Diversity and clustering analysis of *Sterculia quadrifida* R.Br. from Kupang, East Nusa Tenggara, Indonesia based on morphological and anatomical characters

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Abstract. This study aimed to determine the diversity and clustering of Faloak (*Sterculia quadrifida* R.Br) from Kupang District based on morphological and anatomical characters. This research used descriptive qualitative and quantitative methods. To show the clustering relationship, we used the UPGMA method similarity by the Nei Li coefficient through the MVSP software program version 3.2. Results showed that there was a relationship of Faloak plants from both locations in Kupang district forming 4 groups including Group I (Kupang Barat populations); Group II (Taebenu 3 population), Group III (Kupang Barat 3 and Taebenu 2 populations) and Group IV (Kupang Barat 2 and Taebenu 1 populations). The anatomical characters showed a clear similarity on the epidermal cell, thick inner wall, guard cell, and stomatal pore. The type of trichome on the leaves of Faloak plants from these two sub-districts also showing a similarity, namely non-glandular trichome type.

1. Introduction

Faloak (*Sterculia quadrivida* R.Br.) was a medicinal plant that widely used to treat hepatitis, typhus, ulcers and stamina enhancer in East Nusa Tenggara [1, 2]. Faloak belongs to the Sterculiaceae family which widely distributed in East Nusa Tenggara including Belu (6.25 trees/ha), North Timor Tengah (1.4 trees/ha), South Timor Tengah (14.16 trees/ha), Kupang District (7.95 trees/ha) and Kupang City (4.84 trees/ha) [3]. Since Faloak is have important role in health especially in East Nusa Tenggara [4, 5] the overuse of these plants has getting increase in year after year. This condition will result in disruption of faloak sustainability due to the use of these plants that are not environmentally friendly since the local people only used the bark [6]. Therefore, conservation effort is needed to protect and preserve this plant from extinction.

Most conservation efforts only focus on population growth without considering of genetic and morphological diversity. In fact, genetic and morphological diversity is important effect for species survival [7, 8]. Hence, the assessment of genetic and morphological diversity is required not only for crop breeding in developing new varieties but also for conservation strategies. In order to determine genetic diversity, variety of morphology, anatomy, biochemistry and molecular approaches are generally used [9-11]. However, utilization of morphological character for genetic diversity detection was primarily used because of its efficiency and simplicity [12].
Plant morphology or phytomorphology is study or techniques for determining genetic variation between and within species through physical form and external structure of plant [13]. Not only a morphological character but also anatomical characters are required as a valuable approach in taxonomy and identification of plant [9]. In the recent years the study of morphology and anatomy has been the most widely used in the classification of higher plant especially in taxonomy, evolution, and ecology in plant species [12, 14].

In the wild, Faloka grows quite large, reaching a height of 20 m with a spread canopy [15]. This plant grows well in dry soil, degraded land, tolerant to sea breeze and high light intensity. Because of its speciality, Faloka grows widely in East Nusa Tenggara especially in Kupang. However, the wide spread of Faloka cause phenotypic variation within species of Faloka due to influence of genetic and environmental factors [16].

Until now no study was available on assessing diversity or phenotypic variation of Faloka based on Morhological and anatomical. Therefore, the aim of this study was to evaluate morphological, anatomical variability among Faloka (Sterculia quadrifida R.Br.) in Kupang East Nusa Tenggara, Indonesia. This study also expected to be used as basic information for conservation, crop breeding and development of the Faloka plant in East Nusa Tenggara Province.

2. Methods

2.1. Materials

The plant material was collected from local people garden and forest in two districts in Kupang regency including Taebenu district (3 locations) and Kupang Barat district (3 locations). The morphological character includes the leaves, flowers and fruit while anatomical character include epidermal cells of leaves.

2.2. Morphological and Anatomical characters’ analysis

The observation of Morphological characters of Faloka was conducted according to Tropical Fruits Descriptors [17]. Morphological character used includes 14 characters of both qualitative and quantitative characters in vegetative and generative organs (Table 1). All morphological character was photographed using a digital camera.

Observation of leaf stomata used the method of Rompas [18]. The anatomical characters were observed from epidermal cells through slicing of the leaves of the Faloka. Then, each slice is placed on a slide and dropped with water. To get clear results, a drop of 0.05% safranin was being added, then, photographed under the aid of stereo microscope. Stom mata density was deterimed according the formula proposed by Lestari [16].

