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PLANT SEGREGATION AND POLLEN CHARACTERISTICS OF HIGHLAND PAPAYA (*VASCONCELLEA PUBESCENS* A.DC.) BASED ON SEX TYPES

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SUMMARY

Highland papaya (*Vasconcellea pubescens* A.DC.) is a member of the family Caricaceae and native to Ecuador. The plant's introduction and cultivation were successful in specific highland areas of Indonesia but with limited growing areas. *V. pubescens* has monoecious and dioecious characteristics and allows fruit production from female dioecious and monoecious plants. This plant is valuable, and the knowledge about its sex pattern, though still limited, is crucial for its efficiency, productivity, and development. Hence, the presented study aimed to explain the patterns of plant segregation, as well as, pollen morphology and germination based on sex in *V. pubescens*. The investigations began by recognizing the total population of *V. pubescens* in its cultivated area for grouping and mapping according to sex. Observations on the pollen characteristics, such as, morphology and germination, used light microscopy and a Scanning Electron Microscope (SEM). The results revealed that the basis for plant segregation pattern depended on sex in male and female dioecious and monoecious plants of *V. pubescens* (1:5:1). In both sexes, the pollen morphological characteristics showed that the unit of monads, prolate spheroidal, radial, tricolporate, isopolar, reticulate, and P/E index of male dioecious pollen was 0.70, while monoecious was 0.52. Anthers and pollen grains from the male plant were round, but those of monoecious elongate more. However, no significant differences occurred among the sexes for pollens. The pollen grains from male flowers on male dioecious plants showed faster germination than monoecious individual plants, having a difference of 25.14 days.

Key words: *V. pubescens,* sex types, plant segregation, pollen morphology, pollen germination

Key findings: *V. pubescens* are of three types growing in Indonesia, i.e., male dioecious, female dioecious, and monoecious. SEM observations showed that the shape of anthers and pollen of flowers in male plants were round, while those in monoecious elongated. Based on germination and the time required for pollen tube formation, pollen from male flowers of male dioecious reached the ovule stage faster than monoecious, with a time difference of 25.14 days.

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INTRODUCTION

Highland papaya (*Vasconcellea pubescens* A.DC.) is a well-known member of the family Caricaceae in addition to common papaya (*Carica papaya* L.) in Indonesia. This plant is native to Ecuador and can be dioecious and monoecious. It is also an essential plant in South America, specifically Chile (Chong-Pérez *et al*., 2018). In Indonesia, *V. pubescens* introduction has been successful in a limited growth area and thrives only in the highlands around volcanoes at an altitude of 1400 to 2400 masl (Minarno, 2016).

A population of *V. pubescens*, a species of papaya, is known to grow at an altitude of 1606 masl around Mount Arjuna in Batu, East Java, Indonesia. An important source of food, pharmaceutical, and cosmetics, the said species is very beneficial. *V. pubescens* contain flavonoids, polyphenols, tannins, and triterpene saponins (Minarno, 2016). Some of the biological properties of *V. pubescens* include antioxidant (Indranila and Ulfah, 2015), antibacterial (Vega-Galvez *et al*., 2020), antidiabetic (Laily and Khoiri, 2016), and analgesic (Sasongko *et al*., 2016), as well as, promote wound healing (Tonaco *et al*., 2018). Some uses of *V. pubescens* by the community include a traditional crop in the Andes for food and medicine (Peñafiel *et al*., 2016), as well as a fruit used in a ceremony to celebrate the arrival of the rainy season (Acosta-Solís *et al.*, 2018).

The sex-based segregation patterns of *V. pubescens*, a dioecious and monoecious species, need further understanding. Plant segregation based on sex is an important tool for improving crop yields and protecting endangered species, as it allows farmers and scientists to select the plants that best suit their needs (Brown, 2014). Studies on sex ratios have primarily focused on dioecious plants (Munné-Bosch, 2015). However, particular sex expressions observed have also risen in monoecious trees (Leslie *et al*., 2013). Investigations have shown several factors alter sex expression in monoecious plants, including sex allocation (Pannell, 2017), environmental pressure, and adaptation to wind pollination (Walas *et al*., 2018). The tree size also significantly impacts sex expression, with females and monoecious trees often found in higher-diameter classes, which can compensate for a more reproductive investment (Khanduri *et al*., 2021).

