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by Asri Mahulette

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Morphological Character of Clove “Raja” (*Syzygium aromaticum* L.), Endemic to Maluku, Indonesia

Anna Yuliana Wattimena, Asri Subkhan Mahulette*, Marlita Herlin Makaruku, Muhammad Fajri Aditthia Lating

Agrotechnology Study Program, Faculty of Agriculture, Pattimura University, Poka Campus, Ambon 97234, Indonesia

*Corresponding author; email: mahulette_07@yahoo.co.id

Abstract

Clove “Raja” is one of the local Maluku clove germplasm, which has characteristics similar to cultivated cloves and wild types. Until now, very limited information that are available on the morphological character of clove “Raja”. This study aims to provide basic information on the morphological diversity of “Raja”. The study was conducted at the location of the distribution of cloves “Raja” in Mamala village, Leihitu sub-district, Central Maluku district, Maluku province, in June-August 2022. The descriptors used referred to Tropical Fruit Descriptors, with some modifications. The clove “Raja” that were characterized belonged to the farmers and consisted of 30 accessions of cloves that were over twenty years old. The characterization variables included the characters of trees, stems, branches, leaves, flowers, fruits, and seeds. The results of Hierarchical Cluster Analysis (HCA) on 30 plant samples based on 54 morphological characters obtained two accession groups with a dissimilarity coefficient of 41%. The results of the Principle Component Analysis (PCA) got a total diversity of 70.5% where the first group consisted of accessions of cloves “Raja” group I having identifiable characters in the form of leaf area, leaf length, leaf width, and leaf tip shape; while the second group of clove accessions of “Raja” group II in the form of stem circumference, petiole length, flower length, fruit length, fruit width, and fruit weight. Characteristics of accession of “Raja” cloves group II had distinctive morphological traits in the form of crown shape, upper surface of leaves, lower surface of leaves, leaf thickness, leaf texture, old leaf colour, shoot colour, leaf tip colour, leaf aroma, leaf spiciness, flower stalk length, and seed colour.

Keywords: Ambon, biplot, cluster analysis, dendrogram, endemic

Introduction

Cloves are native species to Indonesia, originating from the Maluku Islands (Milind and Deepa, 2011; Mahulette et al., 2022; Hariyadi et al., 2020a). Cloves are classified as essential oil-producing plants of the Myrtaceae family, which are widely used as raw materials for cigarettes and as spices. As a crucial oil-producing plant, cloves are commonly used in pharmacology as a raw material for medicines, cosmetics and perfume industries, food shields and food preservatives (Ulanowska and Olas, 2021; Kaufman, 2015; Nejad et al., 2017; Hassan et al., 2020).

Based on data from Ditjenbun (2020), the area and production of cloves in Indonesia continue to increase, but the increase in scope and output of cloves has not met the growing demand for cloves. The area of cloves in Indonesia in 2018 was recorded at 569,052 ha, then increased to 570,353 ha in 2020. Clove production in Indonesia also increased from 131,014 tons in 2018 to 137,758 tons in 2020. The increase in area and production has not met the growing demand for cloves, so clove imports are carried out to meet the shortage of supply. According to data from the Ditjenbun (2020), the volume of Indonesian clove imports from 2015 to 2018 has increased from 11 tons in 2015 to 13.373 tons in 2018. To overcome these problems, increasing the volume of clove production in Indonesia is necessary, including using potential germplasm of local cloves growing in their distribution area.

The Maluku Islands are known as “the centre of origin” for the distribution of cloves in the world (Mahulette et al., 2019; Mahulette et al., 2022; Milind and Deepa, 2011; Alfian et al., 2019), and store a relatively high genetic diversity of cloves. According to data from the Ditjenbun (2020), Maluku has been the largest clove-producing province in Indonesia since 2015, with an average contribution of 15.37%. According to

BPS Maluku (2018), the area of clove plantations in Maluku in 2016 was recorded at 43,620.3 ha, then increased to 43,780.1 in 2017, with total production increasing from 20,805.6 tons in 2016 to 21,159.6 in 2017. Although the area of development is not as comprehensive as other provinces in Indonesia, the clove productivity in Maluku is higher, affecting the production. One of the causes of the increased productivity of cloves in Maluku is the high diversity of superior clove germplasm, which is supported by the suitability of the agro-climate.

