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Identification of Red Meranti Group (*Shorea* spp., Dipterocarpaceae) Saplings Based on Variations in the Morphological Features of Quantitative Leaves

(Identifikasi anakan Kelompok Meranti Merah (Shorea **spp., Dipterocarpaceae)** *Bedasarkan Variasi Ciri Morfologi Daun Kuantitatif)*

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ABSTRACT

Shorea is the largest genus within the family of Dipterocarpaceae, a major timber tree dominating tropical forest in South East Asia. The genus of Shorea has many similarities, and species identification is often tricky. Most Shorea (Dipterocarpaceae) species perform as a big emergent tree; thus, species discrimination at seedlings level will benefit practical use in the field scale. A study of variations of leaf morphology and color on Red Meranti seedlings growing in an experimental nursery. A total of 450 individuals of 29 species of the Red Meranti were measured for their leaf characters. Data analysis was performed using the application R Statistics 3.6.0, RawTherapee 5.5, ImageJ 1.32, nixsensor, and encycolorpedia.id to obtain the leaf color of the observed species. Cluster analysis (Hierarchical Cluster Analysis) and Principal Component Analysis (PCA) were executed using IBM SPSS Statistics 25. The results showed that 11 of 13 observed leaf characters were, all variables support the grouping and species kinship, and those can be as determinants, except for AS and BS. Leaves color may be helpful in species determination only if transformed into digital color. This study result supports current taxonomical grouping based on flower and fruit characteristics.

1. Introduction

Shorea is one of the dominant tropical tree species that have high economic, ecological and environmental functions. The largest genus within Dipterocarpaceae can be classified into four timber groups, i.e., red, white meranti, yellow meranti, and *Balau* group. The red meranti is the largest group consisting of more than 70 species with wide distribution covering Malaya Peninsula, Southern Thailand, Sumatra, Kalimantan, Moluccas and the Philippines (Soerianegara & Lemmens, 2002).

Leaves-based morphological species identification has been the most general practice carried out at the field scale as they are visible. Thus, the variations could be assessed quickly when compared with other characters (Hartvig, Czako, Kjaer, Nielsen, & Theilade, 2015). This identification is also common within *Shorea* red meranti group. However, the identification is still challenging due to many morphological similarities (Yusniar & Kustiyo, 2014). Nevertheless, this technique has also been known to have weaknesses as this marker strongly

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influenced by the environment (Paria & Bose, 2017).

According to Ashton (1982), the red meranti is grouped into five sections based on their different flower and fruits, namely the *Brachypterae*, *Mutica*, *Ovalis*, *Pachycarpae* and *Rubella* sections. In addition, Newman et al. (1996) reported that Section *Brachypterae, Mutica, Ovalis, Pachycarpae* and *Rubella* are considered as light hardwood, while the balau group (Section *Shorea*) and the yellow meranti (Section *Richetioides*) are considered as heavy hardwood.

In general, *Shorea* is known for its emerging tree with cylindrical buttressed bole. The diameter may reach 45-50 cm (Ashton, 1982) with a height up to 70-80 m that make the trees occupy the top layer (stratum A) at a forest landscape. However, some species stand as the canopy layer in stratum B (Newman et al., 1996). Therefore, species identification at the field scale based on leaf morphology is highly constrained due to their height. Seedlings identification from the offspring growing near the mother trees becomes an alternative and an indirect approach to identifying the intended mother tree. Therefore, it is essential to develop an alternative identification method on seedlings to assist identification of the target parent trees.

2. Methodology

2.1. Study site

The study was conducted at the Nursery of Forest Research and Development Centre, Bogor City, Indonesia, from September to November 2019.

2.2. Samples

A total of 450 nursery-grown seedlings of 29 *Shorea* species belonging to the red meranti group were measured, consisting of 5-20 individuals for each species. Thirteen leaf characters were measured on 3 to 5 leaves collected from the upper part of each individual, summing 1500 leaves in total.

