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Various macro and micro-morphological characters of three sex types of highland papaya (*Vasconcellea pubescens*) in Java, Indonesia

AINUN NIKMATI LAILY[1,♥], BUDI SETIADI DARYONO[1], AZIZ PURWANTORO[2], PURNOMO[1]

[1]Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika South, Sleman 55281, Yogyakarta, Indonesia.
Tel./fax.: +62-274-580839, ♥email: ainunnikmati2020@mail.ugm.ac.id
[2]Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora No. 1, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia

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Abstract. *Laily AN, Daryono BS, Purwantoro A, Purnomo. 2022. Various macro and micro-morphological characters of three sex types of highland papaya (*Vasconcellea pubescens*) in Java, Indonesia. Biodiversitas 23: 6238-6246.* Highland papaya (*Vasconcellea pubescen*s A.DC.), locally called *karika*, is a fruit plant successfully introduced to Java, Indonesia. *V. pubescen*s plays an essential role as it contains various substances that can be used as ingredients in the food, pharmaceutical, and cosmetic industries. This plant is *dioecious* and *monoecious* as it contains three sex types, namely female, male, and *monoecious*. It grew from seed and began to bear fruit two years after sowing. The consumer preference for fruit is produced from female plants because female plants produce fruit faster, more abundantly, and with maximum fruit size under suitable environmental conditions. Sex differentiation is important for farmers to ensure the growth of female plants. Therefore, this research aimed to investigate the variation in macro and micro-morphological characters. The results showed that leaf and flower differ in macromorphological characters among the three sex types. Similarly, there were considerable differences in the trichomes of female and *monoecious* plants based on micro-morphological characteristics. There was no significant difference in seed shape between those. Seeds from female and *monoecious* plants differed in macro and micro-morphological characters. Macro and micro-morphological characters might have potential applications in early sex determination in *V. pubescens*. Macro and micro-morphological characters have many potential applications in early sex determination in *V. pubescens*.

Keywords: Indonesia, Java, morphology, sex, *Vasconcellea pubescens*

INTRODUCTION

Vasconcellea spp. belongs to the *Caricaceae* family. This plant, specifically the *Vasconcellea pubescens* A.DC., is widespread in South America and is known to originate from Ecuador (Salvatierra-González and Jana-Ayala 2016). It was introduced to Indonesia as a raw material for the pharmaceutical industry. Due to its economic value, it is cultivated by the natives of the Dieng Plateau in Central Java. Subsequently, this plant spread to two other areas with similar altitudes and natural conditions, namely Bromo and Cangar Batu in East Java. *V. pubescens* plays an essential role as it contains various compounds that can be used as ingredients in the food, pharmaceutical and cosmetic industries, such as phenols (Rahayu et al. 2019). *V. pubescens* has antifungal properties (Torres-Ossandón et al. 2019), anti-inflammatory (Albuquerque 2020), and wound healing effects (Silva 2020).

*Vasconcellea pubescen*s is mainly *dioecious* and *monoecious* in that the female and male flowers grow on different plants. However, a low percentage of *monoecious* plants with pistillate and staminate flowers on the same plant were discovered. Salvatierra-González and Jana-Ayala (2016) reported that *V. pubescen*s is a *trioecious* plant in Ecuador, Colombia, and Peru, but *dioecious* in Chile and *monoecious*. This corresponds to the research by Laily et al. (2021), which stated that three variations of *V. pubescen*s were observed in *dioecious* plants. Farmers often expect the female plant to produce spherical fruit with thick flesh and short stalk, while information regarding the use of male plants remains limited. The existence of male, female, and *monoecious* plants causes problems in the commercial cultivation of *V. pubescen*s. Farmers have difficulty distinguishing the sex of these plants, specifically at the seed stage and when they are young.

Sex determination in plants is a developmental process that promotes allogamy to support their fitness and living fitness (Lloyd 1982). The family of *Caricaceae* is a very interesting family for the study of sex determination because it has unisexual flowers on *monoecious* and *dioecious* plants. Despite *V. pubescens* being grown as a plantation crop for a long time in Indonesia, there is still limited scientific literature on the fundamental aspects of macro and micro-morphology in male, female, and *monoecious* plants. Sex expression in this crop provides useful information in the development of plant cultivation protocols to increase fruit production. Knowledge of macromorphological characteristics, such as the majority of organs and trichomes as species-specific identifiers, will also be of great importance in the direct use of plant seeds for agricultural production. Furthermore, it is useful in the selection of male or female plants in hybrid populations and the cultivation of strong F1 females. Gong (1995) studied the leaf shape, crown layer, bark and mature plants of *Populus talassica* in summer and winter, and suggested morphological parameters to match the gender. Lian et al.

(2000) measured the leaves of *Hippophae rhamnoides* and discovered that female plants usually have a higher ratio of leaf length to width than males.