Cloves in Maluku consist of many types with specific characteristics, but the most traded are "Tuni", "Hutan", and "Raja" cloves. Cloves "Tuni", "Hutan" and "Raja" are the local names of cloves in Maluku. (Mahulette et al., 2019; Hariyadi et al., 2020b; Alfian et al., 2019). According to Mahulette et al. (2022) and Hariyadi et al. (2020a), "Tuni" are classified as aromatic cloves, which have been released by the Indonesian Ministry of Agriculture as a superior variety since 2013, while "Forest" and "Raja" are classified as non-aromatic cloves. While the status of "Forest" cloves is temporarily in the stage of preparation for release as a variety, the study of clove "Raja" is still limited.

Information on the morphological diversity of "Tuni" and Forest cloves has been widely published, but is still minimal on "Raja" cloves. Clove "Raja" is a non-aromatic clove with an intermediate character; the morphological shape resembles cultivated type cloves from the aromatic group while the flowers have similarities to wild non-aromatic cloves, or the wild types (Alfian et al., 2019; Mahulette et al., 2022; Pool et al., 1986). According to Pool and Bermawie (1986) and Alfian et al. (2019), the "Raja" clove is probably the result of a cross between the cultivated and the wild type cloves so it is likely to have specific morphological characters.

Based on the description above, a study was conducted to inform on the morphological character of clove "Raja" to provide the basic information in the context of the future development of clove "Raja" in Maluku.

Material and Methods

Place and Time

The morphological characterization of "Raja" cloves was carried out at the distribution location of the "Raja" cloves, namely in Mamala village, Leihitu sub-district, Central Maluku district, Maluku province (S: 03033'34.8", E: 128011'41.7", altitude 18.3 mdp) in

June-August 2022.

Materials and Methods

The material used in the morphological observations consisted of thirty accessions of "Raja" clove plants that belonged to farmers. Thirty plants of clove "Raja" were selected and measured; the trees more than 20-years-old with the criteria for healthy growth and the similar crown shape. The characterization was carried out in the village of Mamala, the location of "Raja" clove distribution with a total area of ± 10 ha. Equipment for morphological observation consisted of a Haga meter to measure tree height; a tape measure for measuring the length, width and circumference of the rod; a measuring ruler to measure leaf length, flower length, fruit and seed length; a caliper to measure flower, fruit, and seed diameters; digital scales for weighing flowers, fruit and seeds; easy leaf area software for leaf area measurement; Royal Horticultural Society (RHS) colour chart 2015 for determining the colour scale of leaves, fruit, seeds.

Research Methods

The morphological characterization of "Raja" cloves used a survey method, and the selected sample was determined randomly. Observation of morphological characters refers to Tropical Fruit Descriptors (IPGRI 1980), Mahulette et al. (2019) dan Mahulette et al. (2022), which was modified.

Research Implementation

The morphological characters measured included the surfaces of trees, stems, branches, leaves, flowers, fruits, and seeds (Table 1). Morphological characterization was carried out on thirty samples of "Raja" clove plants, where ten pieces of leaves, flowers, fruit, and seeds were taken for measurement from each plant. The criteria for the leaves measured were selected from the fourth leaf, which was counted from the shoots (Ruhnayat, 2007; Mahulette et al., 2022; Mahulette et al., 2019) and free from pests and diseases, while ripe flowers picked were selected from flower arrangements with criteria that one or two flowers had bloomed (Tresniawati dan Randrian, 2011). Measurement of fruit and seeds was carried out when the fruit had entered physiological maturity, which was marked by a change in the colour of the fruit to purple-black.

Morphological characters are characterized as nominal or measurement variables. The nominal variable is then scored for Hierarchical Cluster Analysis (HCA) and Principle Component Analysis (PCA).

