Research Article

Leaf Morphometric and Chlorophyll Content Study of Bisbul (*Diospyros discolor* Willd.) at the Bogor Botanical Garden

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ABSTRACT

Bisbul (*Diospyros discolor* Willd.) is one of the collections from the Bogor Botanical (BBG). The wide distribution of this plant in Southeast Asia's tropical forests creates many morphometric variations of this species. The diversity of leaf morphometric variations of a plant species can be the basis for grouping various species. This study aimed to determine the morphometric diversity of Bisbul leaves from various locations of origin. The study took samples of bisbul leaves from Bogor Botanical Gardens from three accessions, namely the Philippines, West Java, and Papua. All the trees are over 30 years old. The leaves are taken based on a horizontal position, vertical crown, and leafage. The results showed that the origin of the location gave different multiplier values to calculate the area, namely the Philippines $y = 0.733x + 0.034$; West Java $y = 0.765x - 2,949$; and Papua $y = 0.758x - 1.389$. The length to leaf width ratio also has differences, namely, the Philippines, which is 2.64, West Java 2.65, and Papua 2.81. The chlorophyll content in young leaves increases in old leaves. The samples from Papua also have the highest chlorophyll content compared to the Philippines and West Java. This difference indicates morphometric variations between the three, even within one species. Some environmental conditions may affect shade areas and tree age. In addition, DNA research from accession *D. discolor* is also needed to determine the cause of the morphometric variation.

INTRODUCTION

Bogor Botanical Gardens (BBG) is an ex-situ conservation area with more than 12,370 collected plants covering 3,555 species, 1,202 genera, and 191 families (Ariati et al. 2019). These collections are planted on a land area of 87 hectares in the center of Bogor City. BBG is located at an altitude of 260 m asl with a humidity level of 80-90% and an average rainfall of 3,000-4,000 mm per year (Statistics Bogor Regency 2014). This Botanical Garden focuses on conserving lowland humid and wet plant species. BBG collections originate from Indonesia and abroad. One of the plants that the BBG has successfully collected is the Ebenaceae family (Ariati et al. 2019).

The Ebenaceae family collected in the BBG has only one genus, namely Diospyros. Ebenacean diversity centers are Southeast Asia, Madagascar, Central Africa, and South America. The Ebenaceae family species has reached 1,698 species, and only 751 species have valid names (GBIF 2019; POWO 2019). The genus *Diospyros* has 500 - 600 species scattered in tropical forest areas. The genus *Diospyros* is known to have anti-diarrhea, antibacterial, anti-protozoal, anti-fungal, molluscicide, and anti-inflammatory properties (Howlader et al. 2012). This genus is an essential component of forest vegetation composition in Africa and Asia (Bakhuizen van den Brink 1933; White 1988). The number of these species decreases over time as deforestation increases. However, the Bogor Botanical Gardens have successfully conserved 198 specimens and 32 species of *Diospyros* spp. (Wanda et al. 2019). These collections come from exploration activities in almost all major islands in Indonesia and exchange seeds with botanical gardens from other countries.

Diospyros discolor is a species of the Ebenaceae family widespread in Southeast Asia. This plant is known as the Bisbul Tree, Butter fruit tree, and velvet apple tree. *D. discolor* is synonyms with *Cavanillea philippensis* Desr. These are scattered in Sumatra, Java, the Philippines, Taiwan, and the Malay Peninsula (Bakhuizen van den Brink 1936; GBIF 2019). Bisbul trees grow in primary and secondary forests with an altitude of up to 800 m above sea level. Besides producing edible fruit and becoming a food commodity, Bisbul also has potential as wood and an ornamental plant (Rauf et al. 2017).

Bisbul tree has tree habitus; up to 32 m high, free branches up to 10 m, diameter up to 100 cm, and conical. Elliptical leaves; varied in size between 8 -30 x 2.5-12 cm; rounded base; tapered leaf tips, hair with grey color on the underside. Male flowers; 3-7 in cymes; Solitary female flowers, tubular corollas, white. Globose-shaped fruit; 7-10 cm in diameter, brown to dark red, velvety; edible fruit (Bakhuizen van den Brink 1936; Knapp & Gilbert 2002). The seeds are dark brown and flattened. Some vegetative characters, such as leaves in this plant, vary in size, marked by the leaf's size range. Genetic and environmental factors can cause this difference. Ecological differences will cause differences in plant species diversity or plant plasticity (Koch et al. 2006).