2.3. Measurements

Measurement of morphological data was carried out as those developed by Kremer et al. (2002) with some modifications to the method of Wu et al. (2007) and Ellis et al. (2009). The measured characteristics included lamina length (LL), petiole length (PL), leaf width at its broadest point (LW) Kremer et al. (2002), the length between the largest leaf point with the base of the leaf (LP) Ellis et al. (2009), and an angle formed between the primary and the secondary vein on the right or left sides at the broadest leaf point (SD) (Kremer et al., 2002; Ellis et al., 2009). The number of leaf veins, the shape of leaf tip (AS) and the leaf base (BS) were following (Ash et al., 1999). The measurement of leaf width (WL), the circumference of the leaf (CL), aspect ratio (AR), form factor (FF), and perimeter ratio of diameter (PR) was based on Wu et al. (2007), with the following formula:

$$WL = \frac{1}{2} \text{ x } \boldsymbol{\pi} + (\boldsymbol{LW} \text{ x } \boldsymbol{LL}) \quad (1)$$

$$CL = \frac{1}{2} \text{ x } \boldsymbol{\pi} + (\boldsymbol{LW} + \boldsymbol{LL}) \quad (2)$$

$$AR = \frac{LL}{LW} \quad (3)$$

$$FF = \frac{4\pi \, X \, WL}{CL^2} \quad (4)$$

$$PR = \frac{CL}{LW} \quad (5)$$

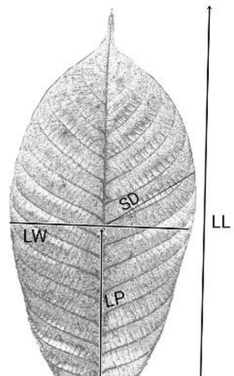


Figure 1. Measurement of leaf morphological characteristics

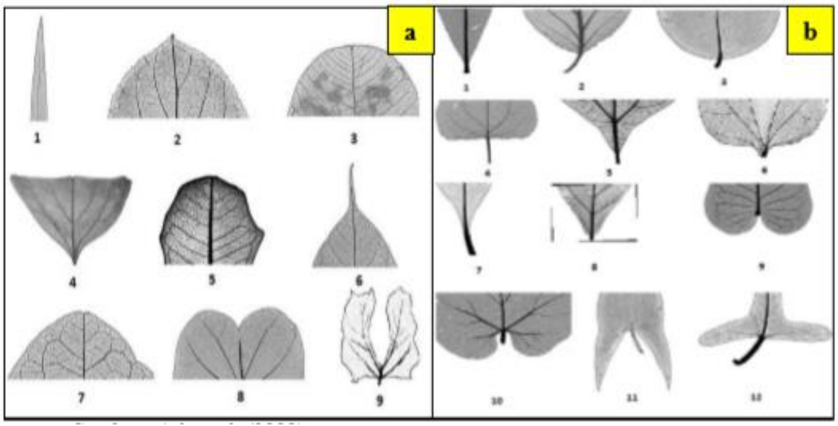


Figure 2. The shape of leaf tip (a) and the shape of leaf base (BS) (b) based on Ash et al. (1999)

Leaves' color was determined by capturing their picture and transforming them into a Munsell chart value based on a technique developed by Kendal et al. (2013). Chlorophyll content was measured using of SPAD-502 Chlorophyll Meter.

2.4. Data analysis

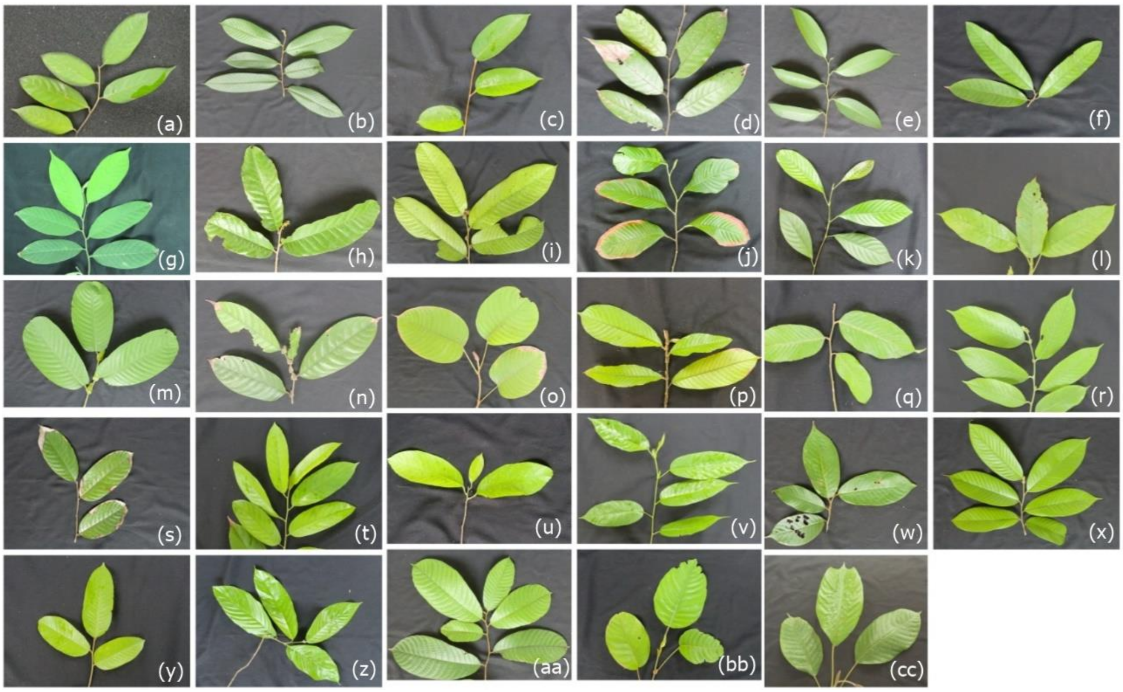
The morphological dimensions were analyzed by using comparative and multivariate analysis. The comparative test was carried out using a *one-way ANOVA F independent* test to quantify the differences and the significance of the relationships between variables. At the same time, multivariate analysis was performed with Principal Component Analysis (PCA) to simplify the complex data by transforming it into simple dimensions. The results of transformation were displayed in the form of biplots. In addition, kinship analysis and similarities among the red meranti group species were performed using the Hierarchical Cluster Analysis method in the IBM SPSS Statistics 25. The analyzed data was a combination of morphological leaf characteristics and leaves color.