Table 1. Variables measuring the morphological character of clove germplasm

Plant parts	Observation variable
Tree	Height, habitus, canopy shape, canopy width (north to south and east to west).
Stem	Stem circumference, main stem shape (single/dividing).
Branch	Branching direction, branch angle, lowest branch length.
Leaf	Leaf size (index), leaf length and width, leaf shape, old leaf colour and shoot colour, leaf tip and base shape, leaf stalk length and colour, leaf surface texture, leaf edge shape, leaf veins, leaf thickness.
Flower	Number of flowers per stalk, flower length and diameter, flower shape, flower stalk length, the colour of flower buds, colour of ripe flowers picked, colour and shape of flower crowns, the weight of mature flowers picked.
Fruit	Fruit length and diameter, fruit weight, shape and colour of ripe fruit.
Seed	Seed length and diameter, seed weight, seed shape and colour.

Data Analysis

Analysis of the data from the morphological characterization used Hierarchical Cluster Analysis (HCA) to obtain the percentage of morphological similarity among all samples of the "Raja" clove plants. The data from the description was followed by Principle Component Analysis (PCA) to obtain the distinctive characteristics of Clove "Raja" (Descriptor of Clove "Raja"). Hierarchical Cluster Analysis (HCA) and Principle Component Analysis (PCA) were performed using R Stat 3.1.0 software.

Result and Discussion

Morphological Characters of Clove "Raja" In Mamala Village, Central Maluku Regency Based on Hierarchical Cluster Analysis (HCA)

Morphological characterization of "Raja" clove accessions in Mamala Village, Central Maluku Regency, was conducted against 54 morphological characters. The results of the overall grouping of clove accessions based on Hierarchical Cluster Analysis (HCA) are presented in Figure 1. According to Hartati et al. (2022) and Mahulette et al. (2022), Hierarchical Cluster Analysis (HCA) can be used to characterize the morphology of clove plants and can be used to select high-yielding parent trees. Furthermore, according to Ahmadzadeh and Felenji (2011) and Wang et al. (2014), HCA is widely used in breeding studies, especially to identify morphological characters and yield components, especially in the selection of accessions with high production potential.

Based on Hierarchical Cluster Analysis (HCA) results, "Raja" cloves in Mamala Village, Central Maluku Regency, can be divided into two large groups with 41% dissimilarity coefficients or only 59%

similarity. The first group consisted of 20 accessions grouped with a coefficient of dissimilarity of 14% (similarity 86%). The second group consisted of 10 accessions with a coefficient of dissimilarity of 6% (similarity of 94%). Accessions to the second group on the dendrogram were also divided into two groups, where the first group consisted of 11 accessions with a dissimilarity coefficient of 8% (92% similarity), and the second group consisted of 9 accessions with a dissimilarity coefficient of 10% (90% similarity). According to Maji and Shaibu (2012) and Rosmaina et al. (2021), plant accessions can be grouped into the same group if they have many characteristics in common. Furthermore, according to Jan et al. (2012) and Karuwal et al. (2021), accession groups that have many similarities are accession groups that tend to have a closer kinship. In contrast, accession groups that have many differences are accessions that tend to have somewhat distant kinship relationships.

The differences in morphological characters in the "Raja" clove population obtained in the study are thought to be caused by several factors, including genetic and environmental factors and the interaction between the two. Clove "Raja" has cross-pollination properties, so natural pollination in the long term can cause the emergence of new variants in the population. According to Mahulette et al. (2019c) and Singh et al. (2013), variations in a plant population can provide information on the diversity of genetic traits and the level of variability to assist in selecting superior genotypes.

The morphology of the clove "Raja" accession group is shown in Figure 2-4. In contrast, the results of the measurement of the morphological character of the "Raja" clove accession group in Mamala Village, Central Maluku Regency, are presented in Table 2.