In Angiosperm, the morphological characteristics of generative organs (flowers) are widely used as information on species identification and morphometric analysis. However, flowers are temporary, and not all plants can have flowers, resulting in identification using other plant parts, such as vegetative organs. Vegetative organs that can provide information about a plant include leaves, roots, and stems. Of the three organs, leaf organs have the most knowledge and can be the right solution to replace the identification process if no flower organs are found (Stuessy 2009). This condition can be seen from the identification process of *D. discolor,* which rarely uses generative characters because the organ is not always found in every season.

Meanwhile, other characters such as leaves are often found but are neglected because of their plasticity. Apart from leaf morphometrics, leaf chlo-

rophyll content can also be used as information for species identification. One of the causes of leaf color variation is the presence of chlorophyll pigment in leaf tissue. Chlorophyll is a green color-giving pigment in plants, which plays a role in plants' photosynthesis process. The intensity and levels of chlorophyll will be expressed in various green leaves (Buschmann et al. 2012; Ma et al. 2018).

Several studies using leaves for identification have been carried out. Afrinawaty (2007) found many morphological variations in the leaves of the Tabat Barito plant (*Ficus deltoidea*) in West Sumatra. Jawati (2006) also found morphological variations in Andalas (*Morus macroura*) plants in West Sumatra. Haq (2019) studied the Nepenthes pitcher in Central Kalimantan and West Kalimantan and found that morphometrics can be used to distinguish species in Nepenthes. Using a morphometric study, Utama et al. (2012) distinguished five Macaranga species in the Forest Biology Research and Education (HPPB) Padang. However, no studies use leaves to see the morphometric variations of bisbul (*D. discolor*). The use of morphometrics in taxonomy helps to improve scientific rigor in the description of crucial features of biodiversity's phenotypic dimension (Viscosi & Cardini 2011).

Leaf morphometrics can be used as one of the morphological characteristics describing the diversity of phenotypes. Genetic and environmental factors determine the phenotypic diversity of a living thing. Phenotypic properties can be observed in plain view in shape, color, and size (length and width). Leaf morphometric ratio and imaginary leaf area (intact) can determine leaf length and width, while the amount of chlorophyll can be associated with leaf color characters. This research will study the variation of leaf morphometric of *D. discolor*, which grows in the same environment (landscape) but has different origins or genotypes in leaf morphology. Leaf morphometric identification was chosen because it is easy to observe and vital for plant survival. Besides, morphometric studies and chlorophyll content of the Bisbul species have never been carried out.

MATERIALS AND METHODS
Materials
All samples are collections of Bogor Botanical Gardens. The samples used in this research were *Diospyros discolor* leaves from three accessions, namely the Philippines, West Java, and Papua. All the trees are over 30 years old.

Methods
The study was conducted at Bogor Botanical Gardens-BRIN from January 2018 - to May 2020. The study method used a survey, observation, and purposive sampling. Microclimate data retrieval was done using several tools, including termohygro, Soil pH meter, altimeter, and camera.

Morphometric Identification
Morphometric identification is made by taking leaf samples with the criteria

of young and old leaves at; 1) the four cardinal directions; 2) the position of the leaves on the tree (bottom, middle, top), and 3) the inside and outside of the canopy (Figure 1). Each leaf sample was taken in three replications so that the number of each individual was 48 leaf samples. Each *D. discolor* species from each accession (the Philippines, West Java, and Papua) used a sample from one tree. The parameters observed in this study include leaf length and width to determine the leaf morphometric ratio, imaginary leaf area (intact), and the amount of chlorophyll.
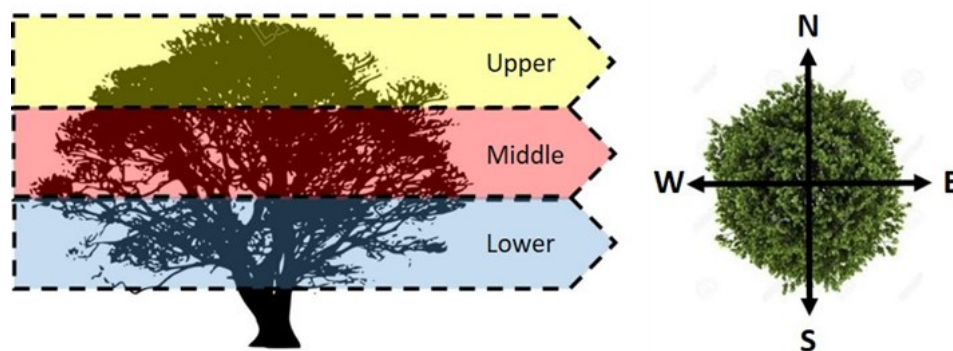
Figure 1. Leaf sampling.