3. Results and Discussions

The results of morphological observations based on leave color of 29 red meranti (*Shorea* spp.). The color variations between species are showed in Figure 2.

This study showed that digital leaves color could be used as a standard model for identifying red meranti seedlings. All leaf colors were normally distributed following the result of the Kolmogrov-Smirnov test for distribution normal Gaussian at the 0.05 confidence level. In addition, color characterization determined that the leaves of the seedlings had a high green color and brightness with a somewhat yellowish leaf (Table 1).

The morphological differences of leaves color among these *Shorea* spp. might be due to differences in chlorophyll content. Chlorophyll is a green pigment found in chloroplasts at the palisade and leaf sponge parenchyma. They are very important in converting light into chemical energy that is further stored in plants. Chlorophyll content directly determines the potential photosynthesis rate and primary production (Croft & Chen, 2017). The highest chlorophyll content among the group was showed by *S. leprosula* at 46.7 nmol/cm2, while the lowest was *S. macrophylla* of at 18.6 nmol/cm2. Statistical analysis showed that the chlorophyll content differences among species were significantly different at level 0.05.



Remarks: (a) *S. acuminata*, (b) *S. amplexicaulis*, (c) *S. balangeran*, (d) *S. beccariana*, (e) *S. curtisii*, (f) *S. curtisii* subsp. *grandis*, (g) *S. dasyphylla*, (h) *S. fallax*, (i) *S. hemsleyana*, (j) *S. johoriensis*, (k) *S. leprosula*, (l) *S. macrantha*, (m) *S. macrophylla*, (n) *S. martiniana*, (o) *S. mecisopteryx*, (p) *S. ovalis*, (q) *S. palembanica*, (r) *S. parvifolia*, (s) *S. parvistipulata*, (t) *S. pinanga*, (u) *S. platycarpa*, (v) *S. platyclados*, (w) *S. rugosa*, (x) *S. scaberrima*, (y) *S. selanica*, (z) *S. singkawang*, (aa) *S. smithiana*, (bb) *S. stenopthera*, (cc) *S. teysmaniana*

Figure 3. Actual leaf color of 29 *Shorea* spp. of the red meranti species

Table 1. Digitally-modeled color of 29 *Shorea* red meranti species, and their chlorophyll content

No	Species	RGB			Munsell Charts	Digital color	Chlorophyl content (nmol/cm2)
		R	G	B			
1	*S. leprosula*	160	187	101	5GY 7/6		46.7
2	*S. rugosa*	161	190	101	7.5GY 7/8		43.4
3	*S. curtisii*	153	180	98	7.5GY 7/6		40.5
4	*S. acuminata*	163	202	102	7.5GY 8/10		41.9
5	*S. singkawang*	160	209	100	7.5GY 8/10		39.3
6	*S. smithiana*	162	198	111	7.5GY 7/8		38.2
7	*S. teysmaniana*	161	205	111	7.5GY 8/8		37.6
8	*S. parvifolia*	154	199	110	7.5GY 7/8		39.8

