

Morphological and Molecular Identification of Ramie Plants (*Boehmeria nivea* L. Gaud) in Indonesia

Reni Mayerni^{a,1}, Sari Rukmana Okta Sagita Chan^b, Gustian^a

^a Agrotechnology Department, Agriculture Faculty Andalas University, Limau Manis, Padang, 25163, Indonesia
E-mail: ¹renimayerni@agr.unand.ac.id

^b Politeknik Pertanian Negeri Payakumbuh, Lima Puluh Kota, 26271, Indonesia
E-mail: sari.rukmanasagita@politaniptk.ac.id

Abstract— This research has been conducted at the Experimental Farm of Agricultural Faculty of Andalas University and the Laboratory of the Department of Agronomy and Horticulture Agricultural Faculty, IPB University, Bogor. The purpose of this study was to determine the genetic diversity with similarity analysis of the ramie plant collection of Experimental Farm, Agricultural Faculty of Andalas University. This research was conducted employing morphological and molecular characterization. Morphological characterization of the ramie plant is done by observing the character 17 qualitative and 15 quantitative characters ramie plant. While the molecular characterization using RAPD markers (Random Amplified Polymorphic DNA) with ten primers. The results of morphological characters, which serve as distinguishing characteristics of the five clones, is the color of the leaf. The results of the qualitative character of phenotypic variance analysis of variability obtained plants with broad criteria contained in the 4-character observation (the color of the leaf petiole, the leaf color, flower color, and female sex flowers). Similarity analysis based on morphological characters is qualitatively classified into two groups with the degree of similarity coefficient from 0.34 to 0.75. Similarity analysis of qualitative and quantitative combined data grouped into two groups with the degree of similarity coefficient from 0.02 to 0.20. The similarity analysis based on molecular characters is grouped into two groups with a similarity coefficient level of 0.27 to 0.75.

Keywords— ramie plants; genetic Diversity; characterization; morphology; RAPD.

I. INTRODUCTION

Ramie fiber is a natural fiber taken from the stem of the ramie plant (*Boehmeria nivea* (L). Gaudich) which is a nettle plant, also known as China grass. It is spread across eastern and southern Asia [1] and is commonly grown in China, India, and other countries in South Asia [2]. The ramie plant has high protein content [3], and the roots and the leaves can be used as fodder [4]. Fibers derived from the ramie plant can be used in the textile industry [5]. The fiber made from ramie plants has been known for more than 6,000 years [6]. In Egypt, ramie fiber was used to make material for wrapping mummies because the fiber is resistant to attack by fungi and bacteria [7]. Currently, ramie fibers are widely used in technological innovations such as in the automotive industry and for biodiesel production [8].

Ramie fiber quality is high compared with other fibers [5]. Ramie fibers can be used for various purposes [9]. The fibers are longer and shinier than cotton fibers and fibers from other plants. Ramie fibers can also be blended with other fibers such as wool, cotton, or polyester [10]. Ramie plants

can grow in the tropics and subtropics. This is a perennial plant that is easy to grow [11]. These plants will grow in Indonesia if planted in the middle land area to the highlands (500-1500 m asl), plants can be cultivated from the lowlands to the highlands (10-1500 m above sea level) [12]. Five ramie plant clones are known to grow and adapt to the condition at Andalas University (located 350 meters above sea level): Indochina, Padang 3, Ramindo 1, Lembang A, and Padang 3. These five clones can produce a good amount of fiber, including the Ramie clone [13].

One problem encountered in the ramie plant breeding program is that there is still minimal information about the genetic variability of ramie germplasm grown at this location. According to Ref. [14], limited information exists regarding genetic analysis causing it difficult to draw any conclusion about its genetic diversity, resource protection, hybridization breeding, or screening for excellent germplasm.

Genetic variability is necessary for a population of plants to cope with environmental change. If there is no genetic diversity in a population or species, it is very likely to become extinct because it cannot withstand changes in the environment [15]. Genetic diversity is also very important

for plant breeders to establish and enhance the desired characters of a cultivar [16]. Demonstration of genetic Diversity and structure in rare plant species is often crucial for the formulation of conservation and management strategies because it provides valuable insights into the vital aspects of demography, reproduction, and ecology [2].

Molecular characterization observations can understand genetic diversity, information about the molecular character is needed as a differentiator between plant accession and the estimation of distance or genetic diversity in kinship analysis so that it can be used as the primary material for improving ramie plants [17].