Regression analysis was used to determine the relationship between length, width, and leaf area (Stowe 1995). Here is the formula:

$Y = ax + b$

Information:

Y: leaf area (cm2)

a: coefficient x

x: (length (cm) x wide (cm))

b: constant

Morphometric measurements

Morphometric measurements were carried out by scanning the leaf area and measuring the leaves' size using the Epson L210 printer scanner with the Epson scan software. Image details use JPG format with a resolution of 300 dpi. Leaf scanning results were processed using ImageJ 1.46r software to determine the leaves' length, width, and area. The length and width of the scanned image area are 2481 x 3509 pixels. The calculation starts by calibrating the size to A4 paper size (21.0 x 29.7 cm). The calibration results obtained a scale of 118.14 pixels/cm. The image is then adjusted for color through the threshold technique and selected to calculate the leaf's length, width, and area.

Chlorophyll content measurements

Chlorophyll content measurements were carried out on all leaves taken at each location of origin. The adaxial sides of the leaves were scanned using the Chlorophyll Meter SPAD-502 Plus tool.

Data Analysis

The effect of *D. discolor* origin on the morphometric variation and analyzed using the Analysis of Variance (ANOVA) method. If a real impact is found, continue with the Duncan's Multiple Range Test (DMRT) 5% to determine the difference. Leaf morphological characteristics data on different land-scapes were processed with Microsoft Office Professional Plus 2016 (Excel) and Minitab 16.1.1 software.

RESULTS AND DISCUSSION

The results of *D. discolor* leaves' observations found that the three plants of *D. discolor* originating from different locations had different leaf morphometrics (Table 1). The statistical results showed that plant origin differences significantly affected the variables of leaf area, leaf length, and leaf width. From the recapitulation of the results of variance, it was found that the *D. discolor* leaves that had the most significant length, width, and leaf area were *D. discolor* leaves originating from West Java (length: 24.11 cm; width: 9.12 cm; area: 171.20 cm²), and the smallest leaves are *D. discolor* from the Philippines (length: 19.02 cm; width: 7.25 cm; size: 107.40 cm²). *D. discolor* from Papua is 22.34 cm long, 7.99 cm wide, and has a surface area of 137.99 cm². The results indicate that the difference in origin (parentage) of a plant species also means that the species have a different genotype characterized by different appearances of phenotypes.

The microclimate conditions around the sample are relatively the same, with 28-29 °C of air temperature, 67.6% of air humidity, 5.8 soil pH, and 85-100% of soil humidity. Phenotypic differences that can be seen from these leaf morphometric variations still appear even though these species grow and develop in relatively the same environment. However, there may be differences in the micro-environmental conditions where *D. discolor* grows, which causes differences in leaf phenotypes. Variations in plants due to environmental conditions indicate that a plant adapts. Some environmental conditions that may affect shading area and tree age include *D. discolor* from the Philippines in open areas, while *D. discolor* from West Java and *Diospyros* from Papua are grown in shading areas. In addition, the age of *D. discolor* from the Philippines is younger than the other two *Diospyros*. In response to shade,

Table 1. *Diospyros discolor* leaf morphometric variations based on the origin and position of leaves in plants.

| Category	Origin	Leaf area (cm²)	Length (cm)	Width (cm)	Length: Width (cm)	Chlorophyll (SPAD)
Origin	Philippines	107,40^a ± 54,76	19,02^a ± 4,88	7,25^a ± 1,87	2,64^a ± 0,22	41,95^a ± 16,83
	West Java	171,20^c ± 59,57	24,11^c ± 4,81	9,12^c ± 1,77	2,65^a ± 0,20	41,22^a ± 23,70
	Papua	137,99^b ± 47,13	22,34^b ± 4,07	7,99^b ± 1,47	2,81^b ± 0,24	45,29^b ± 21,77
The position of the leaves on the tree	Lower	139,07^{ab} ± 57,86	21,79 ± 4,77	8,20^{ab} ± 1,86	2,68 ± 0,25	41,03^a ± 21,22
	Middle	152,72^b ± 63,02	22,90 ± 4,73	8,54^b ± 1,83	2,69 ± 0,19	44,99^b ± 21,19
	Upper	124,79^a ± 56,06	20,79 ± 5,45	7,63^a ± 1,83	2,72 ± 0,26	42,44^{ab} ± 20,50

Note: The number in the same column, followed by a different letter, differs significantly from Duncan's Multiple Range Test (DMRT) 5%.

plants showed functional changes in physiological and morphological aspects. These functional changes can be seen in the plant *Enterolobium contortisiliquum* (Vell.) Morong (Souza et al. 2017). As a form of adaptation to natural conditions or environmental stresses, plants can experience phenotypic plasticity, which is the ability of an individual to modify some specific traits during their development period (Jones & Luchsinger 1987). Understanding genetic diversity and its origin distribution are fundamental to conservation and sustainable use.