The morphological characters of plants are valuable inputs for the preservation of genetic resources. According to Wahyuni and Bermawie (2015), nutmeg's male and female plants (*Myristica fragrans*) are distinguished based on morphological characteristics. The male has protrusions or horns at the end of the seeds, while female nutmeg seeds tend to be smoothly rounded. Indonesia lacks complete information on this fruit's morphological character for determining sex. Therefore, macro and micro-morphological characters of *V. pubescen*s should be explored to complete the information. This research aimed to determine the macro and micro-morphological characteristics of vegetative and generative organs for sex determination on *V. pubescen*s in Java, Indonesia.

MATERIALS AND METHODS

This research was conducted from April to September 2022. Identification and sex determination of morphological characters were performed in the field. *V. pubescen*s was sampled in three regions of Java, Indonesia, namely Dieng Plateau in Central Java, as well as Batu and Bromo in East Java.

The morphological data was measured using descriptor books, such as the International Board for Plant Genetic Resources (IBPGR) in 1988 and the International Union for The Protection of New Varieties of Plants (UPOV) in 2014 for papaya, sewing meters, rulers, pencils, scissors, digital camera, GPS (Global Positioning System) Garmin eTrex, digital pH meter, soil tester, garden temperature and sunlight monitor measuring tool, Stereo Olympus SZX7 microscope, Scanning Electron Microscopes (SEM), as well as Munsell color chart.

V. pubescens from Dieng, Batu, and Bromo, with a total of 27 trees, were used as plant material. Table 1 shows the accession. On flowering, various macro and micro-morphological characters of *V. pubescen*s were observed (Tables 2 and 3).

Plantation lands in Dieng Plateau, Batu, and Bromo are located at S 07°10'57.36" E 109°53'28.04", S 07°44'24.43" E 112°32'04.13", and S 07°55'11.43" E 112°52'38.59" with soil pH of 6.5, 6, and 5.5 at an altitude of 1.822 meters above sea level (m a.s.l.), 1.606 m a.s.l., and 1.903 m a.s.l. as well as have the temperature of 21°C, 20°C, and 16°C, respectively. In each field, *V. pubescens* was discovered in three sex expressions of female, male, and *monoecious* plants. Figure 1 shows the location.

Table 1. Accession of *Vasconcellea pubescens* on Java Island

No. of accession	Location	Vernacular name	Height (m asl.)	Latitude	Longitude	pH	Temp. (°C)	Collection sources
1	Kepakisan Village, Batur Sub-district, Banjarnegara District, Central Java	*Karika*	1.822	07°10'57.36"S	109°53'28.04"E	6.5	21	Farmer
2	Sumberbrantas Village, Bumiaji Sub-district, Batu District, East Java	*Karika*	1.903	07°44'24.43"S	112°32'04.13"E	6	20	Yard owner
3	Ngadirejo Village, Tutur Sub-district, Pasuruan Dstrict, East Java	*Carikaya, Karika*	1.606	07°55'11.43"S	112°52'38.59"E	5.5	16	Planted land officers

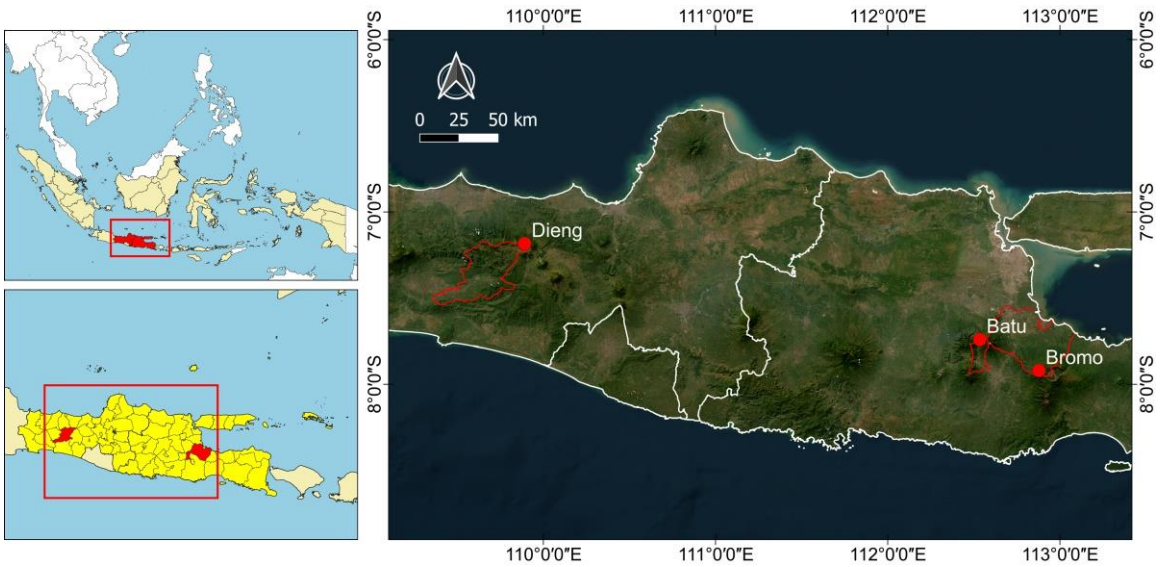

Figure 1. Distribution and sampling locations of *Vasconcellea pubescens* in Java Island, Indonesia