Morphological markers include observations of the growth and development (qualitative and quantitative characteristics), resistance to pests and diseases, and adaptation to the environment [18]. Identification of genetic Diversity by morphological characters is strongly influenced by environmental factors as well as the stage of plant growth and development [19]. Therefore, DNA polymorphism can also be used. The use of molecular characteristics can display the variation for all of the genomes, whether expressed or not [20].

Randomly Amplified Polymorphic DNA markers (RAPD) are frequently used [21]. These markers are simple to use in the laboratory, cost-effective, use only small amounts of plant material which can be obtained at any stage of plant growth [22], and produce a high-value polymorphism [23].

RAPD markers using multiple chain reaction or PCR with an oligonucleotide primer [24]. Single primer can amplify genomic DNA, and polymorphism can be detected by PCR amplification products [25]. The use of morphological markers and molecular basically can analyze the grouping of a plant. The characterization of morphological and molecular is subject to be identifiers, especially for the five clones of ramie plant. Hence, it can provide the data and information about the identity of the plants that can be used for breeding efforts and the development of the ramie plant. The purpose of this study was to determine the genetic diversity with similarity analysis of the ramie plant collection in Experimental Farm of Agricultural Faculty of Andalas University.

II. MATERIALS AND METHODS

A. Time and Place

This research was conducted from August to October 2017 at the Experimental Farm, Agricultural Faculty, Andalas University, and the Laboratory of the Department of Agronomy and Horticulture Agricultural Faculty, IPB University, Bogor.

B. Materials

A Munsell color chart was used as a reference for descriptions of plant material. Fresh leaf samples were collected from five ramie clones: Ramindo 1, Indochina, A Lembang, Bandung A, and Padang 3. CTAB extraction buffer (Tris-HCl (1 M, pH 8), NaCl (1.4 M), EDTA (0.5 M, pH 8)) was used for DNA extractions. DNA Taq Polymerase was used for amplification reactions.

C. Methods

1) Morphological Observations

- Trunk. (1) Type of plant growth, (2) Plant height (cm), (3) The diameter of the stem (cm), (4) The number of tillers per pot, (5) The surface of the stem, (6) The color stems of young plants, (7) The color of the old plant stem.
- Leaves. (1) The shape of the leaves, (2) The shape of the leaf margins, (3) The shape of the tip of the leaf, (4) The form of the leaf base, (5) Vein pattern (7) Leaf length (cm), (8) Leaf width (cm), (9) Petiole length (cm), (10) Leaf size, (11) Leaf hairs, (12) Leaf angle (0), (13) Leaf petiole color, (14) Vein color, (15) The color of the leaf blade (upper surface), (16) The color of the leaf blade (lower surface), and (17) Bud color.
- Flowers. (1) Gender, (2) Time to flowering, (3) The color of male flowers, (4) Color of female flowers, (5) Type of flowering.

2) Molecular Characteristics (Random Amplified Polymorphic DNA)

- Sample Collection. Samples of five clones of ramie plant or fresh leaf samples were collected. Sampling is done on a leaf segment 3-5 to 2 pieces or as much as 15-20 grams. Leaf samples were sterilized using 70% alcohol and washed using distilled water.
- DNA Isolation. DNA isolation was performed using the CTAB method.
- DNA Amplification. DNA amplification was performed using PCR. Reaction mixtures contained: Taq polymerase reaction mixture 6.25 μ L, DDH₂O 4.25 μ L, ramie DNA 1 μ L, and primer 1 μ L. The primers used were from either Ref. [26] or Ref. [7]. [OPC 02 (S62), OPC 19 (S79), OPH 01 (S101), OPH 07 (S107), OPF 04 (S124), OPF 05 (S125), OPX 01 (S301), OPX 02 (S302), OPX 08 (S308) and OPX 17 (S317)] and were purchased from QIAGEN. PCR conditions were as follows: an initial denaturation at 94 $^{\circ}$ C for 5 min followed by 45 cycles at 94 $^{\circ}$ C, 1.5 min at 37 $^{\circ}$ C, 1 minute at 72 $^{\circ}$ C and a final extension reaction at 72 $^{\circ}$ C for 5 minutes.
- DNA Electrophoresis. PCR products were analyzed by agarose gel electrophoresis following staining with ethidium bromide.
- Data Analysis: Fragments were sized concerning the size markers and scored (present/absent). Phenotypic variance (S²) and its standard deviation (SD) were calculated [27].

$$S^2 = \frac{\sum xi - \bar{x}}{n-1} \quad SD = \sqrt{S^2} \quad (1)$$

Assessment of phenotypic variability as broad or narrow [17] where $S^2 \geq 2 SD$ means broad phenotypic variability and $S^2 \leq 2 SD$ means narrow phenotypic variability. The similarity coefficient was calculated, and dendrograms produced using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) and the NTSYSpc software (version 2.02) [28].