Another result was obtained when the leaf morphometric variations were compared based on the leaves' position on the tree, namely the bottom, middle, and top. From the ANOVA statistical results, differences in leaf position significantly affected leaf area and leaf width variables. From the recapitulation of the effects of variance, it was found that the leaf area of the *D. discolor* leaves in the middle position (152.72 cm) was wider when compared to the bottom (139.07 cm) and upper (124.79 cm). This leaf area variation is supported by differences in leaf width, although the length and ratio of height to width are not significantly different. Physiological and environmental factors cause these variations, and tree age and shading area are suspected to be the causes of this condition. Leaves are one of the organs that develop pretty quickly and are classified as sensitive. Cox & Moore (1980) stated a correlation between climate and leaf character. Leaf size and leaf margins can explain plants' adaptation to average rainfall and temperature.

The leaf's length and width ratio describe the leaf's general shape. The leaf shape, formed from the rate of leaf length to width, also shows a significant difference from Papua (Figure 2). The most considerable leaf length and width ratio value was that of *D. discolor* from Papua (2.81). Then followed by *D. discolor* from West Java (2.65) and the Philippines (2.64). The length and width ratio did not significantly differ between the leaves at the top, middle, and bottom positions (Table 1). The value of the ratio of the length and width of the leaf affects the shape of a leaf. The L/W ratio has an intimate association with the geometrical features and the self-similarity of leaf shape (Shi et al. 2021). *Diospyros discolor* leaves from 3 locations, namely the Philippines, West Java, and Papua, have the same morphological shape. Based on the identification results, *D. discolor* from the 3 locations has elliptical leaves with a rounded base; symmetrical; the leaves' tips are tapered and hairy with grey color on the underside. Young leaves have a lighter green color than the fully developed leaves with darker colors. From the ANOVA statistical results, differences in plant origin and leaf position on trees significantly affected leaf chlorophyll content (Table 1). The leaf chlorophyll content is what causes color variations in *D. discolor* leaves. Colour variations in leaves are caused by chlorophyll, carotenoid, and anthocyanin pigments in the leaf tissue. The chlorophyll content in dark green leaves is greater than that of light green leaves (Pandey & Sinha 1979).
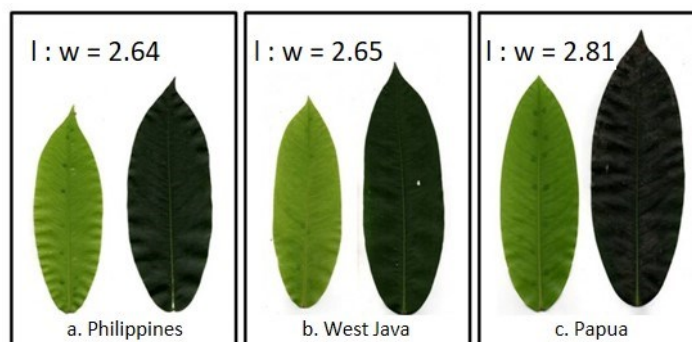
Figure 2. Length and width ratios of *Diospyros discolor* leaves from three locations. note: p = length; l = width; The color of the lower leaf surface is dark green, whereas the color of the top leaf surface is light green.

Chlorophyll content

Internal and external factors of plants influence the difference in the content of chlorophyll pigment. Internal factors include genetics, while external factors include light, nutrients, water, temperature, and soil pH. Internal factors in different genes on the chromosome will regulate chlorophyll's biosynthesis process (Kurniawan et al. 2010). These external factors will later affect pigment synthesis (Mlodzinska 2009; Juneja et al. 2013) and enzyme activity involving chlorophyll synthesis (Raharjeng 2015).