Detailed studies on environmental factors are necessary to discuss their possible impacts on populations, such as, light intensity and temperature. Temperature-sensitive sex determination (TSD) and light-sensitive sex determination (LSD) are two mechanisms to verify sex in plants (Chen *et al.*, 2014). In TSD, the sex validation of a plant is by the temperature at which it is grown. In LSD, sex determination is by its exposure to the amount of light. The study found that cooler temperatures led to an increase in gene expression that promotes male development, and warmer temperatures led to an increase in gene expression that fosters female development in rice (Li *et al*., 2018).

Flora diversity studies can start through pollen morphology (Noflindawati *et al*., 2019). However, information on *V. pubescens* as a monoecious plant with variations in pollen characteristics and germination is limited. Variations in the form of pollen in plants relate to its function in the distribution space (Qodriyyah *et al.*, 2015). Yet, generally, the pollen with small size comes from plant species with unattractive flowers. According to Fidianinta *et al.* (2015), no relationship between the diameter and length of the pollen tube exists with germination in papaya pollen (Caricaceae). Pollen morphology of *V. pubescens* has had brief discussions on regeneration processes from anther culture. Previous studies have shown that most pollen grains in the early uninucleate/vacuolate and binucleate stages ranged from 15 to 25 mm in flower buds (Chong-Pérez *et al*., 2018). Furthermore, pollen from male flowers, from both ambisexual (it has both male and female reproductive organs) and male plants, germinates 75% in summer, yet, germination decreases to 56% in spring (Salvatierra-González and Jana-Ayala, 2016).

The study on plant characteristics related to sex needs carrying out, especially on foremost commodity crops, including dioecious and monoecious plants. Therefore, the pertinent research aimed to determine the pattern of plant segregation, pollen morphology, and germination based on the sex of *V. pubescens* in Indonesia. The knowledge of sexes in *V. pubescens* can be applicable in several places based on growing criteria to achieve high productivity.

MATERIALS AND METHODS

Plant segregation patterns based on sex

This study on *V. pubescens* trees transpired at the Cangar Agro Techno Park (ATP) in Batu, East Java, Indonesia. The ATP location is at 07 44‘23.37" S 112̊32‘02.93" E and has an altitude of 1,604 masl. When monitoring the soil, it had an average temperature of 23 °C and a pH of 6.5. The land covered an area of 374.76 m^2. Identifying all *V. pubescens* individual trees by sex based on the flower type's appearance, followed by grouping and mapping.

Pollen morphology based on sex

The harvest of *V. pubescens* flowers of different sexes that bloomed partially started around 8:00–09:00 AM, then put into a cool box. A one-day survey of dioecious and monoecious populations ensued to collect flower samples. In each harvest, marking and labeling female and male flowers from dioecious and monoecious plants followed. The anther removal used a razor blade, then placing the anthers on a slide gained cutting to obtain a pollen mass. Upon transfer into a tube, the pollen mass had a fixative solution (formaldehyde, glacial acetic acid, and ethyl alcohol) added, with the samples stored for 24 h. Subsequent anther crushing used a needle, followed by the trials centrifuged for 5 min at 2000 rpm. Removing the fixative solution had the glacial acetic acid for replacement, then poured with concentrated sulfuric acid in a ratio of 9:1, followed by immersion in a water bath at 45 °C for 10 min. The samples continued centrifuging for 10 min at 800 rpm, then rinsed twice with distilled water and dried for 30 min. Specimen staining used a 1% safranin solution for 3 min, followed by distilled water rinsing twice. The transferred specimens onto a glass slide had a drop of glycerin covering them. Applying cover glass to the samples consisted of clear nail polish applied around the edges of the cover glasses to secure the slide. Observations followed using an Olympus BX 51 microscope.

Analyzing fresh anther and pollen samples employed the SEM. The SEM analysis required dehydrating the material, with critical points dried and plated with gold. Observations and electron micrographs ran with an SEI SS40 SEM at 10 kV. The evaluation of the pollen structure consisted of the length (P), equatorial diameter (E), P/E index, unit, shape, size, and aperture (Gusmalawati *et al*., 2021). Powder preparation and the extract observed used a microscope-integrated camera for documentation.