Table 2. The descriptor of macromorphological character

Character	Scoring /note
Tree habit	1 = single stem, 2 = multiple stems
Stem color (adult trees)	1 = greenish or light grey, 2 = greyish brown, 3 = green and shades of red-purple (pink), 4 = red-purple (pink)
Stem pigmentation	1 = only or mostly basal, 2 = only or mostly lower, 3 = only or mostly median, 4 = only or mostly upper, 5 = indiscriminate
The general shape of mature leaf teeth	1 = straight, 2 = convex, 3 = concave
Leaf waxines	0 = absent, 1 = present
The general shape of petiole sinus	1 = open, 2 = slightly open, 3 = slightly closed, 4 = strongly closed
Type of sex	1 = monocious, 2 = female, 3 = male
Type of flowering	1 = flowers solitary (single borne), 2 = inflorescences, 3 = both
The color of inflorescence stalk	1 = greenish, 2 = purplish/pinkish, 3 = dark red-purple/pink
Predominant inflorescence	3 = small, 5 = intermediate, 7 = large
Flower size	3 = generally small, 5 = generally intermediate, 7 = generally large
Corolla tube color	1 = white, 2 = white yellow (cream), 3 = yellow, 4 = deep yellow to orange, 5 = greenish, 6 = dark green, 7 = yellow/green and red-purple shades, 8 = red purplish (pinkish), 9 = dark red-purple (pink)
The density of inflorescences on the trunk	3 = sparse (few infloresences), 5 = intermediate, 7 = dense (many inflorescences)
Inflorescence density	3 = sparse (flower), 5 = intermediate, 7 = dense (many flower)
Sex change of flowers during growth	0 = no, 1 = yes
Fruit shape	1 = globular, 2 = round, 3 = high round, 4 = elliptic, 5 = oval, 6 = oblong, 7 = oblong-ellipsoid, 8 = oblong-blocky, 9 = elongate, 10 = lengthened cylindrical, 11 = pear shaped (pyriform), 12 = club, 13 = blossom end tapered, 14 = (heart shaped), 15 = reniform, 16 = turbinate inferior
Fruit skin color	1 = yellow, 2 = deep yellow to orange, 3 = red/purple, 4 = yellowish green, 5 = green
Fruit flesh color	1 = light yellow, 2 = bright yellow, 3 = deep yellow to orange, 4 = reddish orange, 5 = scarlet
The skin color of immature fruits	1 = yellow, 2 = light green, 3 = green
Stalk end fruit shape	1 = depressed, 2 = flattened, 3 = inflated, 4 = pointed
Blossom end scar	3 = smooth, 5 = intermediate, 7 = rough (ridged)
Ridging on the fruit surface	3 = superficial (low depression), 5 = intermediate (moderate depression), 7 = deep (usually 5 distinct ridges)
The shape of the central cavity	1 = irregular, 2 = round, 3 = angular, 4 = slightly star shaped, 5 = star shaped
The thickness of fruit skin	3 = thin, 5 = intermediate, 7 = thick
Flesh aroma	3 = mild, 5 = intermediate, 7 = strong
Flesh density	1 = very low (spongy), 3 = low (crumbly), 5 = intermediate, 7 = dense (crisp), 9 = very dense (firm)
Flesh fibrousness	0 = absent, 1 = present
Placental tissue	3 = little, 5 = intermediate, 7 = much
Seed color	1 = generally tan, 2 = generally grey-yellow, 3 = generally grey, 4 = generally brown black, 5 = generally black, 6 = variable
Seed germination in ripe fruit	0 = absent, 1 = present
Seed surface lustre	1 = generally dull, 2 = generally intermediate, generally glossy
Seed shape	1 = generally round, 2 = generally spherical or ovoid
Seed surface type	1 = generally translucent, 2 = generally opaque
Seed mucilage	1 = almost absent, 3 = small amount, 5 = intermediate amount, 7 = large amount

Table 3. The descriptor of micro-morphological character

Character	Scoring /note
The density of trichome on the leaves	1= low, 2 = middle, 3 = high
The contour of the seed coat	1= tend to be simple, 2 = tend to be complicated
The color of the seed coat	1= generally tan, 2 = generally grey-yellow, 3 = generally grey, 4 = generally brown black, 5 = generally black, 6 = variable

Macromorphological characters

Vasconcellea pubescens was counted, and healthy plants with complete and adequate organs were selected. Based on the sex type, the plants were classified as female, male, *monoecious*, or monoecious. The morphological characters of vegetative and generative organs with the scoring and coding guidelines of *V. pubescen*s were observed, recorded, and compiled based on the descriptor book. Finally, plant organ sampling was performed on

leaves, flowers, and fruit by marking each sample. Table 2 shows the descriptor.

