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Leaf morphological traits of nine major tropical trees of *Shorea* species (Dipterocarpaceae)

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Abstract. *Mufarhatun N, Susilowati A, Hilwan I, Arrofaha N, Yulita KS, Dwiyanti FG, Hidayat A, Kamiya K, Rachmat HH. 2023. Leaf morphological traits of nine major tropical trees of* Shorea *species (Dipterocarpaceae). Biodiversitas 24: 1704-1712.* *Shorea* is the largest genus in the Dipterocarpaceae family and has high leaf morphological variations among its species, which causes difficulties in field identification. Therefore, information on the specific characteristics of the leaf morphology of each species is needed. This study aimed to examine and discriminate leaf morphological traits at both mature and sapling stages of nine *Shorea* species, namely *Shorea balangeran* (Korth.) Burck, *S. leprosula* Miq., *S. mecistopteryx* Ridl., *S. multiflora* (Burck) Symington, *S. ovalis* (Korth.) Blume, *S. pinanga* Scheff., *S. platyclados* Slooten ex Endert, *S. selanica* (Wight & Arn.) Blume, and *S. stenoptera* Burck. The leaves of 90 mature trees growing in the Dramaga Research Forest (DRF) and Gunung Dahu Research Forest (GDRF) as well as the leaves of 180 saplings growing in the nursery of Forest Research and Development Center (FRDC) were observed. Leaf traits, leaf color, and chlorophyll content were assessed on 3 leaves from each mature tree and 5 from each sapling collected. Furthermore, comparative analysis using F independent test in the one-way analysis variance (ANOVA), multivariate analysis using Principal Component Analysis (PCA), and Hierarchical Cluster Analysis were used in this study. The results showed that 8 of the 11 measured morphological traits were identified as the quantitative leaves morphological differentiators, namely Leaves Width (LW), Lamina Length (LL), the length between the largest Leaves Point (LP) with the base of the leaves, angle of leaves vein (SD), Petiole Length (PL), number of leaves vein (LB), breadth of the leaves (WL), and the Circumference of the Leaves (CL). In addition, the results of cluster analysis showed the nine *Shorea* spp. are clustered into two major groups. Group 1 consisted of species, *S. stenoptera,* and *S. mecistopteryx,* while the remaining are included in Group 2. Our findings conclude that the eight leaf morphological traits obtained from this study are useful as additional characters to distinguish the nine *Shorea* species in the field.

Keywords: Cluster analysis, leaves color, leaves morphology, morphology traits, *Shorea*

INTRODUCTION

Dipterocarpaceae is a large family of flowering plants that has a pantropical distribution (Shi et al. 2014; Ghazoul 2016), with approximately 510 species, where 470 species are found in Southeast Asia (Ng 1991; Appanah and Turnbull 1998). In Indonesia, the Dipterocarpaceae family has uneven geographical distribution across its islands, and the diversity decreases in the eastern part of the country (Appanah and Turnbull 1998). Most of the distribution of dipterocarp is in Kalimantan (200 species; 57.5%) and Sumatra (111 species; 31.9%), while to the east it is not more than 4% on each island, i.e Papua (12 species; 3.45%), Java (9 species; 2.59%), Sulawesi (7 species; 2.01%), Maluku (6 species; 1.72%), and the poorest dipterocarp species is Nusa Tenggara (3 species; 0.86%) (Purwaningsih 2004).

Shorea as the largest genus in Dipterocarpaceae is known to produce light hardwood (Ashton 1982) and some species in this genus have also produced resin that is used in the pharmaceutical and other chemical processing industries (Soni et al. 2013; Chatterjee and Ganguly 2019; Sari et al. 2022). Like other dipterocarp species, the *Shorea* group experiences a high rate of exploitation due to its high economic value, and altogether with habitat loss has led to population reduction and the extinction of the genus. About 115 dipterocarp species were listed as endangered, 224 species were critically endangered and 2 species were extinct in the wild on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species due to severe forest loss (Malik 2019). Currently, of these dipterocarps, there are 37 *Shorea* species listed as endangered, and 40 species are critically endangered (IUCN 2023).