![Table 1. Morphological characters of Faloka plants used in the study](image)

| No. | Morphological Character Parameters | Code | No. | Morphological Character Parameters | Code |
|-----|-----------------------------------|------|-----|-----------------------------------|------|
| 1.  | Leaf shape                        | LS   | 8.  | Fruit weight                      | FW   |
| 2.  | Leaf margin                       | LM   | 9.  | Fruit color                       | FC   |
| 3.  | Leaf tips                         | LT   | 10. | Fruit texture                     | FT   |
| 4.  | Leaf veneration                   | LV   | 11. | Presence of seed                  | PS   |
| 5.  | Leaf width                        | LW   | 12. | Seed weight                       | SW   |
| 6.  | Petiole length                    | PL   | 13. | Seed color                        | SC   |
| 7.  | Leaf-blade length                 | LBL  | 14. | Seed texture                      | ST   |

2.3. Data Analysis

Morphological and anatomical data were analyzed descriptively to find out the variation of Faloka in Kupang Regency. Clustering analysis was performed to compare the differences of Faloka. The morphological data were transformed to a binary characters coded as 0 (absence) and 1 (present) for
each character. All matrices were used to construct the dendrogram. The dendrogram was constructed on the basis of a similarity matrix based on a morphological marker by UPGMA (Unweighted Pair Group Methods using Arithmetic averages) [19] on the MVSP (Multivariate Statistical Program) v.3.1 software.

3. Results and Discussion

3.1. Diversity of Morphological Characteristics of Faloak (Sterculia quadrifida R.Br)

Morphological characteristics of the Faloak showed varying in leaf, fruits and seeds (Table 2). Faloak showed a general characteristic of cordate (heart-shape) of leaf shape (Table 2) and pinnate of leaf venation. In other hand, apical leaf of faloak showed two variations, i.e. acuminate and apiculate (Figure 1. A1-A3). In addition, the Faloak fruit has variations in color i.e orange and red in Kupang Barat and orange in Taebenu (Figure 1. B1-B3 & C1-C3). The number of seed in each fruit can be used to differentiate between Faloak in Taebenu and Kupang. The number of seed of Faloak in Kupang has 5-19 seeds while Taebenu has 11 seeds (Figure 1. B1-B3 & C1-C3).

Faloak fruits also has variation based on the weight. The highest number of fruit weight were Taebenu 2 (40 gr) while the lowest was Taebenu 3 (17 gr) (Table 2). The color of fruits is dominated by orange color. However, there is red color in Kupang Barat 1 and 2 (Figure 1. B1 & B2). In the texture of the fruits, all of the samples have the same rough characters. The highest number of seeds was shown by the samples from Kupang Barat 3 (19 seeds) and the fewest were shown by samples from Kupang Barat 1 (5 seeds). The highest weight of seeds was shown by samples from Taebenu 2 and Kupang Barat 3 (7 g), while the lowest was shown by samples from Kupang Barat 1 (0.7 g) (Table 2). The color variation of Faloak seeds showed by 2 variations including black and light brown. The seeds were dominated by black color, except for the samples from Kupang Barat 2, which is light brown color (Figure 1. B2).

All of these differences indicated the high variation on the Faloak plants. It was noticeable from the appearance of either vegetative or generative traits both quantitatively and qualitatively. This tremendous variation, attribute to the genetic diversity that might be contributed by local adaptation to specific geographical conditions for centuries, especially in species where the genetic diversity is considered low, manifested in slight variations, vice versa [10] According to Uslan & Aiman [6] that there were differences in the morphological character due to several factors including various types of flat to fraudulent slopes, where Faloak plants were growing. Additionally, the morphological traits are also showed differences such as fruit color, fruit weight, and seeds character.