Micro-morphological character

Using Olympus microscope SZX10 and Scanning Electron Microscopes (SEM), leaf and seed samples were analyzed for trichome characters and seed surface contours, respectively. The collected leaf sample was the first lobe from the right on mature leaves obtained from the center of the stem and then the organ incision. Subsequently, the images of the trichomes were documented. The seed contour of *V. pubescens* was observed on each sample by examining the grooves and density of the contour on the seed coat surface. These were performed using a microscope, and documentation was conducted in the same field of view.

Growth at the seedling stage

Seed samples were prepared from fruit produced by female and *monoecious V. pubescens* plants. The germination of seeds was conducted in a 20 x 20 cm plastic container containing a thoroughly mixed planting medium. This was performed by placing the seeds in a regular row inside a closed plastic tube and maintaining humidity by watering. After five weeks, the seeds were transferred to the field.

Data analysis

Macro and micro-morphological data of vegetative and generative organs in the form of leaf trichomes and contours are described to determine the characters in different sex expressions specifically.

RESULTS AND DISCUSSION

Macromorphological characters

This research revealed undescribed macro-morphological characters identified in three different sex types of *V. pubescen*s in Indonesia. The morphological characterization of this plant does not explain the sex determination. The macromorphological observations of *V. pubescens* in female, male, and *monoecious* plants showed differences in several organs (Table 4). This occurred at locations for plants growing at Dieng Plateau, Batu, and Bromo. *V. pubescens* develop excellently in these three places because they have more or less the same natural conditions, such as altitude in the range of 1.600 - 2.000 m a.s.l., soil pH in the range of 5.5 - 6.5, and they occupy areas around volcanoes. Dieng plateau is a volcanic area with clear geological traces in the form of many craters, including Sileri, Sikidang, and Sinila Craters in Central Java. Batu is located in a volcanic area around Mount Arjuna in East Java. Meanwhile, Bromo is an active volcano with a phreatomagmatic eruption in East Java. These seem special places where *V. pubescens* grows, which are not found in other areas on Java Island.

The characteristics of *V. pubescens* observed in male, female, and *monoecious* plants are identical. In all three types of sexual expression, the tree height ranges from three to six meters and shows marked branching. Trees can develop branches by cutting the main trunk. Two branches are generated when the main trunk is cut. This will certainly increase plant productivity, specifically for female and *monoecious* plants. Farmers of *V. pubescens* explained that trees derived from reproduction by seeds have a much better resistance quality than their reproduction by cuttings. Plants originating from generative reproduction survive and produce well for about eight years, while those from vegetative reproduction need to be replaced after two years. However, crops that undergo vegetative propagation are faster in producing fruit. The plants on the Dieng Plateau land have been widely cultivated and are a mixture of vegetative and generative reproduction. Meanwhile, those in Batu and Bromo that have not been widely cultivated are derived from generative reproduction. Stem color does not differ significantly in the three sex expressions of the plant. They exhibited greenish or light grey color. Similarly, all three with their stem pigmentation only show colors only or mostly the upper stem. Figure 2 shows tree habit, stem color on adult trees, and stem pigmentation on three sex expressions of *V. pubescens*. Differences in tree habit, stem color on adult trees, and stem pigmentation were not identified. As a result, macromorphological characters in tree habit, stem color on adult trees, and stem pigmentation in *V. pubescens* were not significantly expressed in plants of different sexes.

Leaf shape, the general shape of petiole sinus, and the general shape of mature leaf teeth of male, female, and *monoecious* plants of *V. pubescens* showed differences. Maturity in plants is marked by the appearance of flowers as a means of sexual reproduction. However, there seem to be differences in vegetative organs in this plant. The young leaf tends to be oval-shaped with an equilateral, isosceles, and equilateral and isosceles triangle tendency in female, male, and *monoecious* plants, respectively. In general, the leaves of this plant are round-shaped (*orbicularis*). The leaf's base on the petiole sinus's shape shows variations, such as concave, convex, and strighty in *monoecious* plants. Similarly, the general shape of mature leaf teeth in *V. pubescens* looks slightly open on males and females and straightly closed on *monoecious* plants. Despite flowers being the indicator of the plant's adult phase and thus sex type, there are leaf organs in both young and old plants that can be used in sex determination. This is because the leaf organ's development is related to the differentiation of flower organs. The dominant stem color is strong yellowish green mixed with deep purplish pink in different proportions. Leaf shape is fascinating research in itself. Figure 3 shows the differences in *V. pubescens*'s leaf.