III. RESULTS AND DISCUSSION

A. Morphology Characters

1) *Trunk*: Plant height, stem diameter, number of tillers per pot as well as the type of plant growth showed variation among clones. The average height of plants was as follows from the shortest to tallest: Lembang A (66.7 cm), Indochina (80.5 cm), Bandung A (90.5 cm), Ramindo (92.8 cm) and Padang 3 (94.7 cm). The average stem diameters were: Bandung A (6.9 mm), Indochina (7.2 mm), Ramindo (8.3 mm), Lembang A (9 mm), and Padang 3 (9.2 mm). The number of tillers per plot was: Lembang A (19 tillers/pot), Padang 3 (25 tillers/pot), Ramindo (27 tillers/pot), Indochina (31 tillers/pot) and Bandung A (33 tillers/pot). Plant growth was classified as determinate for four clones (Lembang A, Ramindo, Indochina, and Bandung A) and as indeterminate for one clone (Padang 3). Genetic factors will not have a significant effect on these quantitative characteristics unless all the environmental requirements of the plants are met [29]. The environment is all non-genetic factors that may affect plant growth. Different clones will respond differently to environmental conditions. External environmental conditions (including precipitation, light intensity, temperature, altitude, and availability of nutrients) significantly affect the growth of the plant. No differences between clones were observed for the character of the stem surface (generally very hairy) nor the color of young and old stems (green and brownish, respectively).

2) *Leaves*: Ramie leaves consist of two parts: the petiole and the leaf blade. The average leaf length and width were, from longest to shortest: Bandung A (13 cm and 10.7 cm, respectively), Padang 3 (11.2 cm and 9.6 cm), Ramindo (10.9 cm and 10 cm), Indochina (10.9 cm and 9.1 cm) and Lembang A (10.5 cm and 9.1 cm). Based on leaf length, the ramie plant can be classified as a narrow-leaved plant (leaf length of less than 14 cm). For observations on the length of the leaf, petiole obtained an average length of each leaf clone, Indochina: 5.1 cm, Lembang A: 5.4 cm, Bandung A: 6.2 cm, Ramindo: 5.7 cm and Padang 3: 5.9 cm. The length of the leaf petiole highest in Bandung A clone and clone lowest petiole length of Indochina. Their quantitative character variation in the average observation of leaf length and width of leaves can be caused by environmental factors such as the intensity of the light received. The light intensity is also influenced by a canopy of trees and shrubs system [30]. In observation of the ramie plant, ramie plant, and shaped herb that has many leaves that cover each other resulting in the intensity of light received by the leaves of the protection below. The color of the buds and leaf petioles varied between clones. Bud color and petiole color can help to distinguish between the 5 clones (Figure 1).

3) *Flowers*: The average time to flowering for each clone was: Ramindo (39 days), Padang 3 (39 days), Indochina (39 days), Lembang A (47 days), and Bandung A (51 days). Two clones (Ramindo and Lembang A) produce both male and female flowers. Male flowers appear first; hence the male flowers are located on the lower stem segments, and female flowers are found on the upper stem segments. The other clones (Indochina, Padang 3, and Bandung A) produce only female flowers. Female flower

color differs between ramie clones. Indochina, Lembang A, Bandung A, and Padang 3 all produce green female flowers, whereas Ramindo produced red female flowers (Figure 2). Male flower color did not differ between the two clones that produced them. Both produced green male flowers.

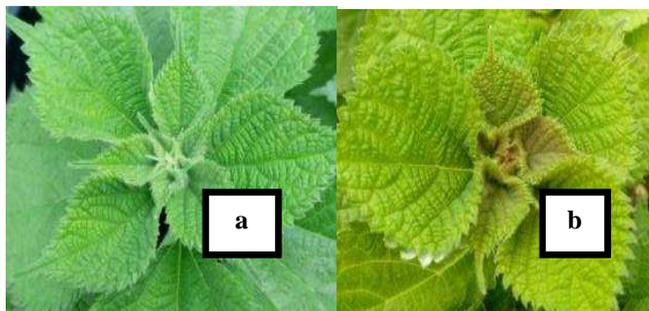


Fig 1. Bud color a). green, b) green colored reddish

There is no variation was observed in the leaf shape, leaf margins, leaf tip, leaf base, the leaf vein pattern, color of the leaf blade (upper and lower surfaces), leaf hairs and leaf vein color.