No	Species	RGB			Munsell Charts	Digital color	Chlorophyl content (nmol/cm2)
		R	G	B			
9	*S. dasyphylla*	169	198	116	7.5GY 7/6		30
10	*S. johoriensis*	178	202	110	7.5GY 8/8		35
11	*S. balangeran*	176	213	115	7.5GY 8/8		32.4
12	*S. fallax*	176	215	109	7.5GY 8/8		35.8
13	*S. scaberrima*	183	214	107	7.5GY 8/8		34.3
14	*S. parvistipulata*	181	219	113	7.5GY 8/8		30.5
15	*S. palembanica*	174	211	124	7.5GY 8/6		32
16	*S. platyclados*	175	225	101	7.5GY 8/8		36.4
18	*S. selanica*	174	208	85	7.5GY 8/10		28.8
17	*S. curtisii* subsp. *grandis*	170	215	88	7.5GY 8/10		34
19	*S. macrantha*	160	204	81	7.5GY 8/10		35.6
20	*S. stenopthera*	165	204	83	7.5GY 8/10		34.5
21	*S. hemsleyana*	170	200	89	7.5GY 8/10		35.5
22	*S. platycarpa*	155	204	73	7.5GY 8/10		32.3
23	*S. amplexicaulis*	172	201	138	7.5GY 8/6		32.6
24	*S. ovalis*	189	212	87	5GY 8/8		31.8
25	*S. pinanga*	191	231	95	5GY 8/8		29.3
26	*S. mecisopteryx*	200	217	120	5GY 8/6		26.3
27	*S. beccariana*	193	214	147	7.5GY 8/6		23.2
28	*S. martiniana*	197	211	157	5GY 8/4		23.2
29	*S. macrophylla*	198	227	161	7.5GY 9/4		18.6

Plants with higher chlorophyll values will perform more optimum photosynthesis than lower chlorophyll content (Wang, Li, Liu, Lv, & Wang, 2017; Yustiningsih, 2019). Chlorophyll is essential in photosynthesis by absorbing light and producing energy (Putri, Suedy, & Darmanti, 2017). The optimal photosynthesis process will produce sugar and oxygen, which acts as food to support plant growth (Limantara, Dettling, Indrawati, Indriatmoko, & Brotosudarmo, 2015). Sufficient food support is beneficial for vegetative organs (Hendriyani, Nurchayati, & Setiari, 2018) and will cause relatively more leaf growth and relatively faster growth (Zhang, Huang, Bian, & Zhao, 2013).

S. leprosula is a moderate growing tree with high ability to adapt to various environment condition (Mawazin & Suhaendi, 2012; Prameswari, Supriyanto, Saharjo, Wasis, & Pamoengkas, 2015; Erizilina, Pamoengkas, & Darwo, 2019) and also known as light-demanding species in the early stage of growth (Abdurachman, Apriani, & Noor, 2013; Erizilina et al., 2019). *S. leprosula* is widespread and generalist species (Achmad, 2017; Kit, Ng, Lee, Tnah, & Ng, 2020) and compare to other Dipterocarps species, it is categorized as fast growing meranti (Mashudi, Pudjiono, Rayan, & Sulaeman, 2012; Ngatiman & Fajri, 2018; Tirkaamiana, Partasasmita, & Kamarubayana, 2019) and those listed as one of priority target to be massively planted on Sistem Silvikultur Intensif (SILIN/intensive silviculture technique) with diameter growth at the range 1.15-2.20 cm/year across various experimental result (Mawazin & Suhaendi, 2011; Widiyatno, Naiem, Hardiwinoto, & Purnomo, 2011; Pamoengkas & Prasetia, 2014; Widiyatno, Soekotjo, Naiem, Purnomo, & Setiyanto, 2014). The high chlorophyll content may become support factors to its ability both in its adaptation capability and fast growth. Some species that showed high chlorophyll content with faster growth than others are *S. leprosula* dan *S. platyclados*. However, this particular result is not meant to propose that chlorophyll content may become the key determinant in identifying or classifying species within *Shorea* in the red meranti group.

In general, the quantitative leaf morphological characteristics of 29 species of red meranti (*Shorea* spp.) have an elongated shape. This is because the value is AR > 1. In addition, the leaf oval and roundness rates of all observed species had almost the same value. It can be seen from the large FF and PR values. Meanwhile, the longest leaf is *S. martiniana* and the shortest is *S. dasyphylla* (Table 2).

Table 2. Quantitative leaf morphological characteristics data on 29 species of red meranti (*Shorea* spp.)