Because of the continuous pressures on *Shorea* group, conservation is paramount to sustain their existence. In doing so, accurate identification of *Shorea* species is inevitable to support the conservation effort. Knowledge of specific morphological traits plays an important role in the identification process of plant species (Bhandarkar et al. 2014; Le et al. 2014; Yusniar and Kustiyo 2014) and also prevents misidentification of species, which causes serious negative impacts such as the accidental extermination of endangered species (Wäldchen et al. 2018). Besides for conservation purpose, the sustainable utilization of trees such as *Shorea* also requires knowledge of plant species identification to effectively make use of forest resources. However, the high level of species diversity of the *Shorea* genus which results in high morphological variations among the genus causes difficulties in identifying species in the field. Although several tree species tend to have morphological differences between the sapling and tree phases, some morphological traits persist from the sapling to the tree phases.

Species identification at different growth stages, such as sapling and mature tree phases, is necessary to determine the changes and differences between species and analyze kinship relationships. General identification aims to determine the critical traits of plant species originating from various growing places (Paria and Bose 2017). A study by (Rangkuti et al. 2021) proposed species identification of the *Shorea* genus can be carried out by measuring leaf morphological traits, such as shape and color, which are easier to observe compared to other traits.

Studies on leaf morphology provide useful information on population and intrapopulation variability and are often combined with other determination tools such as chemotaxonomic, cytological, and molecular analyses (Lind-Riehl 2014; Aykut et al. 2017; Batos et al. 2017; Esfandani-Bozchaloyi and Sheidai 2018). A quantitative study on leaves morphological traits of four *Shorea* species reported seven morphological traits which can be used to identify the species grouping (Rosdayanti et al. 2019). Maya-García et al. (2020) considered some essential morphological variables for *Quercus* spp. Further studies also identified some leaves traits determinants for *Q. dentata* Thunberg and *Q. aliena* (Liu et al. 2018).

Morphological traits of species may later be employed as an initial step in the technological development of sophisticated algorithms for application-based digital species identification. The future digital identification technique is hoped to be able to facilitate faster species identification for wider users by minimizing the dependency on classical species identification that rely on the existence of highly qualified botanist and taxonomist. To date, several smartphone-base applications have been created for plants identification, such as e-KeyPlant (Puspa et al. 2021), PictureThis (Mahonski et al. 2022), Pl@ntNet (Joly et al. 2016), PlantSnap, iNaturalist, Plantifier, etc, and have been increasing in availability, accuracy, and utilization.

In this study, we aimed to (i) collect comparative morphological data of nine *Shorea* species i.e., *Shorea balangeran* (Korth.) Burck, *S. leprosula* Miq., *S. mecistopteryx* Ridl., *S. ovalis* (Korth.) Blume, *S. pinanga* Scheff., *S. platyclados* Slooten ex Endert, *S. selanica* (Wight & Arn.) Blume, *S. stenoptera* Burck, and *S. multiflora* (Burck) Symington, (ii) test the possibility of morphological differentiation among the nine *Shorea* species, (iii) identify morphological leaf determinants, and (iv) examine certain leaf morphological traits of the nine *Shorea* species at sapling and tree phases to determine the species variation and morphological traits determinant. We expect the results of this study will provide basic information on the key leaf traits of each *Shorea* species so that it can assist in identifying these species in the field.

MATERIALS AND METHODS

Research materials and samples sources

The research focused on eight species of the red *Shorea* group (*S. balangeran*, *S. leprosula*, *S. mecistopteryx*, *S. ovalis*, *S. pinanga*, *S. platyclados*, *S. selanica*, and *S. stenoptera*) and one from the yellow *Shorea* group, namely *S. multiflora*. Leaves samples for each species were collected from both sapling and mature trees. Leaf samples at the tree stage were collected from Dramaga Research Forests (DRF) and Gunung Dahu Research Forests (GDRF), while leaf samples at the sapling stage were measured from the nursery of the Forest Research and Development Center (FRDC). All sites are administratively located in Bogor-West Java Province, Indonesia. A total of 180 sapling individuals and 90 mature trees were observed. Three leaves were collected and measured from each sapling individual and five leaves were from a mature tree, totaling 540 and 450 leaves from saplings and mature trees, respectively.