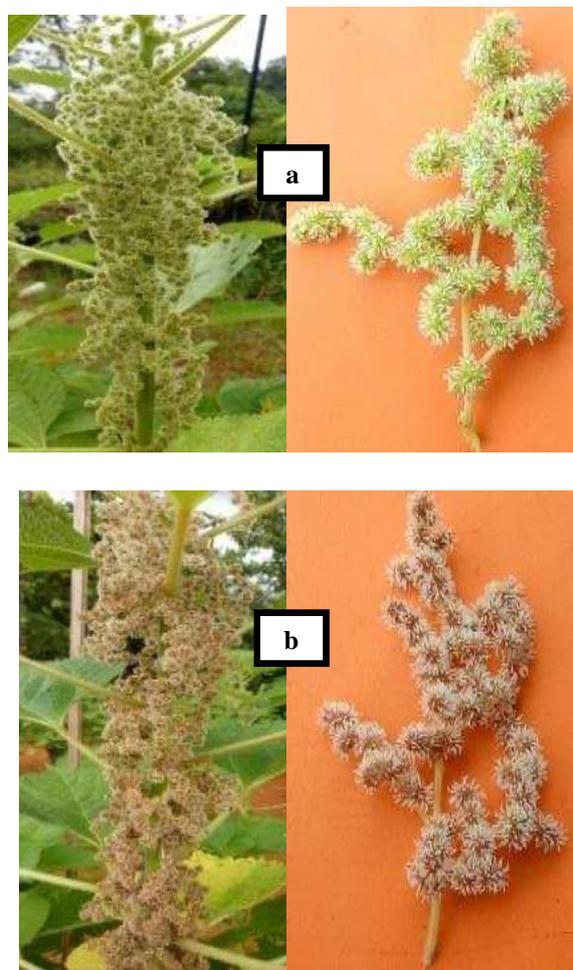


Fig. 2. Female flower color, a. Dark green (Indochina, Lembang , Bandung A and Padang 3), b) Red (Ramindo)

4) *Phenotypic Analysis*: Phenotypic variability was calculated based on the qualitative character of the ramie plant. Based on the observations (Table 1), found wide

variability in the four characters' qualitative petiole color, leaf color shoots, flowers, and colors gender female flowers. Thirteen other qualitative characteristics showed no variability. Broad variability is very important in plant breeding activities. In the absence of wide variability, plant breeding activities will not be effective. The wide variability in phenotypic appearance indicates that environmental factors more influence the character. Narrow phenotypic variability in morphological observation characters cannot be used as a basis for selecting plant breeding activities, because selection will be successful if the plant population to be selected has wide variability.

5) *Qualitative data similarity analysis*: The observed qualitative character consists of 4 parameters of observation. Quantitative data of five clones were analyzed using the 2:02 NTSYS program. Based on the analysis results obtained ramie plant grouping into two large groups (Figure 3).

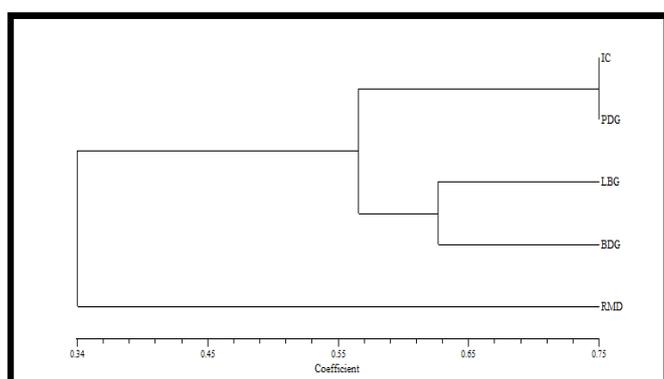


Fig 3. Dendrogram for the five ramie clones based on the qualitative morphological data: IC (Indochina), LBG (Lembang A), RMD (Ramindo), PDG (Padang 3) and BDG (Bandung A).

It is known that the degree of similarity coefficient of 0.34 to 0.75, or 34% -75%, and the overall character of the coefficient 0.34 unite and form two groups: I and II (Figure 3). Where the four clones, namely Indochina, Padang 3, Lembang, and Bandung A, are in group I. Then, I grouped into 2 groups: Ia and Ib are split on the value of the coefficient of about 0.56 or 56%. Whereas in group II there were only Ramindo clones alone. From the dendrogram (Figure 3), clone ramie plants that have similar phenotypic is closest to Indochina and Padang clone 3 with the similarity coefficient of 0.75.