Figure 2. Macromorphological characters of three sex types of *Vasconcellea pubescens* in Java Island, Indonesia: A. Tree habit, B. Stem color on adult trees and pigmentation: 1. female plant, 2. male plant, 3) *monoecious* plant

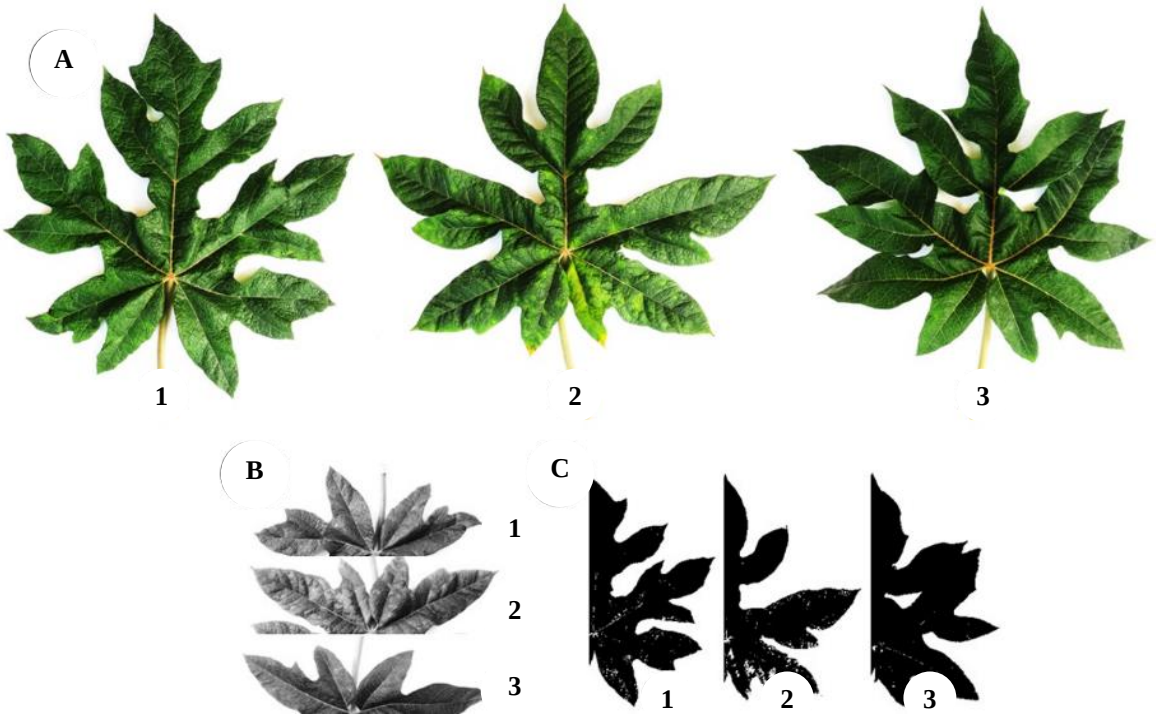

Figure 3. Macromorphological characters of three sex types of *Vasconcellea pubescens* in Java Island, Indonesia: A. Leaf shape and petiole color, B. The general shape of petiole sinus, C. General shape of mature leaf teeth: 1. female plant, 2. male plant, 3. *monoecious* plant

The advent of flowers with a clear morphological distinction between male and female in *V. pubescens* comes approximately two years after planting and facilitates the sex determination of the plant. The plant's stamens and pistil are stored in slender male and elongated round bell-shaped female flowers, respectively. In male plants, only male flowers are identified with short stalks. Similarly, only female flowers are identified with short stalks in female plants. This situation, therefore, places *V. pubescens* in the group of *dioecious* plants. There are also *monoecious* plants with male and female flowers in one inflorescence, usually having long stalks. Hence, this species also produce *monoecious* plant. A similar tri-sexuality phenomenon occurred with *Jatropha curcas*, which can be monoecious, andromonoecious, and trimonoecious (Dasumiati et al. 2017). Furthermore, there are differences between male flowers on *monoecious* and *dioecious* plants. The stamens are more submerged in *dioecious* plants. Also, there is a difference between female flowers on *monoecious* and *dioecious* plants. The flower petals are more curved in *dioecious* plants. Morphological variations within species are also common with *Curcuma soloensis,* which differs in habit, stem color, leaf shape, rhizome shape, rhizome flesh color, and tuber shape (Jalil et al. 2020). It occurs due to different physiological processes in various sex expressions. Meanwhile, in fern (*Cibotium barometz*) there is a correlation between gametophyte size, shape and sex expression in relation to population density (Praptosuwiryo and Isnaini 2017). Figure 4 shows the variation of flower morphology in *V. pubescens*.