Morphological data

The morphological data were measured following Kremer et al. (2002) with a few modifications as described by Wu et al. (2007) and Ellis et al. (2009). The measured traits (Figure 1) included Lamina Length (LL), the length between the largest Leaves Point (LP) with the base of the leaves, Leaves Width (LW) at the widest point (Kremer et al. 2002), Petiole Length (PL), number of leaves vein (LB) and the formed angle between the vines of the primary leaves with a secondary vine on the right or left sides at the widest point (SD) (Ellis et al. 2009). Calculated variables included the Width of the Leaves (WL), the Circumference of the Leaves (CL), the Aspect Ratio (AR), the Form Factor (FF), and the Perimeter Ratio (PR) of diameter (Wu et al. 2007).

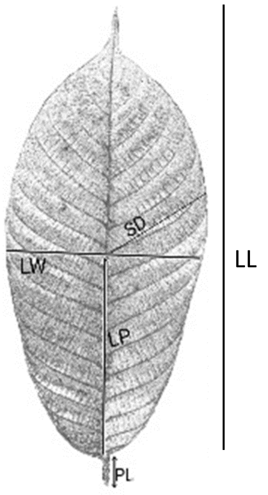


Figure 1. Measurement of leaves' morphological traits

Data analysis

The color of the leaves was measured by capturing their picture and transforming them into a Munsell chart value based on a technique developed by Kendal et al. (2013). To minimize the difference in leaves' age that may affect leaves' color determination, we selected leaves as follow: 1) seedlings: leaves from the second branch of the even-age seedlings; 2) mature individuals: leaves whose position lies on the branch in the upper third of the crown. Colorimetry was then quantified under color organization systems, called the CIELAB color space, which was standardized by the Commission Internationale de l'Eclairage (CIE) in 1976. The CIELAB system has a three-dimensional color space consisting of three axes namely L*, a*, and b* (Ly et al. 2020). The L* value indicates color lightness, while a* value indicates green to the red color of the leaves. The more negative (-) of the a* value leads to greener leaves, and vice versa. Meanwhile, the value of b* signifies the blue to the yellow color of the leaves. The more negative (-) of the value b* yields a bluer color of leaves and vice versa (Kendal et al. 2013). Furthermore, the leaves colors of the nine *Shorea* species were re-displayed by transforming the value of the results in the CIELAB system into an RGB (Red Green Blue) model to be used as a digital visual display and in the form of a Munsell Chart for direct display and to facilitate the species identification based on leaves color analysis in the field (Figure 2). In addition, the chlorophyll content was also measured using SPAD-502 CHLOROPHYLL METER.

The results on the leave morphological dimension were then analyzed using comparative and multivariate analysis. The comparative test was carried out using F independent test in the one-way analysis variance (ANOVA) to determine the differences in data and the significance of the relationships between variables. The multivariate analysis was conducted using Principal Component Analysis (PCA) to simplify the complex data by transforming them into simple dimensions and the results were displayed in a biplots diagram. Similarity among samples was estimated by performing Hierarchical Cluster Analysis with IBM SPSS STATISTICS 25 (IBM 2017). The data analyzed included a combination of morphological leaf traits and color.

RESULTS AND DISCUSSION

Results

The morphological observations based on leaves color of the studied samples of nine *Shorea* spp. showed significant differences among species, growth stages, and growing sites (Figure 2). Digitally extracted leaves' color was re-displayed as a color space value in L* a* b* format (CIELAB) with a normal distribution. Subsequently, the normality test was carried out using the Kolmogorov-Smirnov test Ghozali (2011) to determine whether a variable has normally distributed data. The result showed that the Kolmogorov-Smirnov value was higher than the 5% significance level, indicating that the digital color extract was sufficient to model the actual color. Therefore, the values generated from the CIELAB system were then transformed into an RGB model. The RGB values in the leaves were displayed as the color of the digital modeling results and the Munsell Chart (Table 1).