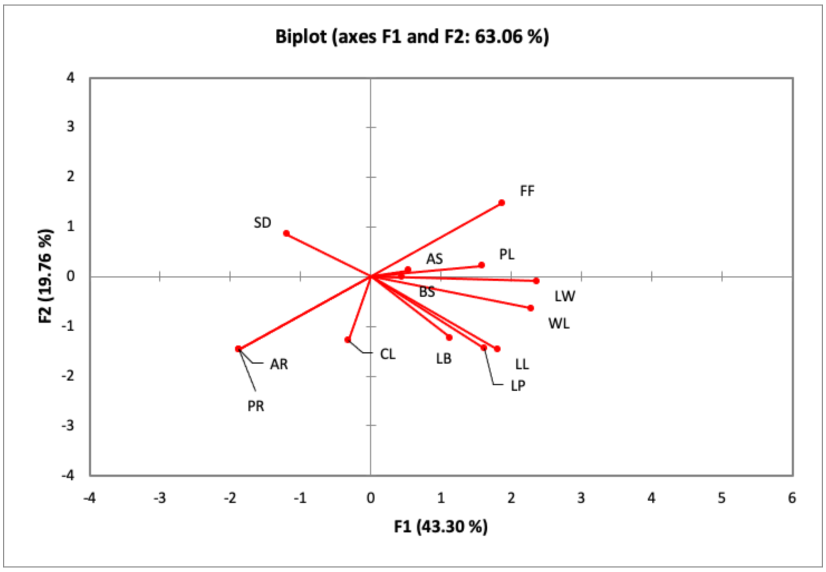
Species	LL (cm)	LW (cm)	SD (º)	LP (cm)	PL (cm)	LB	WL	CL	AR	FF	PR
S. acuminata	15.04	6.24	43.00	7.59	1.24	23	147.32	33.41	2.41	1.66	5.35
S. amplexicaulis	9.23	5.09	37.55	4.18	0.79	20	73.79	22.49	1.81	1.83	4.42
S. balangeran	10.26	3.18	26.18	3.18	1.35	20	51.16	21.09	3.23	1.44	6.64
S. beccariana	20.88	9.82	26.06	9.93	8.32	30	321.84	48.20	2.13	1.74	4.91
S. curtisii	18.39	6.77	36.44	7.61	0.86	31	195.36	39.49	2.72	1.57	5.84
S. curtisii subsp. *grandis*	18.73	6.95	32.67	8.32	0.82	41	204.41	40.32	2.70	1.58	5.80
S. dasyphylla	8.91	3.99	28.97	3.93	1.25	22	55.86	20.26	2.23	1.71	5.07
S. fallax	14.96	5.03	33.56	5.71	1.89	21	118.18	31.38	2.97	1.51	6.24
S. hemsleyana	11.30	4.18	29.67	4.77	0.88	20	74.22	24.31	2.70	1.58	5.81
S. johoriensis	11.06	5.85	38.62	5.93	1.26	27	101.64	26.56	1.89	1.81	4.54

Species	LL (cm)	LW (cm)	SD (°)	LP (cm)	PL (cm)	LB	WL	CL	AR	FF	PR
S. leprosula	15.35	6.87	34.17	7.23	0.87	24	165.48	34.88	2.24	1.71	5.08
S. macrantha	10.48	4.02	35.09	3.99	0.74	21	66.05	22.76	2.61	1.60	5.67
S. macrophylla	18.61	15.75	22.37	7.58	7.67	25	460.09	53.94	1.18	1.99	3.43
S. martiniana	29.01	13.13	28.67	14.44	2.30	39	597.93	66.16	2.21	1.72	5.04
S. mecisopteryx	20.42	17.62	19.56	7.26	9.62	31	550.23	59.00	1.19	1.99	3.44
S. ovalis	10.22	4.57	34.94	3.68	1.78	25	73.25	232.10	2.24	1.71	5.08
S. palembanica	11.22	5.51	36.57	6.65	2.24	37	96.97	26.26	2.04	1.77	4.77
S. parvifolia	13.77	5.72	40.00	5.91	1.08	23	123.63	30.59	2.41	1.66	5.35
S. parvistipulata	17.78	5.35	29.33	8.58	1.22	31	149.37	36.32	3.32	1.42	6.79
S. pinanga	26.88	13.72	25.67	14.63	2.50	32	578.94	63.74	1.96	1.79	4.65
S. platycarpa	12.50	5.05	37.81	6.88	1.68	24	99.17	27.56	2.47	1.64	5.45
S. platyclados	21.78	9.97	26.83	9.08	1.13	23	340.86	49.85	2.19	1.72	5.00
S. rugosa	19.07	7.93	32.17	7.92	1.15	26	237.48	42.39	2.40	1.66	5.34
S. scaberrima	14.33	5.40	37.07	8.82	1.04	34	121.46	30.97	2.65	1.59	5.74
S. selanica	19.85	8.87	25.17	8.67	0.92	30	276.33	45.09	2.24	1.71	5.09
S. singkawang	17.22	6.81	28.47	7.34	5.01	29	184.03	37.72	2.53	1.63	5.54
S. smithiana	14.14	8.96	58.72	5.79	2.18	23	198.80	36.26	1.58	1.90	4.05
S. stenopthera	20.38	14.62	19.33	9.31	1.21	32	467.57	54.94	1.39	1.95	3.76
S. teysmaniana	11.59	4.93	39.03	4.56	0.82	27	89.60	25.93	2.35	1.67	5.26

