Morphological characterization of different provenances of Teak (*Tectona grandis* L.)

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Abstract. Teak (*Tectona grandis* L.) is an important hardwood species in Indonesia. Morphological description of Indonesian teak remains inadequate. The purpose of this study was to determine the morphological traits of teak from the different provenances: Malabar, Java and Muna. The study was observed eight macroscopic morphology variables (anthocyanin color, intensity of anthocyanin color, leaf color, leaf size, edge of the leaf, base of blade, top surface texture of leaf, and veins color) and five microscopic morphology variables (adaxial epidermal density, abaxial stomatal density, abaxial stomatal size, trichomes density, and number of trichomes types). The leaf materials were collected from trees sample of each provenance. The Java and Muna provenance presented six similarities of macroscopic morphology of traits. The Malabar provenance materials were the least similar macroscopic morphology, with only one similar trait. Malabar provenance had the highest density of trichomes and stomata. The Malabar provenance had the lowest percentage of similarity coefficient among provenance.

1. Introduction

Teak (*Tectona grandis* L.) is the most valuable tropical timber because of its versatile range of uses and high quality of wood [1]. The teak compounds are used from its wood, root, leaves, until its bark of the trees. This plant is the best manufactured timber for furniture, decorative flooring, wall paneling, door, windows frame, cabinet, and ship. Java Island and Muna are populations native teak in Indonesia [2]. Teak forest area in Indonesia is distributed in the northern, western, central and eastern parts of Java, Kangean Island, Muna, Bali, and Subana [3].

There are four characteristics of teak wood: lime teak with a light-colored stem, oily teak with hard, heavy, shiny wood, wrinkled teak with wrinkled fibers and *doreng* teak with dark brown stripes in the wood [2]. Morphological variations of leaves have an important role to provide resistance to the plants, including teak plants [4]. For example, the hairy leaf of teak plants protects against insect attacks. The arrangement of teak leaves is opposite, broadly elliptical, about 30 - 70 cm long and 25 - 40 cm broad [3]. Teak trees shed their leaves during the dry season and new leaves sprout in the beginning of rainy season.

Plant morphology is the investigation of plant structure and form. This could be macroscopic or microscopic morphology of tress parts. Macroscopic morphologies are external structure of plants, i.e. leaf shape, fruit color and microscopic morphologies are included trichomes, stomata, and epidermal. Trichomes are tiny hair-like components found on the aerial epidermis of plant stems and leaves [6],
they are a modification of epidermal cell in the great variety of form, structure, and function [5]. Stomata area small apertures on the leaf surface that consist two differentiated epidermal cell or guard cell [7]. Stomata have function to regulate transpiration and CO₂ uptake during photosynthesis [8] . Plant epidermal is a tissue that playing important roles in water relation and pollinator attraction [9]. They are morphologically differing in size, shape and function.

Macroscopic and microscopic characteristics of teak leaves have been used to differentiate phenotype in provenance trials. The leaf morphology of teak is varied in shape, size, colour and texture [9]. This variability of leaf morphologies is essential to understand the adaptation and propagation of plants [11]. However, the information of morphological characteristic of leaf is very limited. For example, in the last decade, there are only 6 studies related to morphological characteristics [12][4][1] [13][14][15]. Therefore, the aim of this study was to determine the morphological characteristics of three provenances (Malabar, Muna, and Java) of teak.

2. Materials and Methods

2.1. Plant materials
The sample of leaf teak was collected from one year teak stand in The Genetic Resource Area, Region II Forest Plant Seeding (BPTH Wilayah II), Bellaburi District. The area is located at 119°40'20" E - 119°40'40" E and 5°10'22" S - 5°10'50" S. The altitude of location is 90 - 120 meters above sea level with slope around 0 - 40%. The climate is classified as wet tropical with an annual precipitation 2,653 mm [16]. The genetic material in the study area was grouped into three provenances: Malabar, Muna and Jawa. Malabar provenance were collected from Raha III District; Muna provenance were collected from Barangka District (Muna regency), Sampolawa District (Buton Regency), Pondidaha District (Kendari City) and Sidrap Regency; Java provenance were collected from Ngawi Regency and Padangan District.

2.2. Assessment of morphological traits
The study of leaf morphology was observed for each teak provenance. The sample of macroscopic morphologies was randomly selected from ten trees (2.5 %) in each provenance. The observation of the study was focused on eight macroscopic morphology variables (anthocyanin color, intensity of anthocyanin color, leaf color, leaf size, edge of the leaf, base of blade, top surface texture of leaf, and veins color) and five microscopic morphology variables (adaxial epidermal density, abaxial stomatal density, abaxial stomatal size, trichomes density, and number of trichomes types). Anthocyanin studies were extracted from the top of leaves and characteristics of leaf studies were made from the mature leaves.