Table 2. Morphological characteristics of Faloak leaves based on IPGRI (1980) with modifications.

| Sample            | Leaf Characters*            |
|-------------------|-----------------------------|
|                   | LS  | LM  | LT  | LV  | LW  | PL  | LBL |
| Taebenu district  |     |     |     |     |     |     |     |
| Taebenu 1         | Cdt | Etr | Amt | Pnt | 7.5 cm | 6.5 cm | 13 cm |
| Taebenu 2         | Cdt | Etr | Amt | Pnt | 10 cm | 7 cm | 14 cm |
| Taebenu 3         | Cdt | Udl | Amt | Pnt | 8.7 cm | 5 cm | 13.5 cm |
| Mean              | -   | -   | -   | -   | 8.73 cm | 6.16 cm | 13.5 cm |
| Kupang Barat district |     |     |     |     |     |     |     |
| Kupang Barat 1    | Cdt | Etr | Apc | Pnt | 12 cm | 7.5 cm | 15 cm |
| Kupang Barat 2    | Cdt | Udl | Amt | Pnt | 6.3 cm | 1.5 cm | 16.3 cm |
| Kupang Barat 3    | Cdt | Udl | Amt | Pnt | 11.5 cm | 6 cm | 18 cm |
| Mean              | -   | -   | -   | -   | 9.93 cm | 5 cm | 16.43 cm |

| Fruit Characters* |
|--------------------|
| Sample             | FW  | FC  | FT  |
|-------------------|-----|-----|-----|

Table 2 continued:

The Table 2. Morphological characteristics of Faloak leaves based on IPGRI (1980) with modifications.
### Taebenu district

| Sample       | Seed Weight (g) | Seed Characters |
|--------------|-----------------|-----------------|
| Taebenu 1    | 23 g            | Orange Rough    |
| Taebenu 2    | 40 g            | Orange Rough    |
| Taebenu 3    | 17 g            | Orange Rough    |
| Mean         | 26.67           | -               |

### Kupang Barat district

| Sample       | Seed Weight (g) | Seed Characters |
|--------------|-----------------|-----------------|
| Kupang Barat 1 | 20 g           | Red Rough       |
| Kupang Barat 2 | 26 g           | Red Rough       |
| Kupang Barat 3 | 25 g           | Orange Rough    |
| Mean         | 23.67           | -               |

| Sample       | PS    | SW  | SC   | ST   |
|--------------|-------|-----|------|------|
| Taebenu 1    | 11    | 4 g | Black| Hard |
| Taebenu 2    | 12    | 7 g | Black| Hard |
| Taebenu 3    | 11    | 3 g | Black| Hard |
| Mean         | 11.3  | 4.67| -    | -    |
| Kupang Barat 1 | 5    | 0.7 g | Brown | Hard |
| Kupang Barat 2 | 6    | 3 g | Light Brown | Mushy |
| Kupang Barat 3 | 19   | 7 g | Black | Hard |
| Mean         | 10    | 10.7| -    | -    |

**Figure 1.** Morphological variation of Faloak plants. A. Leaf tips (A1: acuminate, A2: apiculate, A3: variation of leaflet shape); B. variation of fruit and seed (B1-B3 is a Faloak plant from Kupang Barat 1, 2 and 3); C. variation of fruit and seed (C1-C3 is a Faloak plant from Taebenu 1, 2 and 3).
3.2. Clustering analysis of Faloak plants based on morphological character

Clustering analysis based on morphology (phenetic) of 6 Faloak plants shown to be clustered into four groups (Figure 2). Cluster I and II consisted of only one population, Kupang Barat 1 for cluster I and Taebenu 3 for Cluster II. Furthermore, cluster III consists of faloak population in Kupang Barat 3 and Taebenu 2. Meanwhile, cluster IV consists of Kupang Barat 2 and Taebenu 1. In general, this study revealed that the Faloak plants showed high diversity values (77%) and variability in major, based on morphological characters. If morphological characters can show a high diversity value, then these characters can be used as a reference to distinguish variability in accession and considered to be a great potential of raw material for future crop breeding programs [11, 20]. This difference is in accordance with Uslan [21] which states that the spread of Faloak plants are carried by animals (forest doves and bats), as well as geographical conditions of growing areas of Faloak plants and breeding factor (outcrossing) assisted by wind randomly allowing the process of gene flow and migration through pollen transfer and spread to a fairly wide area.

According to Poerba & Martanti [22], this phenomenon indicates the genetic diversity caused by genetic recombination. Karuniawan et al. [23] state that the populations from a similar habitat may not necessarily have a close relationship. It is because a close relationship also existed in genotypes and phenotypes of different origins. This is influenced by environmental factors or the interaction of genotypes or phenotypes by the environment. It is also influenced by the population of the plants tested which have a varying age level that gives rise to a diversity of genotypes and phenotypes [24].