B. Molecular Character

1) *RAPD Analysis*: Across the five clones, the ten primers used produced a total of 101 bands (between 4 and 14 bands per clone, Figure 4). A total of 94 (93%) of these were polymorphic (Table I), showing the high level of diversity among these five clones.

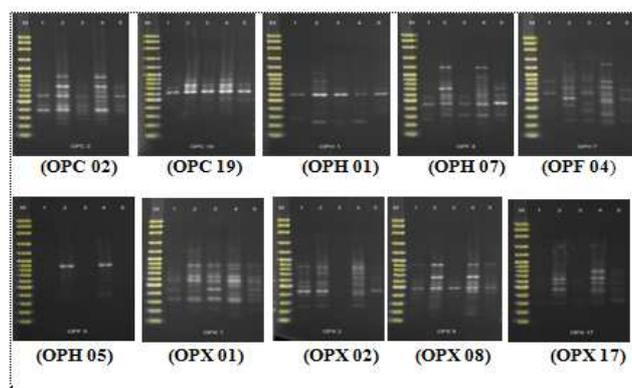


Fig. 4 DNA electrophoresis

TABLE I
RESULT OF DNA ELECTROPHORESIS

Primer Code	Mono morphic	Poli morphic	Total of Bands	Polymorphism (%)
OPC 02	1	10	11	91
OPC 19	1	6	7	86
OPH 01	1	8	9	89
OPH 07	0	13	13	100
OPF 04	1	13	14	93
OPF 05	0	4	4	100
OPX 01	2	11	13	85
OPX 02	0	13	13	100
OPX 08	1	8	9	89
OPX 17	0	8	8	100

2) *Molecular data similarity analysis*: Cluster analysis of the binary data generated from the RAPD profiles produced the dendrogram shown in Figure 6.

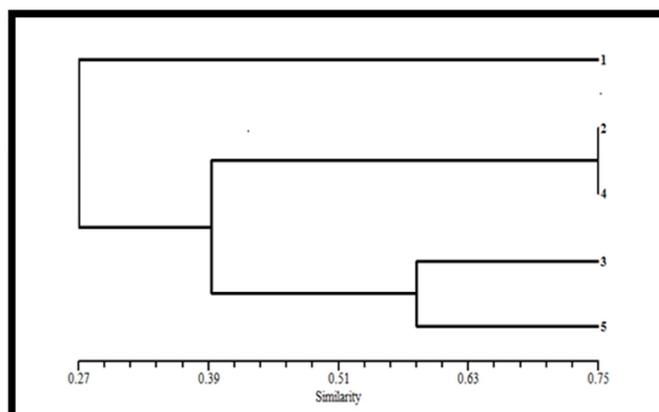


Fig. 5 Dendrogram for the five ramie clones based on the RAPD analysis: IC (Indochina), LBG (Lembang A), RMD (Ramindo), PDG (Padang 3) and BDG (Bandung A)

For these five clones the similarity coefficient is between 0.27 and 0.75, and the overall character of the coefficient 0.27 unite to form two major groups, namely group I and II. Where 1 clone spread in group I, namely clone Ramindo, as

well as in group II there were two clones of Bandung A, Lembang A, Indochina, and the Padang 3.

IV. CONCLUSION

Based on the similarity analysis of qualitative character is known that the degree of similarity coefficient of from 0.34 to 0.75, or 34% -75%, and forms two groups: I and II. Group I consist of clones Indochina, Padang 3, Bandung, and Lembang A, while group II consisted of 1 clone Ramindo only. This group occurs based on the differences in the color of the leaf petiole and leaf color shoots. Based on similarity analysis of quantitative and qualitative data merging of five clones of the ramie plant with 32 characters observations of morphological characters qualitatively and quantitatively, the degree of similarity coefficient of 0.02 to 0.20 or 0% - 20%. and forms two groups: I and II. Group I consist of clones Indochina, Ramindo, and Padang 3.

In contrast, group II consisted of clones Bandung and Lembang A clone A. Overall clustered in the coefficient of 0.02, this grouping occurs because of the similarity in 13 characters qualitative leaf shape, leaf edge, the tip of the leaf, the leaf base, bone leaves, leaf color bone, upper leaf surface color, the color of the lower leaf surface, leaf hairs, the type of leaf size, stem surface, the color of old stems, young stems color. Similarity analysis of molecular-based on five clones character ramie plant produces dendrogram the similarity coefficient level of .27 to .75 or 27% -75. And forms two groups: I and II. Group I consist of clones Indochina, Padang 3, Bandung, and Lembang A, while group II consisted of 1 clone Ramindo only.

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