The microscopic morphology of each provenance was randomly selected from five trees in each location. A total of 105 sample leaves was collected from three leaves sample in each tree. Observation of microscopic morphology was included adaxial epidermal density, abaxial stomatal density, abaxial stomatal size, trichomes density, and number of trichomes types. Atlas of Plant and Animal Histology was referred to determine the types of trichomes [17]. Preparations of leaf anatomy were collected from fresh leaves using the replica method. The preparations of leaf anatomy were taken from adaxial and abaxial leaves at the widest area between major veins of leaves. The number of trichomes, stomata, epidermis, and types of trichomes was determined using nail polish imprints. The desity of epidermal cell was counted only on adaxial epidermal. The stomatal density was observed only on the abaxial. Trichomes, stomata and epidermal were counted under a light binocular microscope in 1 mm² area at a magnification of 200. The size of stomata was observed at a magnification of 400. Images were taken with a microscope digital camera.

2.3. Data Analysis
The similarities coefficient of the macroscopic morphological traits was analyzed using a cluster system. Cluster analysis was performed using Numerical Taxonomy and Multivariate Analysis System (NTSys) software version 2.02 [18]. The similarity matrix were created using the SIMQUAL
(Similarity for Qualitative Data), grouping using SAHN (Sequential Angglomerative, Hierarchical and Nested), and clustering using the UPGMA (Unweighted Pair Group Method with Arithmetic average).

3. Results and Discussion

3.1. Macroscopic morphology

3.1.1. Anthocyanin. Malabar and Muna provenances showed the similar strength of anthocyanin color intensity. The intensity and diversity of anthocyanin was influenced by the number and position of hydroxyl and methoxy groups on the basic skeleton [19]. The anthocyanin color of Malabar provenance was brownish red. This brownish red color indicated that methoxyl groups were more predominant then hydroxyl. Muna and Java provenances teak showed the purplish or bluish red anthocyanin color. This result indicated the predominance of hydroxyl groups on their epidermal leaves. The bluish anthocyanin indicated the dominant of hydroxyl and the redness anthocyanin indicates the dominant of methoxyl [20].

3.1.2. Leaves characteristic. Muna and Java provenances had more similar leaf morphology than Malabar provenance. The leaves color of Muna and Java provenances were yellowish green, whereas the Malabar provenance was dark green. Muna provenance teak had bigger leaf size than Malabar and Java provenances. The Muna provenance had ranges about 40 - 67 cm long and 25 - 52 cm width, Malabar provenance ranges about 10 - 49 cm long and 6 - 32 cm width, and Java provenance had the smallest range about 40 - 49 cm long and 25 - 32 cm width (Table1).

| Characteristic          | Malabar     | Muna        | Java        |
|-------------------------|-------------|-------------|-------------|
| Anthocyanin colour      | brownish red| purplish red| purplish red|
| Intensity of anthocyanin colour | strong      | strong      | medium      |
| Leaf color              | dark green  | yellowish green | yellowish green |
| Leaf size               | small to medium | medium to large | medium |
| 10 - 49 cm long        | 40 - 67 cm long | 40 - 49 cm long |
| 6 - 32 cm width         | 25 - 52 cm width | 25 - 32 cm width |
| Base of blade           | acute       | acuminute   | acuminute   |
| Margo Folii             | entire      | entire      | entire      |
| Top surface texture of leaf | Scaber     | more scaber | more scaber |
| Veins color             | light yellow| yellow      | yellow      |

Table 1. Leaves characteristic for Malabar, Muna and Java provenances

The shape and leaf blade of all provenances had elliptical leaves (Figure 2). Frequency of the base of blade in Malabar teak leaves were 40% acute and 60% acuminute. Muna and Java provenances had similar leaf shape with the base of blade are acuminute (Figure 3). Frequency of the base of blade in Muna provenance were 20% acute and 90% acuminute, Java provenance were 25% acute and 83% acuminute from the sample (Figure 1). The top surface texture of the leaves from Muna and Java provenances were more scaber than Malabar provenance. The edge of the leaf in each provenance was entire, similar among provenance. Veins color of Malabar provenance was light yellow and the vein color of Muna and Java provenance are yellow. Muna and Java provenance had six similarities of leaves characteristics. This result indicated Malabar provenance had the largest differences of leaf characteristics than Muna and Java provenances (Table 1.).
The dendrogram of cluster analysis showed that the macroscopic characteristic of teak was classified into three groups (Muna, Jawa and Malabar) with high similarity coefficient among groups ranges from 76% to 98% (figure 4). The Muna provenance had highest ranges of similarity coefficient.
among locations (92% - 98%). Malabar provenance had the least similar characteristic of macroscopic morphological among provenance with the similarity coefficient 76%.

Figure 4. UPGMA dendrogram of teak provenances based on macroscopic morphological traits

Morphological characteristic of teak is influenced by genetic and environmental factors. This study showed that Java provenance and Muna provenance had the closeness morphological characteristic among groups with similarity coefficient 84%. These two provenances were not classified in the same group, although they are originality from same place. It might indicate that this provenance becomes landrace and has different genetic pattern and structure [21]. However, the Malabar provenance from Raha showed the least similarity of morphological characteristics among groups. It might indicate that genetic factor has more influence to the morphological characteristic than geographic factors.