Remarks: LW (Leaf width), LL (Lamina length), LP (lengthy of the widest leaf), SD (angle of leaf vein), PL (lengthy of leaf stem), LB (number of leaf vein), WL (breadth of the leaf), CL (circumference of the leaf), FF (form factor), AR (aspect ratio), PR (perimeter ratio of diameter)

The analysis results using PCA (Figure 4) showed that the variables of PL, BS, LW, WL, LP, LL, and LB had a positive relationship, which means that high value of one variable will be followed by high value of other variables and vice versa. On the other hand, AR and PR variables negatively correlated with FF, while SD characteristics negatively correlated with LP.

All observed leaf morphological characteristics were statistically significant, except for the variable of tip shape (AS) and leaf base (BS). The two variables have the same value for each observed species, so that these variables cannot be used as a key determinant in species identification or grouping. Rosdayanti et al. (2020) reported that seven of 12 observed morphological characters (i.e., the circumference of leaf, area of the leaf, lamina length, leaf width, aspect ratio, form factor, and perimeter ratio of diameter) could be used as the key determinant variables for the identification of *S. ovalis*, *S. leprosula*, *S. parvifolia*, and *S. guis*o. Meanwhile, García, Miranda, Reyes, & Oyama (2020) considered that the most important morphological variables were specific leaf area, leaf width, and the length of both the lamina and petiole. However, a study on *Quercus dentata* Thunberg and *Quercus aliena* Blume showed that morphological characters of petiole length and the length between the largest leaf point with the base of leaf and leaf width at its widest point have been reported to be the key determinants in identifying species or clustering the group (Liu et al., 2018). Another study on *Quercus alba* L., *Quercus palustris* Muench *and Quercus velutina* Lam. showed that morphological characters of leaf mass per area, petiole length, leaf area and the formed angle between the vein of the primary leaf with the secondary vein on the right or left sides at the broadest leaf point had been reported to be the key determinants in identifying species or clustering the group (Kusi & Karsai, 2019). According to this study and previous study, the variable of leaf, lamina length, leaf width, and petiole length were recorded to be the major and consistent determinants in species determination both for *Shorea* and non-*Shorea*.



Remarks: LW (Leaf width), LL (Lamina length), LP (lengthy of the widest leaf), SD (angle of leaf vein), PL (lengthy of leaf stem), LB (number of leaf vein), AS (shape of leaf tip), BS (shape of leaf base), WL (breadth of the leaf), CL (circumference of the leaf), FF (form factor), AR (aspect ratio), PR (perimeter ratio of diameter)

Figure 4. Relationship of observed morphological leaves characteristics in 29 *Shorea* red meranti species