The results showed that the highest chlorophyll content was *D. discolor* from Papua (45.29), and the lowest was *D. discolor* from West Java (41.22) (Table 2). Based on the leaf position, the middle part contained the highest chlorophyll (44.99), and the lowest was in the lower leg (41.03) (Figure 3). The tree's age is why *D. discolor* from Papua has the highest chlorophyll content. *D. discolor* from Papua has the youngest tree age compared to the other two *Diospyros*. Sunlight is the main factor in determining the amount of chlorophyll content in leaves and is directly proportional to the leaf's age. Early in leaf development, leaf meristem activity causes leaf elongation, and subsequent leaf elongation occurs due to intercalary meristem activity (Hidayat 2008). Chlorophyll biosynthetic ability is thought to be different between species and cultivars. According to Ai & Banyo (2011), pigment formation in plants is influenced by internal factors, namely plant genetics. Kurniawan et al. (2010) stated that different genes on chromosomes regulate chlorophyll biosynthesis.

Table 2. Leaf chlorophyll content of *D. discolor* from three locations based on leaf age.

Leafage	SPAD value		
	Phillippines	West Java	Papua
Young leaves	26,54	18,19	25,04
Old leaves	57,37	64,24	65,53
Average	41,95	41,22	45,29

The chlorophyll content of young leaves and old leaves is other. This difference can be seen in the relatively different green colors of the leaves.

The darker green leaf color may indicate that they contain higher levels of chlorophyll. Also, chlorophyll in leaves is influenced by many things, especially genetics, light, oxygen, water, and temperature. The highest amount of chlorophyll in young leaves was found in *D. discolor* leaves from the Philippines (26.54), and the lowest in *D. discolor* leaves from West Java (18.19) (Table 2). In old leaves, the highest chlorophyll content was found in *D. discolor* leaves from Papua (65.53) and the lowest in leaves from the Philippines (57.37). This condition is influenced by genetic and environmental factors that provide phenotypic variations in the chlorophyll content of *D. discolor* leaves. Besides genetic factors, light, oxygen, water, and temperature, other factors that influence chlorophyll's formation include N, Mg, and Fe as builders and catalysts in chlorophyll synthesis. The chlorophyll content in plants is about 1% dry weight (Dwidjoseputro 1994). According to Sumenda et al. (2011), leaves' ability to photosynthesize increases until the leaves are fully developed and slowly decline. Old leaves that are almost dead become yellow and unable to photosynthesize because of chlorophyll breakdown and loss of chloroplast function.
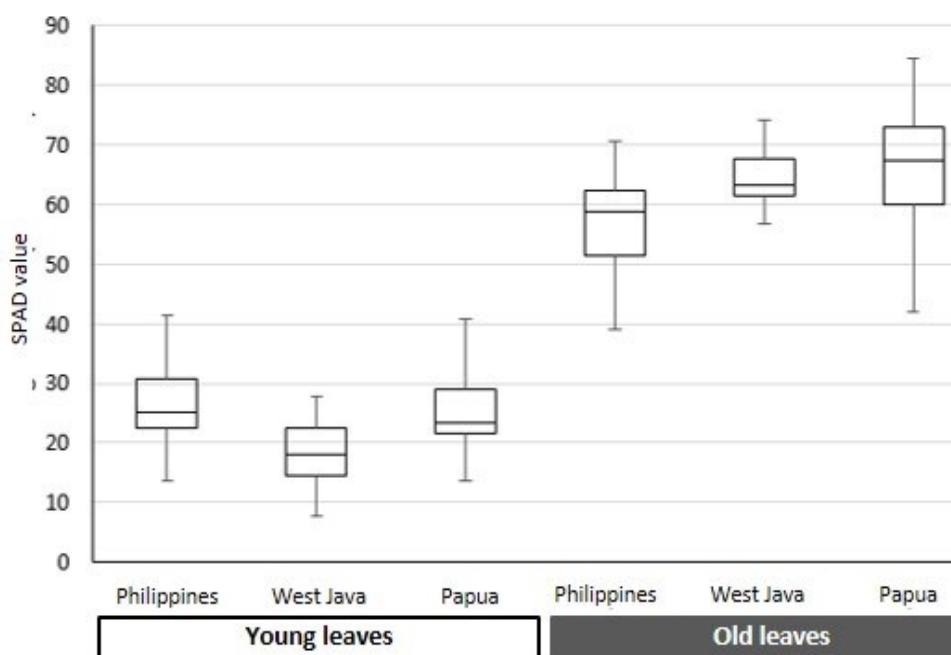
Figure 3. The chlorophyll content of *D. discolor* leaves from three different locations.