Pollen germination based on sex

In the field, the collected *V. pubescens* flowers of different sexes received storage at ± -10 °C before use. A total of 27 pollen grains, observed under the germination media (Brewbaker's and Kwack's media), comprised dissolved 0.01 g H_3BO_3, 0.03 g $Ca(NO_3)_2\ 4H_2O$, 0.02 g $MgSO_4 \cdot 7H_2O$, 0.01 g KNO_3, and sucrose (10% concentration) in 100 mL of distilled water. Using the media immediately after manufacture or storing it at ± -10 °C before use retains its potency. The fresh pollen grains, placed on the object glass, had the germination media dripped on them. The pollen came from raw samples stored in a cool box. Evaluating the germination process in vitro using an Olympus BX 51 microscope every 15 min for 60 min, followed by documentation using a microscope integrated with a camera and the Olympus CellSens Standard software. The viable and germinated pollens were characteristic of a shiny appearance, a filled mass of cells, and a growth of pollen tubes. Non-viable pollen exhibited an opaque appearance, a lack of cell mass, a damaged structure, and no pollen tube growth. Pollen grains germinated were also illustrative of the presence and increase in length of their pollen tube.

Data analysis

Data on plant segregation, pollen morphology, and pollen germination based on various sexes are available in tables and graphs and analyzed descriptively to explain the plant characteristics. The unpaired t-test helped determine the average difference in the pollen tube growth and length in male and monoecious plant pollens through the t-statistics comparison. The quantitative data were statistically analyzed using Microsoft Excel®. Salvatierra-González and Jana-Ayala's research method measured pollen germination, while this study measured the germination of pollen and pollen length of the pollen tube.

RESULTS

Plant segregation patterns based on sex

Male and female flowers, observed in *V. pubescens* populations, had three types of sexes found in the plantations of Batu, East Java, including male dioecious, female dioecious, and monoecious (Figure 1). In *V. pubescens* populations, identification of three plant variants based on sex ensued. These variants appeared similar to each other in their overall appearance. The visible feature of a male plant (Figure 1a) was the presence of a male flower and the absence of fruits. The female plant (Figure 1b) was characteristic of the presence of female flowers and clusters of round and short-stemmed fruit arranged together. The monoecious plant (Figure 1c) exhibited the presence of male and female flowers in one tree and the long-stemmed oval fruit bunches.

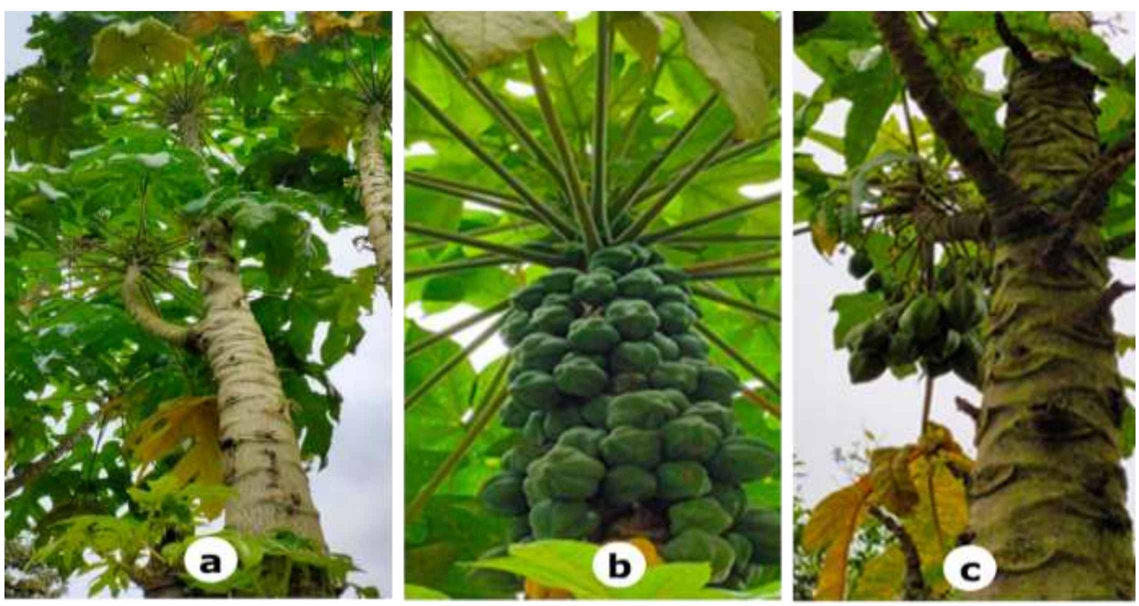

Figure 1. The habit of three sex types in *V. pubescens*, a) male plant, b) female plant, and c) monoecious plant.