The leaves color differences (Table 1) on the nine *Shorea* species were observed, possibly due to the variation in the chlorophyll content. Chlorophyll content in the nine *Shorea* spp. observed in two growth stages and locations also showed different values. The chlorophyll content of *S. leprosula* showed the highest value of 46.7 nmol/cm^2 for sapling in DRF, 64.22 nmol/cm^2 for the mature trees in DRF, and 57.5 nmol/cm^2 for the mature trees in GDRF (Figure 3). Meanwhile, the lowest chlorophyll contents were discovered in the sapling of *S. mecistopteryx* in GDRF (26.3 nmol/cm^2) and the mature tree of *S. selanica* in the DRF (39.04 nmol/cm^2).

A positive relationship among leaf traits was shown by the sharp angle (<90°) and vice versa (Sumertajaya et al. 1997). The biplot of the mature leaves shared similar patterns compared to the sapling (Figure 4) and indicated the consistency of seven leaf traits (PL, LW, WL, LL, CL, LP, LB) regardless of the differences in the growing site. The trait of PR and AR showed the closest positive relationship among the stages, while SD, FF, PR, and AR constantly separated from those seven leaf traits above.

The results of Hierarchical Cluster Analysis showed that the sapling phase growing in FRDC and the mature tree phase growing in both DRF and GDRF had similar dendrogram patterns, and were clustered into two large groups, which separated *S. stenoptera* and *S. mecistopteryx* from the other seven species studied (Figure 5).

The results of the dendrogram analysis showing the formation of two large clusters were then followed by a further bi-plot analysis to see which leaf traits were the cause of the clustering formation. The formation of group 1 in the tree stage at both DRF and GDRF as well as the formation of group 1 in the sapling stage was due to the more dominant values for the LD, LS, PT, JT, LP, FF, KL and PL (Figure 6). Meanwhile, the formation of cluster 2 in tree stage at DRF was due to the dominance of SD and B values, while in GDRF was due to the value of R, G, B, and SD.

Figure 2. Actual leaves color of nine species of *Shorea* spp. observed. A. Sapling phase; B. Tree phase in DRF; C. Tree phase in GDRF. Note: 1. *S. balangeran*, 2. *S. leprosula*, 3. *S. mecistopteryx*, 4. *S. multiflora*, 5. *S. ovalis*, 6. *S. pinanga*, 7. *S. platyclados*, 8. *S. selanica*, 9. *S. stenoptera*

Table 1. Digitally-modeled color of nine *Shorea* species on saplings and mature trees grown in DRF and GDRF, West Java, Indonesia

Species	Sapling phase					Tree phase in DRF					Tree phase in GDRF				
	R	**G**	**B**	**Munsell chart**	**Color**	**R**	**G**	**B**	**Munsell chart**	**Color**	**R**	**G**	**B**	**Munsell chart**	**Color**
S. balangeran	176	213	115	7.5GY 8/8		142	188	103	10GY 7/8		135	176	93	7.5GY 7/8	
S. leprosula	160	187	101	7.5GY 8/8		132	177	91	7.5GY 7/8		134	184	93	7.5GY 7/8	
S. mecisopteryx	200	217	120	#C8D978		134	179	96	7.5GY 7/8		142	187	98	7.5GY 7/8	
S. multiflora	173	211	98	7.5GY 8/10		116	170	88	10GY 6/8		144	180	106	7.5GY 7/6	
S. ovalis	189	212	87	#BDD457		142	173	90	7.5GY 7/8		127	174	91	10GY 6/8	
S. pinanga	191	231	95	#BFE75F		128	175	105	10GY 6/6		136	187	102	10GY 7/8	
S. platyclados	175	225	101	7.5GY 8/8		132	174	101	10GY 6/6		123	176	77	7.5GY 7/8	
S. selanica	174	208	85	7.5GY 8/10		142	182	102	7.5GY 7/6		134	185	88	7.5GY 7/8	
S. stenoptera	165	204	83	7.5GY 8/10		140	181	88	7.5GY 7/8		125	170	77	7.5GY 6/8	