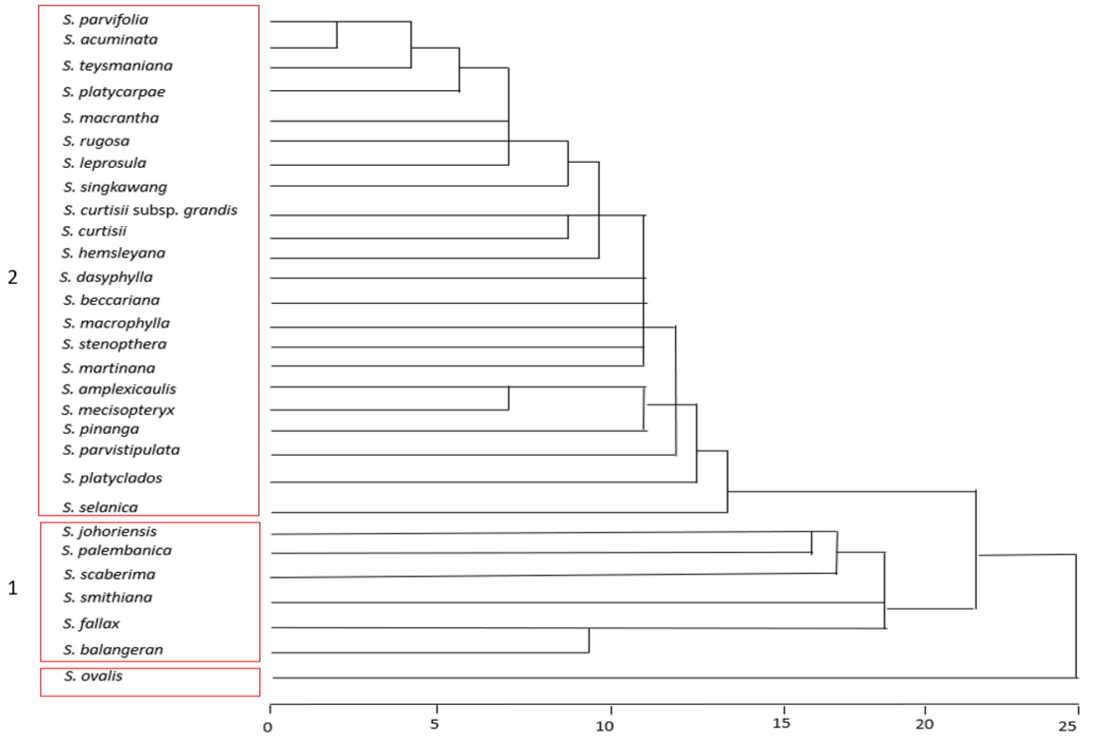
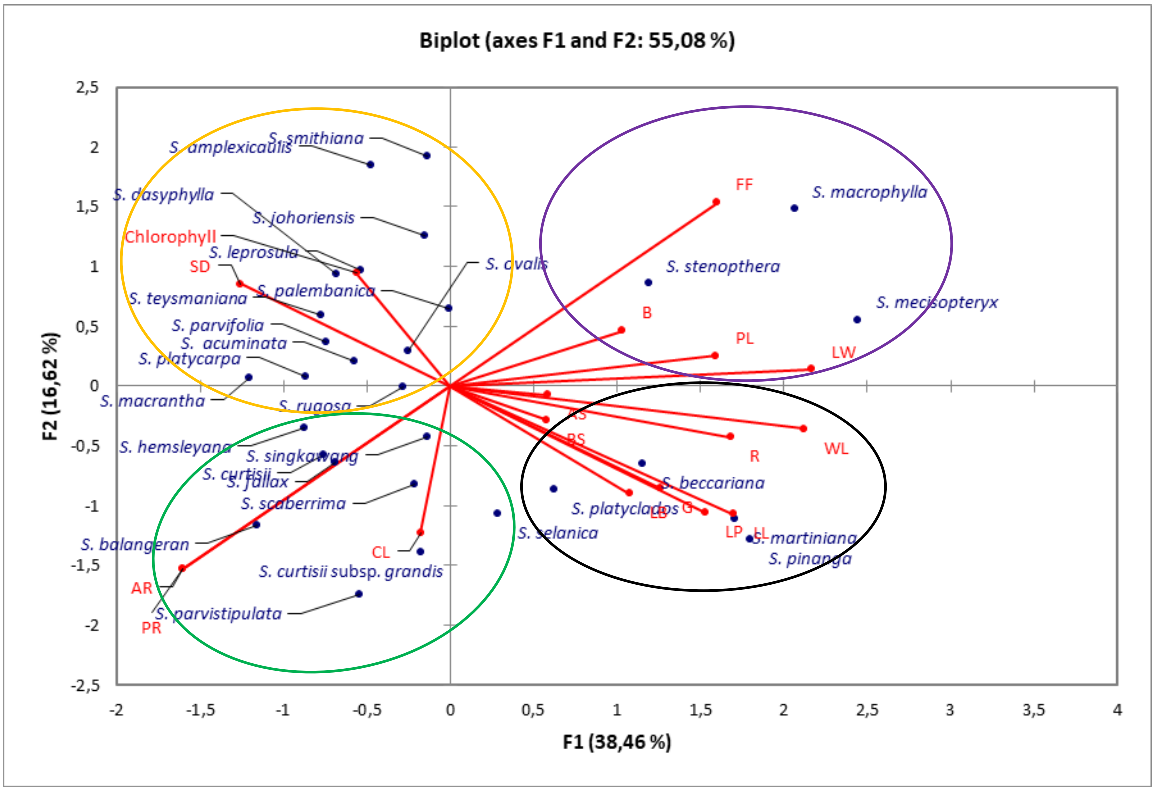