3.2. Microscopic morphology

3.2.1. Epidermal cell density. This work showed that Java provenance has larger adaxial epidermal cell density than the other provenances (Table 2). This result indicates that Java provenance are more resistant to high intensity of light and heat than Malabar and Muna provenances.

Table 2. Microscopic characteristics of teak for each provenance

| Provenance | Adaxial epidermal cell density (mm²) | Abaxial stomatal density (mm²) | Abaxial stomatal size (µm) | Trichomes density (mm²) | Number of trichomes type |
|------------|-----------------------------------|-------------------------------|---------------------------|------------------------|--------------------------|
| Malabar    | 1266                              | 468                           | 15                        | 10                     | 16 104 4 1               |
| Muna       | 1211.25                            | 392.5                         | 12.81                     | 7.81                   | 16 84.75 4               |
| Java       | 1376                               | 411.33                        | 12.92                     | 8.33                   | 16 68.67 3               |

3.2.2. Stomatal size and density. The result showed the absent of stomata on adaxial surface of leaf teak. Hypostomaty occurred even in the tallest tree with a high level of overheat irradiance on the whole crown [22]. Leaf stomatal density affects gas exchange. Stomata were located only on the abaxial surface. Stomatal density among teak provenance range from 392.5 mm² to 468 mm² (Table 2). Stomatal size ranges from 12.81 µm to 15 µm long and 7.81 µm to 10 µm width. Figure 5 shows the comparison length and width of stomata in each provenance. This result contrast with the previous study in 35 rainforest species that showed the negative relationship between stomatal density and
stomatal size [23]. Malabar provenance had the largest size and the greatest of abaxial stomatal density. It might indicate the Malabar had the highest net photosynthesis and biomass. The previous study on Azadirachta indica also showed the positive correlation between stomatal density and net photosynthesis and biomass [24].

Figure 5. Comparison stomatal size among provenances

3.2.3. Trichomes type. The result of this research showed that abaxial trichomes density is varied ranged from 68.67 to 104 mm⁻². Malabar provenance has the largest abaxial trichomes density (104 mm⁻²). The Malabar provenance has the largest abaxial stomatal density, stomatal size, and abaxial trichomes density. The type and density of the leaf trichomes are varied among plant species, population and individual species [25]. The variation is related to many factors that include geography. Trichomes have a role in the interaction between plant and environment. For example, the morphology of trichomes takes important role on regulating leaf absorption of radiation [26].

This study found the Muna provenance has four types of trichomes; unicellular papillary, unicellular branched, pluricellular filiform and glandular trichomes. The Malabar provenance has glandular trichomes types. The Java provenance has three tricome types:glandular trichomes, pluricellular filiform and unicellular papillary (figure 6). This result is similar with another study on Indonesian teak provenance that found three different types of trichomes on the abaxial surface; suberect, erect and glandular trichomes [13].

The Muna provenance has the most complex trichome types. This result indicates that Muna provenence has best resistance against pests, diseases and environmental interactions than other provenances. Malabar provenance, in contrast, only has glandural tricome types. The glandular trichome play more important role on the aspect of plant physiology from production into secretion many variety compounds, especially terpenoids and flavonoids [27].

The Malabar provenance has the largest abaxial glandular trichomes density. The variation of tricome density is influenced by environmental gradients or heterogeneous habitat conditions in which higher tricome density indicates better adaptation in stressful environment [28].

Further, the type of trichomes also can influence the aspect of plant physiology and ecology. For example, the non-glandular trichomes can be formed at various stages of organ development before the organ reaches maturity and influence plant functions by virtue of their physical properties (size, density) [29].

The result showed Malabar provenance has the largest differences of morphological characteristics both of macroscopic and microscopic morphology. This morphological difference might be influenced by the genetic inherent that related to sample collection site. trichome types might be useful for differentiating of teak provenances.
Figure 6. The trichomes types of teak abaxial surface. (A) Muna provenance, granular trichomes (red arrow), unicellular papillary trichomes (yellow arrow), pluricellular filiform trichomes (blue arrow) and unicellular branched trichome (green arrow); (B) Malabar provenance, granular trichomes (red arrow); (C) Java provenance, granular trichomes (red arrow), unicellular papillary trichome (yellow arrow), pluricellular trichome (blue arrow). Images obtained from scanning electron microscopy (SEM) at the magnification of 200.

4. Conclusion
This study concluded that the three provenances (Malabar, Muna, and Java) have difference of morphological characteristic. Further, the Malabar provenance had the largest differences of macroscopic and microscopic morphology. The cluster analysis showed that Malabar provenance had the lowest percentage of similarity coefficient among provenance. The result of the study further emphasizes the important of morphological characteristic for identifying characteristic of teak provenance.

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