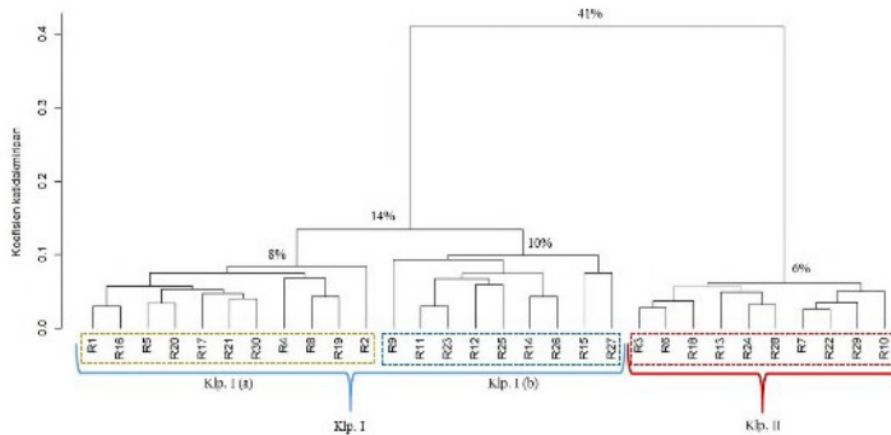


Figure 1. Dendrogram of Grouping of 30 Clove "Raja" Samples (Code: R1-R3) in Mamala Village, Central Maluku Regency, Based on 54 Morphological Characters.

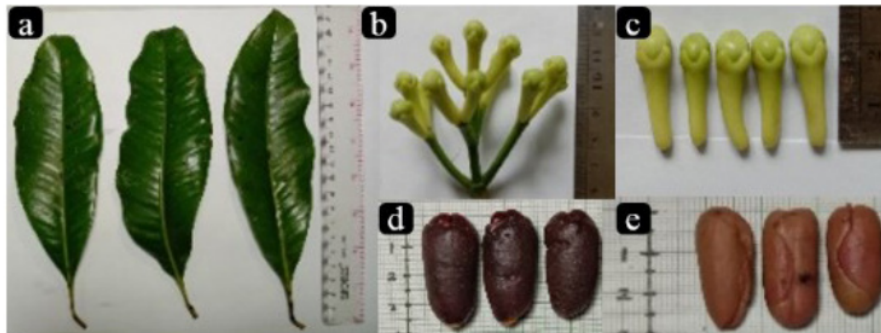


Figure 2. The morphology of cloves "Raja" group I-a, morphological parts: leaf (a), flower arrangement (b), flower bud (c), fruit (d), seed (e).

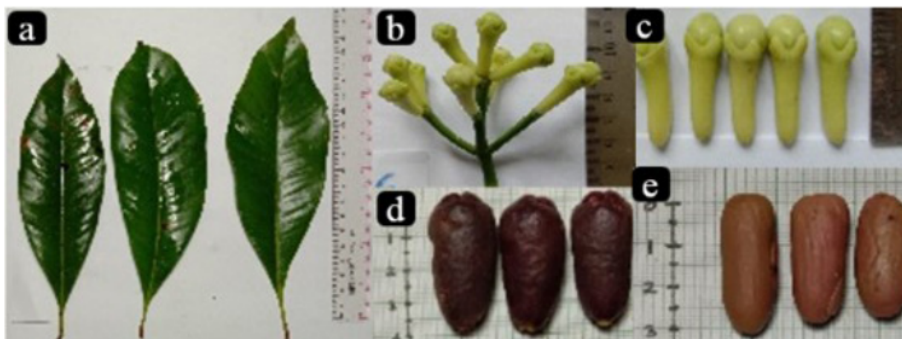


Figure 3. Morphology of cloves "Raja" group I-b, morphological parts: leaf (a), flower arrangement (b), flower (flower bud) (c), fruit (d), seed (e).

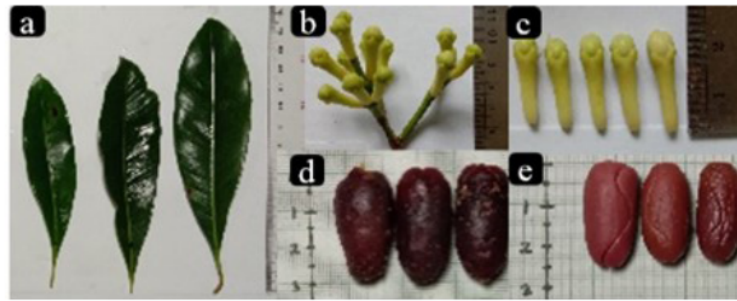


Figure 4. Clove "Raja" Group II, morphological parts: leaf (a), flower arrangement (b), flower bud (c), fruit (d), seeds (e).