Leaf Area Analysis

Figure 4 shows that *D. discolor* originating from West Java has a leaf area formula $y = 0.765x - 2.949$, Papua is $y = 0.758x - 1.389$ and the Philippines is $y = 0.733x + 0.034$. (y) is the actual leaf area value, while (x) is the length measurement value multiplied by the leaf width. The leaf area regression analysis showed that the leaves *D. discolor* originating from West Java had the highest coefficient compared to those from the Philippines and Papua

This coefficient difference value indicates that there are morphometrically based on length, width, and leaf area variations between the three, even
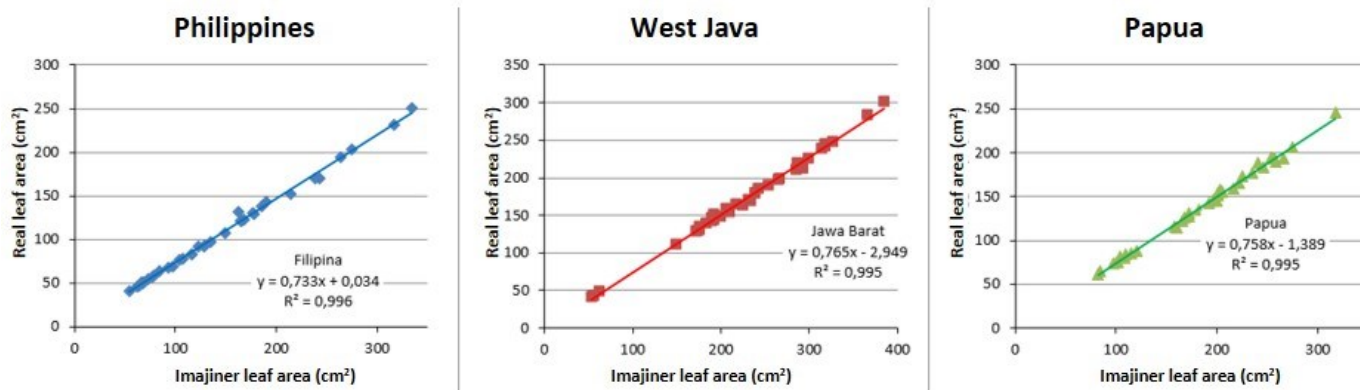
Figure 4. Relationship between imaginary leaf area and actual leaf area in *D. discolor* leaves.

though within one species. The value of the coefficient (a) in the leaf area formula ($Y = ax + b$) can be a reference to distinguish the difference in leaf area from three different locations. The greater the coefficient value, the higher the multiplier for the length and width. The coefficient will be directly proportional to the results of the leaf area calculation; the leaf area difference is thought to be because *D. discolor* underwent morphological adaptations to its environment. Changes in adaptation may cause genetic changes, so further research is needed regarding these changes. This coefficient can be used to reference further research for *D. discolor* living or originating from each location and research for other plant species. This difference is also thought to be caused by one factor, namely the condition of sun exposure. According to Sirait (2008), sunlight has an essential function in photosynthesis, affecting leaf growth.

Leaf morphometric differences can indicate canopy density, biomass, and a determinant of plant evapotranspiration. The similarity of the LAI pattern shown in Figure 4 also has implications for the health aspects of the three types of *D. discolor*, which are classified as the same. Further developments in determining the Leaf Area Index (LAI) can also estimate plant health and plant optimum productivity. LAI values have increased along with plant growth and development. The beginning of growth is marked by young leaves leading to adult development marked by aging leaves. Likewise, the leaf area in the crown and the area of protected land will also increase. Increasing the LAI value will increase the net assimilation result or what is known as the NAR (Net Assimilation Rate) (Zakariyya 2016).

CONCLUSION

The difference in plant origin significantly affects the leaf morphometrics *of D. discolor*, namely the leaf area, leaf length, leaf length and width ratio, and chlorophyll in leaves. The highest chlorophyll content was in *D. discolor* leaves from Papua, and the lowest was *D. discolor* from West Java. Based on leaf area, there are morphometrically variations between the three, even though within one species. Some environmental conditions may affect shade areas and tree age. In addition, DNA research from accession *D. discolor* is also needed to determine the cause of the morphometric variation. Further inves-

tigations into the DNA from *D. discolor* accessions are expected to confirm the taxonomic status of this species.

AUTHORS CONTRIBUTION

The author's contribution: IFW designed the research, collected and examined the data, and wrote the manuscript. ANR designed the research, collected and analyzed the data, and AAO improved the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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