The molecular markers approach was known to have a higher level of accuracy compared to the morphological approach in the identification of plants. Proven by one of the molecular markers which often used in research on genetic diversity in plants, RAPD marker. As carried out by Probojati et al. [25], the genetic diversity in plantains was able to show the high genetic diversity, forming a suitable grouping pattern, beside there are still many other markers that often be used.

The study of genetic variation is still needed in this study to accurately describe the genetic diversity among Faloak plants because the character observed were shown to highly influenced by the environment. Therefore, further research using specific genetic markers are needed to confirm the genetic diversity and clustering pattern accurately.

![Figure 2. Dendrogram of 6 samples of Faloak plants based on the similarity index inferred by UPGMA analysis.](image)

3.3. Anatomical Characters Analysis

A longitudinal cross-section of stomata leaf in 2 sub-district from Kupang district showed identical stomata type i.e actinocytic (Figure 3). It is consisting of the epidermal cell, thick inner wall, guard cell, and stomatal pore. Stomata on the leaves of the Faloak plants have a round shape character. The type of stomata type showed the genetic and evolutionary history of plants [26]. Thus, information
about the structure of a cell has a significant role in plant systematics [27]. Plant species in one family tend to have certain types of stomata, although there are some exceptions in plants that have variations in the type of stomata in one type.

Figure 3. Longitudinal cross-section of stomata character (*Sterculia quadrifida* R.Br) collected from 2 sub-district in Kupang. A. Kupang Barat 1, B. Kupang Barat 2, C. Kupang Barat 3, D. Taebenu 1, E. Taebenu 2, F. Taebenu 3. note: EC= epidermal cell, TIW= thick inner wall, SP= stomatal pore, Trc= trichome.
Furthermore, trichome showed a similarity structure to tomato trichome with a shaped like needles. Supported by Nurhayati et al. [28] which states that *Lycopersicon pimpilellifolium* (tomato plants) have a similar trichome shape to non-glandular trichome found in *Solanum tuberosum* with pointed tips. Trichome also serves a different function because manifested in several shapes. Each trichome has a different function, non-glandular trichome, among others, has a function as a pathogen barrier through the stomata, while glandular trichome has a function to secrete secondary metabolites. The shape, size, and density of the shape and type of trichome also affect the function of the trichome in protecting the leaf organs of a plant [29].

Faloak leaf showed a measurement ranging from 2.9 to 5.6 stomata/μm. The density of Faloak leaf that shows the highest was indicated by the samples from Kupang Barat 1 (5.6 stomata/μm) and the lowest density indicated by the samples from Kupang Barat 3 (2.9 stomata /μm) (Figure 4). The highly varying density of stomata are determined by the process of adaptation of plants, in regards to the environmental conditions (water availability, light intensity, temperature, and CO₂ concentration) [30]. The light intensity affects the ambient temperature. The higher the intensity of the light, the higher the ambient temperature. Consequently, stomatal density may strongly affect the rate of basic physiological processes such as photosynthesis, transpiration besides determining the rate and type of respiration as well [30].

Stomata density is thought to be influenced by the environment of the growth site. In the Kupang Barat 1, the environmental conditions are still maintained naturally, with a few of motorized vehicles in the area and the climate conditions are quite cool and are overgrown by many other types of plants. While, in Kupang Barat 3 which has the lowest stomata density, the environmental conditions have been heavily polluted by motor vehicle fumes, which may contribute to the stomata density reduction in the area.

![Figure 4](image)

**Figure 4.** Graph density Faloak plants (*Sterculia quadrifida* R.Br) stomata collected from the Taebenu sub-district and Kupang Barat sub-district, Kupang District.

4. Conclusions
Analysis of diversity and clustering in Faloak plants indicated the high diversity based on morphological characters. It shows that group I covering the location of Kupang Barat 1, group II covering the location of Taebenu 3, group III covering the location of Kupang Barat 3 and Taebenu 2, while group IV covering the location of Kupang Barat 2 and Taebenu 1. Some of the morphological characters studied are important characters that can be used as indicators of diversity in Faloak plants. Furthermore, the anatomical characters in the stomata shape and stomata density have a variation.
pattern to the environmental conditions in which they grow. Stomata density is an important character that can be used as an indicator of environmental conditions and climate change. However, the clustering and diversity analysis by the means of morphological and anatomical characters still considered being less consistent and accurate. Therefore, further research using more specific genetic markers are needed to confirm the level of genetic diversity and clustering.

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