Group I-a. This group consisted of 11 accessions grouped with 92% similarity (8% dissimilarity coefficient). Members of this group have a leaf length of 13.34 cm, a leaf width of 5.28 cm, and a leaf area of 58.39 cm², and the leaf colour is moderate green/green group (131B). The flower buds of group members have a flower length of 3.13 cm, a diameter of 3.68 mm, a weight of 0.58, and the number of flowers in each arrangement is 13.64. The colour of the ripe flower buds is brilliant yellow-green/yellow-green group (150B). This group has fruit with a length of 3.81 cm, a diameter of 1.61 mm, a weight of 6.68 g, and a fruit colour of dark red/red-purple group (59A). Seeds belonging to this group have a length of 2.70 cm, a diameter of 0.91 mm, a weight of 2.00 g, and seed colour is moderate red/greyed red group (181B).

Group I-b. This group consisted of 9 accessions grouped with 90% similarity (10% dissimilarity coefficient). Members of this group have a leaf length of 11.28 cm, a leaf width of 3.98 cm, and a leaf area of 38.12 cm², and the leaf colour is moderate green/green group (131B). The flower buds of group members have a flower length of 3.09 cm, a diameter of 3.62 mm, a weight of 0.97 g, and the number of flowers in each arrangement is 12.44. The colour of the ripe flower buds is brilliant yellow-green/yellow-green group (150B). This group has fruit with a length of 3.86 cm, a diameter of 1.69 mm, a weight of 7.03 g, and a fruit colour of dark red/red-purple group (59A). Seeds belonging to this group have a length of 3.06 cm, a diameter of 0.88 mm, a weight of 2.05 g, and seed colour is moderate red/greyed red group (181B).

Group II. The second group of the Clove "Raja" population consisted of 10 accessions with a 97% similarity level (3% dissimilarity coefficient). Members of this group have a leaf length of 11.15 cm, a leaf width of 3.87 cm, and a leaf area of 35.54 cm², and the colour of the leaves is deep yellowish green/green group (141B). The flower buds of group members have a flower length of 2.20 cm, a diameter of 3.51

mm, a weight of 0.42 g, and the number of flowers in each arrangement is 15.03. The colour of the ripe flower buds is brilliant yellow-green/yellow-green group (150B). This group has fruit with a length of 3.21 cm, a diameter of 1.39 mm, a weight of 4.11 g, and a fruit colour deep red/red-purple group (60A). The seeds of this group have a length of 2.80 cm, a diameter of 0.85 mm, and a weight of 1.71 g, the colour of the seeds is a deep purplish red/red-purple group (59B).

Characterization of Clove "Raja" in Mamala Village, Central Maluku Regency, Based on Principle Component Analysis (PCA)

Principle Component Analysis (PCA-Biplot and Variable PCA) was conducted on 30 accessions of clove "Raja". The PCA variable was performed on 36 characters with diverse morphological data (Figure 5a). The results show a diversity of morphological characters with a total variety of 70.5%, where the symbol indicates the characterizing nature with the most extended vector. These characters are shown as the characters that contribute the most to the grouping. PCA used can identify and classify groups of plant characters that contribute a lot to the collection. According to Rosmaina et al. (2021) and Yugandhar et al. (2018), PCA can determine the characters that contribute the most to the total variation of characters analyzed. According to Mahulette et al. (2022) and Sharma et al. (2018), principal component analysis can determine independent character traits from the overall analyzed characters and provide different grouping results.

The results of the PCA-Biplot (Figure 5b) show that the characteristics of "Raja" cloves group I are divided into two groups of characters : Clove "Raja" group I-a in the form of leaf area, leaf length, leaf width, leaf tip shape; and Clove "Raja" group I-b in the form of stem circumference, leaf stalk length, flower length, fruit length, fruit width, fruit weight. The characteristics