Table 4. Determination of sex type based on descriptors for papaya (IBPGR and UPOV) in *Vasconcellea pubescens*

Morphological character	Female plant	Male plant	*Monoecious* plant
Stem color (adult trees)	greenish or light grey	greenish or light grey	greenish or light grey
Leaf shape	oval shape with an equilateral triangle tendency	oval shape with a tendency to an isosceles triangle	oval shape with a mixed tendency of equilateral triangles and isosceles triangles
The general shape of mature leaf teeth	concave	convex	straight
The general shape of the petiole sinus	slightly open	slightly open	slightly closed
Type of sex	pistillate flowers only	staminate flowers only	*monoecious* flowers
Sex change of flowers during growth	no	no	yes
Fruit shape	oval	-	elliptic
Seed color	generally brown black	-	generally brown

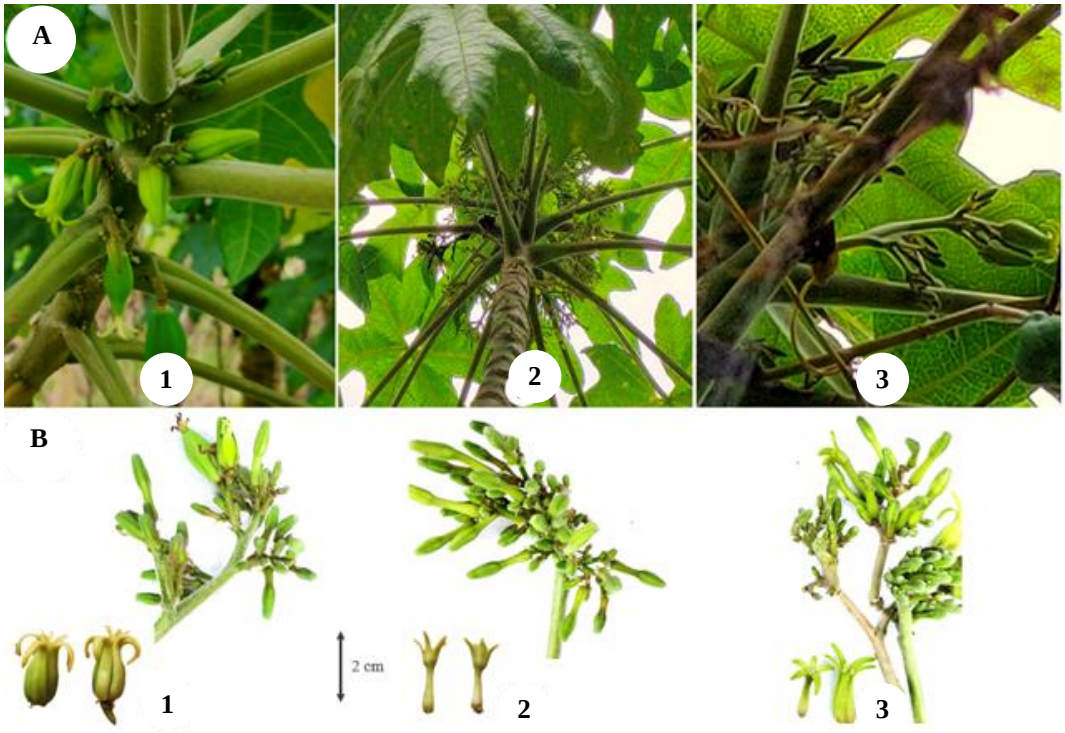

Figure 4. Macromorphological characters of three sex types of *Vasconcellea pubescens* in Java Island, Indonesia: A. Flowers on the tree, B. Flowers and inflorescence: 1. female plant, 2. male plant, 3. *monoecious* plant

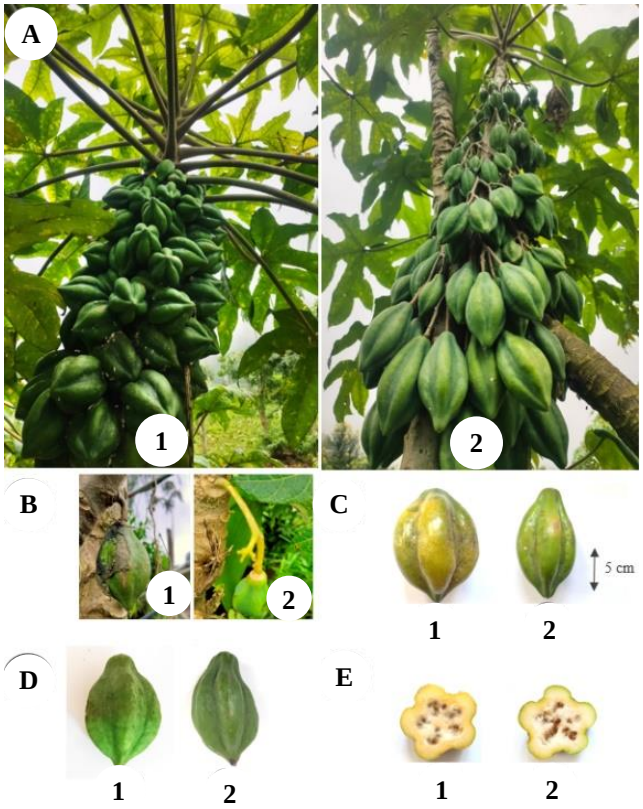

Figure 5. Macromorphological characters of three sex types of *Vasconcellea pubescens* in Java Island, Indonesia: A. Fruit on the tree, B. Stalk end fruit shape, C. Fruit shape and skin color, D. The skin color of young fruits, E. Fruit flesh color: 1. female plant, 2. *monoecious* plant

Vasconcellea pubescens fruits are produced in female and *monoecious* plants. The striking difference between the two is the presence of long stalks and more slender fruit in *monoecious* plants. Therefore, the fruit on the female plant appears to be attached to the stem, with the stalk virtually not visible. It is also more rounded and tightly packed, as shown in Figure 5.