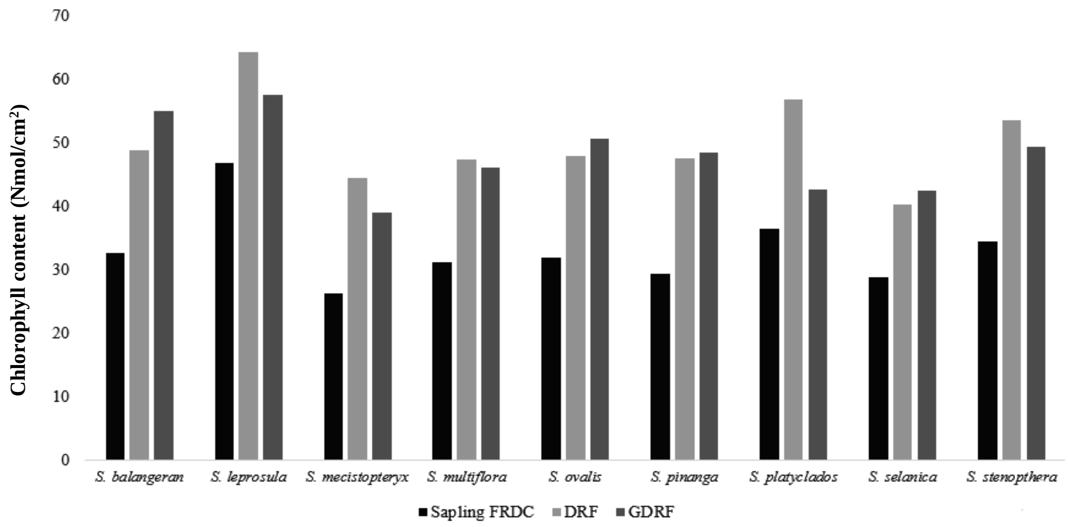
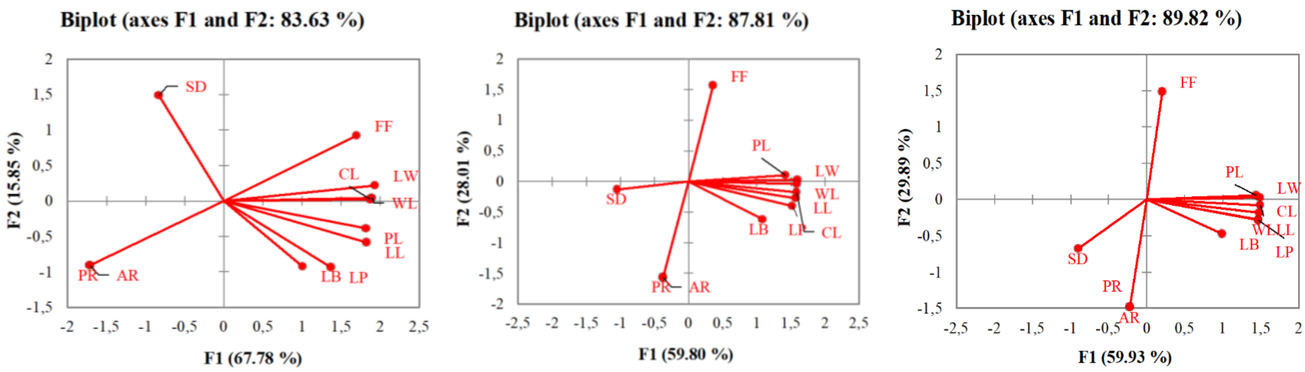
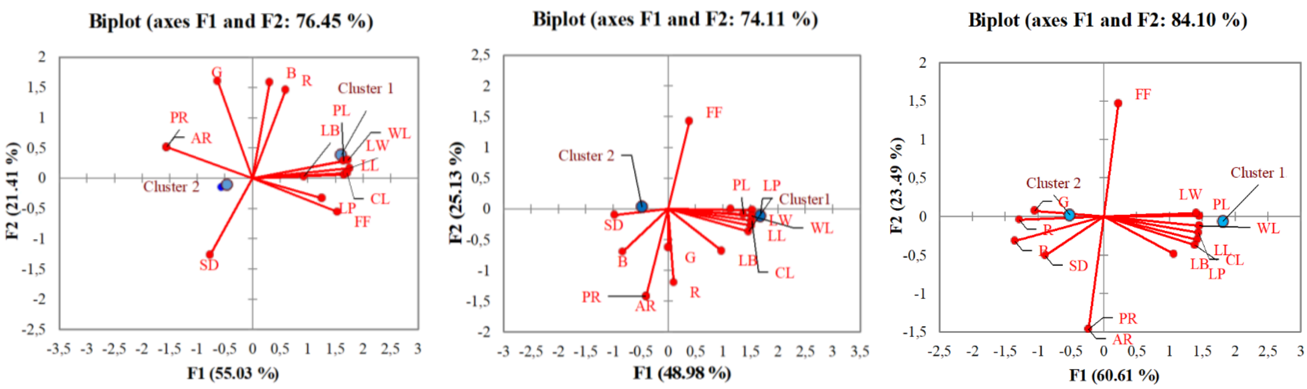