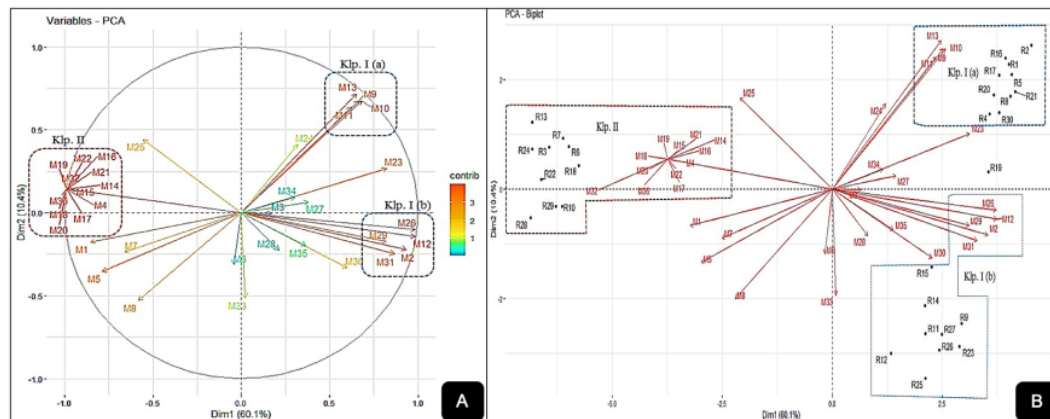


Figure 5. Principle Component Analysis: Variable PCA (5a), PCA-Biplot (5b) 30 clove accession “Raja” in Mamala Village, Central Maluku Regency, based on 36 morphological characters. Accession of Clove “Raja”, R1-R30; Morphological characters, plant height (M1), stem circumference (M2), main stem (M3), crown shape (M4), canopy width U-S (M5), canopy width T-B (M6), lowest branch height (M7), leaf size (M8), leaf length (M9), leaf width (M10), leaf area (M11), petiole length (M12), leaf tip shape (M13), upper leaf surface (M14), lower leaf surface (M15), leaf thickness (M16), leaf texture (M17), dark leaf colour (M18), shoot colour (M19), petiole tip colour (M20), leaf aroma (M21), leaf spiciness (M22), flower stalk length (M23), weight of flower stalk (M24), number of flowers/series (M25), flower length (M26), flower tube diameter (M27), the weight of ripe flowers picked (M28), fruit length (M29), fruit width (M30), fruit weight (M31), mature fruit colour (M32), seed length (M33), seed width (M34), seed weight (M35), seed colour (M36).

of Clove “Raja” group II consisted of morphological characters : crown shape, upper surface of the leaf, lower surface of the leaf, leaf thickness, leaf texture, old leaf colour, shoot colour, leaf tip colour, leaf aroma, leaf spiciness, flower stalk length, seed colour. The characterizing characters obtained in the morphological characterization of “Raja”’s cloves can be used as a reference in describing plant groups. These characters can be used to distinguish the morphological characters of “Raja” cloves in the population. According to Purnobasuki et al. (2014), characterizing characters obtained from PCA results can be used as a reference in “Raja” plant descriptors to assist in identifying certain plant groups. The use of PCA in previous studies has been widely used in studying morphological variations in plantation crops such as cloves (Mahulette et al. 2022, 2019b). These results indicate that although they are still of the same type, there are still differences between accession groups’ characterizing characteristics. This suggests that the clove plant is cross-pollinated, so it is possible for new variants to emerge in the population. PCA can help describe the characters that most contribute to the grouping of “Raja” cloves in the population.

Conclusion

Morphological characterization of clove “Raja” accessions based on Hierarchical Cluster Analysis (HCA) on 30 plant samples based on 54 morphological characters obtained two accession groups with a 41% dissimilarity coefficient. The first group consisted of two accession groups and the second group consisted of one accession group. The Principle Component Analysis (PCA) results obtained morphological diversity in the distribution population of “Raja” cloves with a total variety of 70.5%. The first group consisted of accessions of cloves “Raja” group I having identifiable characters in the form of leaf area, leaf length, leaf width, and leaf tip shape, while the second group of clove accessions of “Raja” group I in the form of stem circumference, petiole length, flower length, fruit length, fruit width, fruit weight. Characteristics of accession of cloves “Raja” group II are distinctive morphological traits in the form of crown shape, an upper surface of the leaf, a lower surface of the leaf, leaf thickness, leaf texture, old leaf colour, shoot colour, petiole tip colour, leaf aroma, leaf spiciness, flower stalk length, seed colour.

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