The following table shows the results obtained based on observations of the morphology of vegetative and generative organs in *V. pubescen*s in Java, Indonesia.

Micro-morphological characters

In this research, in addition to the observation of trichomes, the contours of the seeds are included among the micro-morphological characteristics of *V. pubescens* for sex determination. Seeds from female plants have a brighter color and slightly wider contour than those from *monoecious*. The cocoa brown color of the seeds of female plants corresponds to the Munsell value: 2.5YR3/4, sRGB: R=10, G=61, B=47, and the contours tend to be simple. Meanwhile, the seeds from femalemonocious plants have a color that is proportional to the value. On Munsell value: 2.5YR31/2, sRGB: R=44, G=22, B=19, the contours tend to be more complicated, as shown in Figure 6a. A similar observation was reported by Wahyuni and Bermawie (2015) when comparing male and female nutmeg plants in the early seed stage. The characteristic of the male nutmeg seed is the tip that has a bulge/horn.

The species designation "pubescens" means "feathers" indicating the presence of trichomes on the plant. Trichomes of *V. pubescens* are in most plant organs, including adaxial and abaxial leaves, petioles, flowers, and stalks. The research revealed that trichomes' leaves in male plants were longer than in female and *monoecious* plants. For example, in this observation, the length of the trichomes on male, female, and *monoecious* leaves averages 703.353 µm, 569.86 µm, and 523,706 µm. In contrast, regarding trichome density, there is no tendency for one sex type to have denser trichomes. The density in the abaxial leaves is lower in females. Trichomes on the adaxial surface were only a few, while on the abaxial surface, there were many. On the adaxial surface of the leaf, the veins and lamina were not significantly different in the three sex types, as shown in Figures 6b, c, d.

Micro-morphological observations are expected to support early sex determination of *V. pubescens*, specifically in the vegetative phase before the emergence of flowers. The observations of Susanto et al. (2016) were made based on the anatomical character of leaves in distinguishing male and female plants of snake fruit. The differences in the anatomical characters of male and female snake fruit leaves are in the thickness of the leaves mesophyll of the "pondoh" snake fruit males. In contrast, the thickness of the cuticle, the thickness of the epidermis, the length and width of the stomata, and the number of stomata and trichomes between regions also do not differ. between sexes

Trichomes in *V. pubescens* are non-secretory, long-conical in shape, unicellular, and unbranched. Trichomes that are in the epidermal tissue have special properties as a defense against insects. This property is determined by the presence or absence of glands (glandula), density, length, shape, and straightness of the trichomes. Trichomes reduce leaf temperature, increase light reflectance, prevent drying and reduce frictional forces on leaves (Margineanu et al. 2014). Taxonomically significant is the presence of continuous stomata and sinuous anticlinal walls on the abaxial leaf surface of the non-bitter variant of *Vernonia amygdalina* and in both leaf surfaces (adaxial and abaxial) of *Vernonia cinerea* (Kemka-Evans et al. 2014). The grouping of species in this genus is mainly determined by the existence of trichomes on leaf surfaces and petiole, leaf venation pattern, variegation, and shape (Permata and Susandarini 2021).

Growth at the seedling stage

The seed form of *V. pubescens* is classified as oval based on the seed index, calculated by the length divided by the width. There was no significant difference in seed shape between those from female and *monoecious* plants. Differences between female and monoecious *V. pubescens monoecious* after germination was not obvious either, as shown in Figure 7. The difference in leaf lobes is not a distinguishing feature of plants that will grow into female, male, or *monoecious* plants. *Monoecious* nutmeg, *V. pubescens*, has a slightly bulging seed belly. In contrast, female nutmeg has a rounded seed tip with a slightly flat belly (Bermawie and Wahyuni 2015). Seed size influences seedlings' initial growth but not after three months (Pramono et al. 2019).