Figure 5. Leaves morphological clustering analysis of 29 *Shorea* red meranti species

The result of a clustering analysis (Figure 5) determined three clusters that consisted of two main groups with *S. ovalis* separated from the two groups. Cluster 1 (*S. balangeran*, *S. fallax*, *S. smithiana*, *S. scaberrima*, *S. palembanica*, and *S. johoriensis*) and cluster 2 (*S. selanica*, *S. platyclados*, *S. parvistipulata*, *S. acuminata*¸*S. parvifolia*, *S. teysmaniana*, *S. platycarpa*, *S. macrantha*, *S. rugosa*, *S. leprosula*, *S. singkawang*, *S. curtisii*, *S. curtisii* subsp. *grandis*, *S. hemsleyana*, *S.*, *S. dasyphylla*, *S. beccariana*, *S. macrophylla*, *S. stenopthera*, *S. martiniana*, *S. amplexicaulis*, *S. mecisopteryx*, and *S. pinanga*).

The separation of *S. ovalis* from the main grouping follows the taxonomic treatment of Dipterocarpaceae (Ashton 1982). Ashton (1982) assigned *S. ovalis* as a monotypic species in the sections *Ovalis*. All types of red meranti in cluster 1 came from one section, namely *Brachypterae* Ashton (1982), while cluster 2 has more diverse or mixed members. Species that were included in cluster 2 came from various grouping sections, namely *Mutica*, *Pachycarpae*, and *Brachypterae*.

The infrageneric classification of *Shorea* spp. of the red meranti by Ashton (1982) was based on the flowers and fruits, while the grouping in this study was carried out based on the morphological characteristics of the leaves. However, the results of this study were fascinating as the quantitative morphological characters on the leaves of meranti saplings were in line with the grouping of Ashton (1982).



Remarks: LW (Leaf width), LL (Lamina length), LP (width of leaf width), SD (angle of leaf vein), LP (length of leaf stem), LB (number of leaf vein), AS (shape of leaf tip), BS (shape of leaf base), WL (area of the leaf), CL (circumference of the leaf), FF (form factor), AR (aspect ratio), PR (perimeter ratio of diameter), R (red), G (green), and B (blue).

Figure 6. PCA analysis of leaves morphological characters of 29 *Shorea* red meranti species

Biplot analysis (Figure 6) shows that each species has different leaf morphological characteristics, both dominant and recessive. Some species such as *S. amplexicauli, S. smithiana, S.dasyphylla, S. johoriensis, S.leprosula, S. palembanica, S. teysmaniana, S.parvifolia, S. acuminata, S. platycarpa, S. macrantha, S. regusa* and *S. ovalis* has morphological characteristics that are more dominant in the SD variable and chlorophyll content. These types also have recessive values on the variables WL, R, G, LB, LP, and LL. It is inversely proportional to S. *beccariana, S. platyclados, S. smithiana, S. martiniana,* and *S. pinanga*. These types have morphological characteristics that are more dominant in the variables WL, R, G, LB, LP, and LL. Furthermore, *S. macrophylla, S. stenopthera,* and *S. mecisopteryx* had more dominant characteristics in the FF, B, PL, LW variables and had the smallest values on the AR, PR, CL variables. Meanwhile, *S.hemsleyana, S. singkawang, S. curtisii, S.curtisii* subsp. *grandis, S. scaberrima, S.fallax, S. singkawang, S. balangeran, S. parvistipulata,* and *S. selanica* had morphological characters inversely proportional to *S. macrophylla, S. stenopthera*, and *S. mecisopteryx.*

This study showed that all variables determine the leaves' morphological characteristics in each type of red meranti group except for the AS and BS variables. In the biplot analysis (Figure 6), the two variables did not show a dominant value in either type of observed red meranti group. Thus, it cannot be used as a differentiator between types and in statistical analysis. The variables are also not significantly different at the 0.05 level. In comparison, leaf color can be used to determine the kinship lever only if they are transformed into digital colors in Red, Green, or Blue. The degree of closeness of the relationship variable between characters in the biplot is shown based on the angle and length of the line formed (Figure 6). The longer the line, the more influential the character (Firmansyah, Kadiarsih, & Taryono, 2020).

4. Conclusions

This study showed that all variables could characterize the morphological characteristics of the leaves in each type of red meranti group, except for the variable of tip shape (AS) and leaf base (BS). Those two variables did not support the grouping and species kinship and can be neglected as determinants. In addition, several species with similar dominant morphological characteristics were grouped into the same quadrant. While leaves color needs to be extracted digitally to use in quantifying relationship among *Shorea* red meranti group.

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