickness and transpiration rate

A high correlation coefficient indicates lower transpiration rate and higher stomatal distribution. The relationship between transpiration rate and stomatal distribution showed an inverse relationship and also occurred in the relationship between leaf thickness and transpiration rate (Figure 7a & 7b).

The upper epidermis tends to be thicker than the lower epidermis due to the plant's efforts to avoid high transpiration. Kartasapoetra [21] states that the thick cells of the epidermis are related to their protective function. The lower epidermis is thinner than the upper epidermis. This thickness is related to its function with transpiration [8]. Usually, in the lower epidermis there are stomata [35]. Mesophyll thickness is closely related to the water content in the tissue [3]. In palisade, part of the mesophyll has thickness elasticity. The changes in the number of palisade layers occurred because the same species was exposed to different light intensities [13,42]. *Syzygium paniculatum* Gaertn had a high transpiration rate (Table 2) and a thinner lower epidermis. *Syzygium paniculatum* Gaertn also had a thicker palisade (Table 4) than the other species.

The thickness of the mesophyll was almost the same amongst species (Table 4 and Figure 8) except *Excoecaria cochinchinensis* Lour. *Excoecaria cochinchinensis* Lour had the thinnest palisade (13.75 µm) and thickest sponge (125.62 µm) and its transpiration was included in the lower transpiration rate (Table 2). On the other hand, *Syzygium paniculatum* Gaertn had thickest palisade (Table 4) and higher transpiration rate. From this description it appears that the palisade thickness, the one that influence transpiration rate of a species. However, from this anatomical data have not shown how the difference of palisade thickness due to transpiration rate. According to Canny & Huang [6] palisade due to its elasticity can play a role in response to water lost. Therefore, it is needed to do the further research to understand the relationship between the changes of palisade thickness before and after transpiration. Due to tissue thickness of plant species also varied so it could not be concluded yet from this leaf anatomy.
5. Conclusion
The stomatal distribution, leaf thickness, and transpiration rate were affected by plant species. The stomatal distribution was inversely correlated with transpiration rate in different degree from low to high correlation coefficient. In the same manner, leaf thickness was also inversely correlated to transpiration rate. Syzygium paniculatum Gaertn had higher transpiration rate and thicker palisade also thinner lower epidermis that could be indicate this relationship happen amongst transpiration rate, lower epidermis and palisade thickness. The thickness of leaf tissue in plants varied amongst species.

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