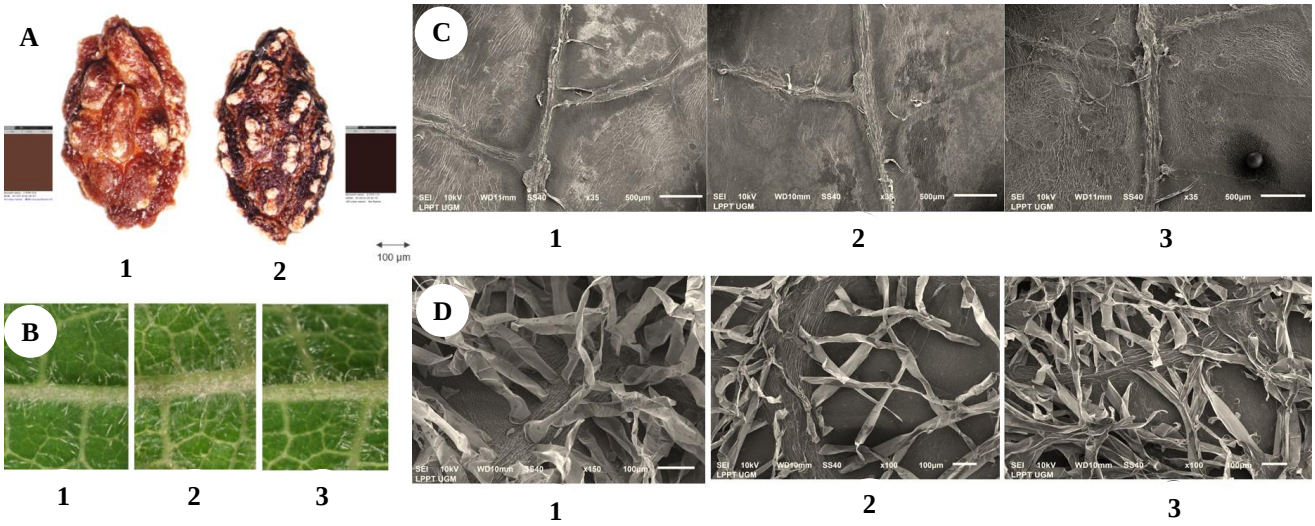

Figure 6. Micro-morphological characters of *Vasconcellea pubescens* in Java Island, Indonesia: A. Contours and colors on the surface of seeds by a stereo microscope, B. Trichomes on abaxial leaf sides by a stereo microscope, C. Nervatio on the adaxial leaf by SEM, D. Trichomes on abaxial leaf sides by SEM: 1. female plant, 2. male plant, 3. *monoecious* plant

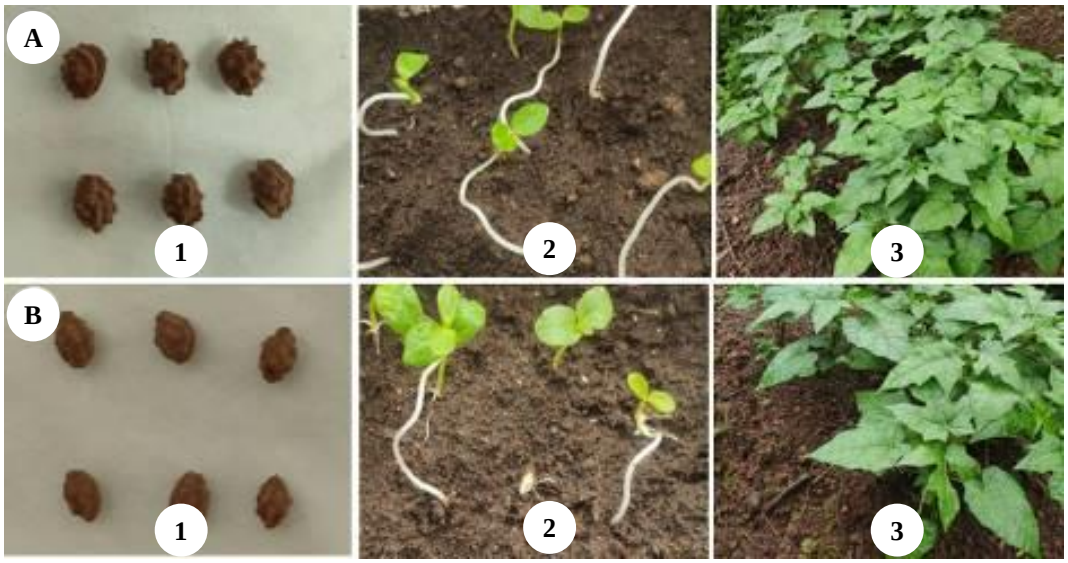

Figure 7. *Vasconcellea pubescens* at the seedling stage: A. Female plants, B. *Monoecious* plants. 1. Seeds, 2. Seedlings at 5 weeks of age, 3. Young plants at 17 weeks of age.

Knowledge of macro and micro-morphological variations of plants that are striking in each sex type is needed as it helps farmers of *V. pubescens* to cultivate more efficient plants. According to Pramashinta et al. (2003), the presence of male and female trees poses a problem in the commercial farming of *Gnetum gnemon*, particularly when it is reproduced by seed, as sex cannot be determined before five to six years after sowing. The most straightforward sex determination is by distinguishing generative organs through morphological characters. However, emerging generative organs are time-consuming. In conclusion, micro and macro-morphological characters might have many applications for early sex determination of *V. pubescens* on Java Island, Indonesia.

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