Figure 3. Chlorophyll content value of nine *Shorea* species for sapling phase in FRDC and tree phase in DRF and GDRF



A B C

Figure 4. PCA analysis of the degree of closeness of the relationship among leaves morphological traits. Sharper angle determined closer/positive relationship. A. sapling phase biplot in FRDC; **B.** tree phase biplot in DRF; C. tree phase biplot in GDRF. Notes: LW (Leaves width at the widest point), LL (Lamina Length), LP (the length between the largest Leaves Point with the base of the leaves), SD (angle between the vines of the primary leaves with a secondary vine on the right or left sides at the widest point), PL (Petiole Length), LB (number of leaves vein), WL (Width of the Leaves), CL (Circumference of the Leaves), FF (Form Factor), AR (Aspect Ratio), PR (Perimeter Ratio of diameter)



A B C

Figure 6. PCA analysis of the dominant and recessive leaf morphological traits of nine *Shorea* species observed that determining species clustering: **A.** Sapling phase in FRDC; **B.** Tree phase in DRF; **C.** Tree phase in GDRF. Notes: LW (Leaves Width at the widest point), LL (Lamina Length), LP (the length between the largest Leaves Point with the base of the leaves), SD (angle between the vines of the primary leaves with a secondary vine on the right or left sides at the widest point), LP (Petiole Length), LB (number of leaves vein), WL (Width of the Leaves), CL (Circumference of the Leaves), FF (Form Factor), AR (Aspect Ratio), PR (Perimeter Ratio of diameter), R (Red), G (Green), and B (Blue)

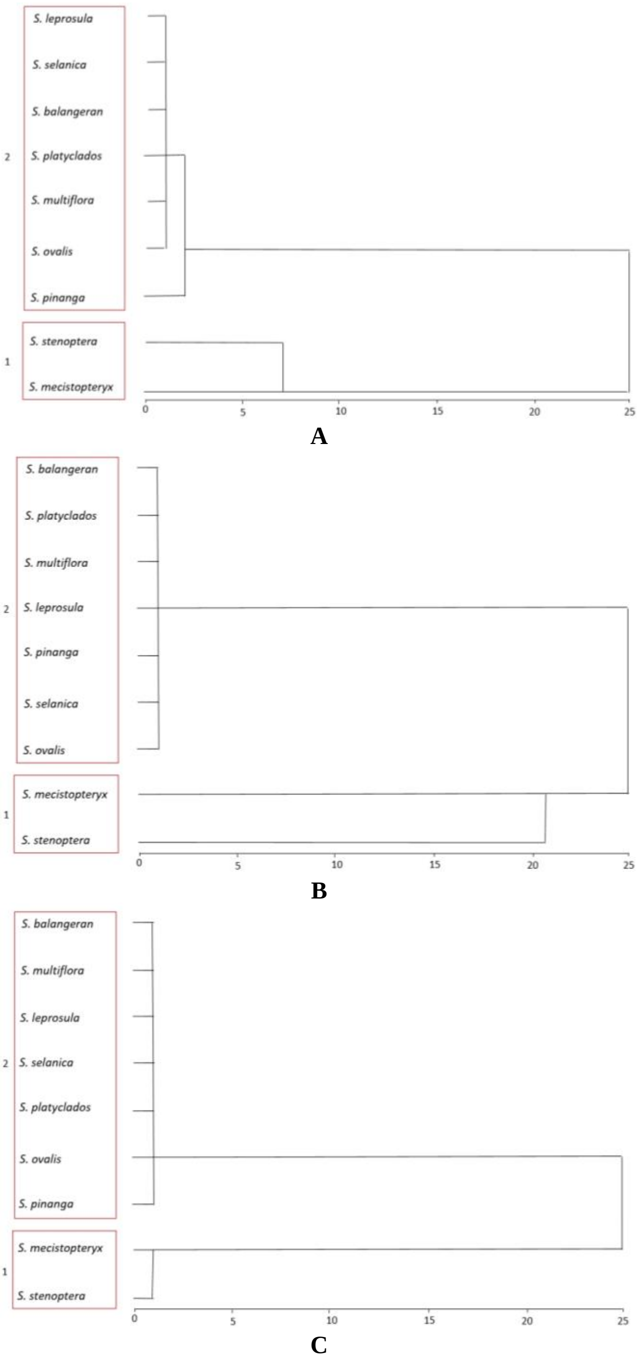


Figure 5. Dendrogram analysis of nine Shorea spp. observed. A. Sapling phase in FRDC; B. Tree phase in DRF; C. Tree phase in GDRF

Discussion

The results from the sapling stage showed that *S. leprosula* had the darkest, while *S. mecistopteryx* had the lightest leaf color (Table 1). The darkest color at a mature stage both in GDRF and DRF was shown by *S. leprosula*, while the lightest in GDRF and DRF was shown by *S. mecysopterix* and *S. selanica*, respectively (Table 1). The darkest color on the leaves of *S. leprosula* in the sapling and tree phases may be caused by the chlorophyll content, which in this study revealed that the chlorophyll content of *S. leprosula* was higher than other species, while the lightest color of the leaves of *S. mecisopteryx* in the sapling and tree phases could be due to the lower chlorophyll content (Figure 3).

In all species observed, the chlorophyll content at the tree phase was higher than that at the sapling phase (Figure 3). This result is in accordance with Harmens et al. (2017) who reported that the chlorophyll content in a leaf increases with the age of the plant due to chlorophyll biosynthesis that occurrs during the development of young leaves into tree leaves. In the mature tree, the chlorophyll content is higher compared to the leaves in the young phase because individual trees require a lot of nutrients for their life as an energy source. Therefore, the chlorophyll biosynthesis process occurs more in the tree phase than in the young phase (Ajiningrum 2019; Solikhah et al. 2019). Plants with a high chlorophyll content carry out the photosynthesis process more optimally than plants with low content (Hidayati et al. 2016; Wang et al. 2017).

Shorea leprosula has a moderate growth rate and easily adapts to a good environment (Erizilina et al. 2019). *S. leprosula* is widely spread and generalist species (Ng et al. 2020). However, when compared to other *Dipterocarp* species, *S. leprosula* is categorized as a fast-growing *Shorea* species (Mashudi 2017; Ngatiman and Fajri 2018). *S. leprosula* is one of the priority target species to be mass planted in the national program called Intensive Silviculture System (SILIN) with diameter growth ranging from 1.15 to 2.20 cm/year on various experimental sites (Naiem et al. 2014; Pamoengkas and Prasetia 2014). High chlorophyll content as shown in this study can be a factor supporting its ability to adapt and grow fast. According to Salisbury and Ross (2010), high chlorophyll causes relatively more leaves and faster plant growth. Several species that also show high chlorophyll content with faster growth than others are *S. stenoptera* and *S. platyclados* (Gustafsson et al. 2016; Mufarhatun et al. 2021), which are also recommended for the Intensive Silviculture (SILIN) system (Regulation of Director General Sustainable Production Forest Management 2018). However, the results of this study are not intended to suggest that chlorophyll content can be the primary determinant in identifying the nine *Shorea* species studied.

The PCA analysis (Figure 6) performed at the sapling phase growing in FRDC and mature tree phase growing in DRF and GDRF illustrated by a biplot diagram showed a similar pattern between phases, which indicated that the PL (Petiole Length), CL (Circumference Leaves), LW (Leaves Width at the widest point), WL (Leaves Width), LP (length between the largest Leaves Point with the base of the leaves), LL (Lamina Length), and LB (number of leaves vein) variables had a positive relationship due to the <90° angle. This showed that when one of these variables has a high value, the other variables follow. Moreover, the AR (Aspect Ratio) and PR (Perimeter Ratio of diameter) variables had a negative relationship with the FF (Form Factor) due to the 180° angle.

The results of the 11 variables of leaves morphological traits observed showed that only 8 variables had a significance value < 0.05, while others had a significance value > 0.05 (Figure 4). This indicated that the observed variables simultaneously had a significant influence on all *Shorea* species and were different from one species to another. Harnelly et al. (2020) and Meinata et al. (2021) suggested that the *Shorea* group had almost the same elongated leaves shape and was not too round, while the AR, FF, and PR variables were physical leaves shape variables that quantitatively determined the shape of the leaves blade, elongation, and roundness (Wu et al. 2007). Therefore, AR, FF, and PR traits showed weak variables as species determinants to discriminate species within the *Shorea* genus. This is supported by Rosdayanti et al. (2019) who reported that the four *Shorea* species in their study had the same leaves tip shape and 7 variables out of the 12 morphological traits influenced leaf morphological traits, namely Circumference Leave (CL), Width of the Leaves (WL), Lamina Length (LL), Length from base to the widest Point (LP), Ratio Aspect (AR), Form Factor (FF), and Perimeter Ratio of diameter (PR). According to Ponton et al. (2004), 2 variables could not distinguish species in the case of young seedlings because they produced 11% misclassification in the sample used such as the variable length of the petiole and the number of leaves vines.

Clustering results based on Dendrogram analysis in this study showed that *Shorea mecisopteryx* and *S. stenoptera* clustered in group 1 and the other seven species clustered in group 2 (Figure 5). *S. mecisopteryx* and *S. stenoptera* are members of Section *Pachycarpae* (Tengkawang group). According to Ashton (1982), the classification of the sections within the genus *Shorea* was based on flower and fruit characteristics. The result from this analysis suggests that the clustering of *S. stenoptera* and *S. mecistopteryx* in group 1 was based on the similar size or shape of the leaves. This is possible to conclude because another member of Section *Pachycarpae* that is used in this study, *S. pinanga,* is included in group 2 which has a smaller leaf size compared to *S. stenoptera* and *S. mecisopteryx*. Group 2 consisted of seven species and belongs to sections of red *Shorea*, i.e. *Section Mutica* (*S. leprosula*), *Pachycarpae* (*S. pinanga*), and *Brachypterae (S. balangeran, S. platyclados,* and *S. selanica)* and *Section Richetioides* of the yellow *Shorea* (*S. multiflora*). The inclusion of *S. multiflora* was due to the quantitative physical shape of the leaves and the leaves color.

Biplot analysis conducted to identify leaf traits determinants showed that group 1 was formed because it had more dominant LP, WL, PL, LB, LP, FF, CL, and LL values. Meanwhile, the formation of group 2 was due to higher AR and PR values, which are almost the same for each species. The formation of group 1 in the mature tree phase both in DRF and GDRF was similar to the sapling phase because it had more dominant values of LW, WL, PL, LB, LP, FF, CL, and LL (Figure 6). Meanwhile, the formation of group 2 in the tree phase in DRF had dominant SD and B values. Furthermore, the tree phase in GDRF formed group 2 since each species had almost the same R, G, B, and SD values. Figure 6 also shows that the leaf color of the *Shorea* group cannot be used as a leaf trait for species determination unless it is processed digitally into RGB form to obtain an absolute value in distinguishing the species. Thus, leaf color can be a good determinant in discriminating species only when further processed into RGB.

In conclusion, this study revealed that eight of eleven variables of leaf morphological traits could be used to quantitatively distinguish nine *Shorea* spp. both at sapling and mature tree. The eight variables are LW (Leaves Width), LL (Lamina Length), LP (lengthy of the widest leaves), SD (angle of leaves vein), PL (lengthy of leaves stem), LB (number of leaves vein), WL (breadth of the leaves), and CL (Circumference of the Leaves). Leaves colors are also possibly used to identify the species, and further extraction of the digital color showed that the tree phase is darker than the sapling phase. These leaf morphological traits derived from this study are expected to be used as additional characters to distinguish nine *Shorea* species in the field.

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