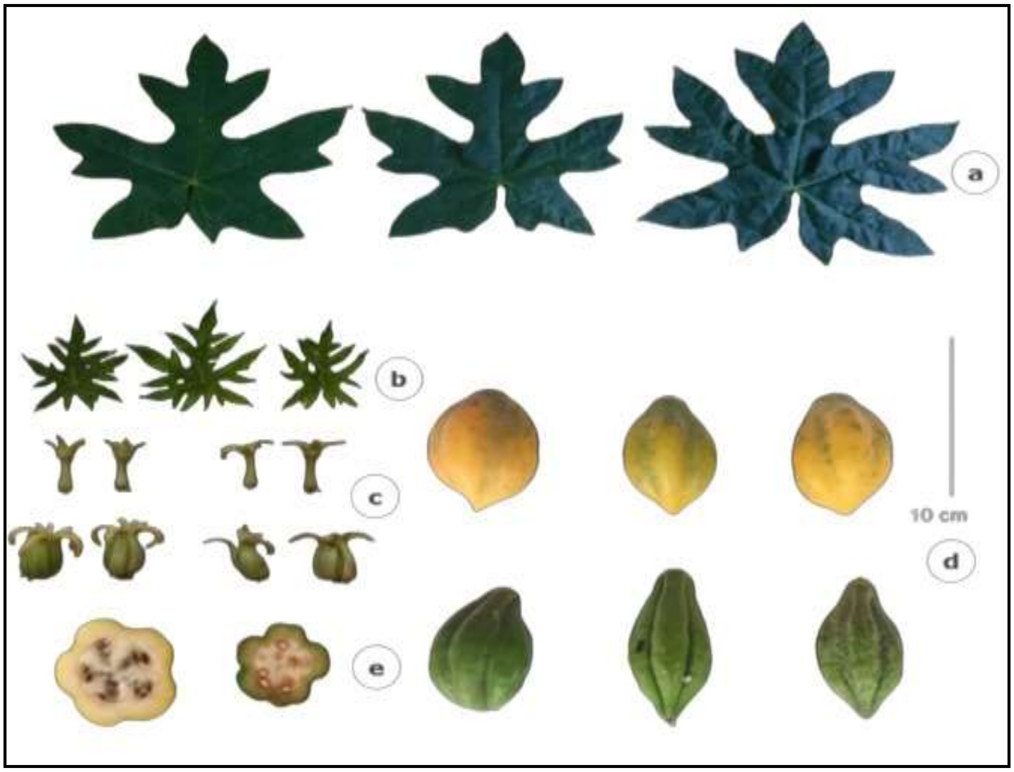

Figure 2. Organs of *V. pubescens* in several sex types: mature leaves (left to right: male, female, monoecious plant) (a); mature leaves (left to right: male, female, monoecious plant) (b); male flower (up) and female flower (bottom) (c); the fruit of female (up) and monoecious plant (bottom) (d); cross-section of fruit from female (up) and monoecious plant (bottom) (e).

In vegetative organs, such as, leaves, the differences between sexes were not apparent. However, a trend showed in young and mature leaves. Male dioecious plants had orbicular leaf shapes with an isosceles triangular inclination, convex in the mature leaf teeth, and slightly open petiole sinuses. The female dioecious had oblong leaf shapes with an equilateral triangular tendency, concavity in the adult leaf teeth, and moderately open petiole sinuses. Monoecious plants occurred to have an oval leaf shape with a mixture of equilateral and isosceles triangles, straight on the teeth of mature leaves, and slightly closed petiole sinuses (Figure 2a and Figure 2b).

In male dioecious, the generative organs were distinctive of the presence of slender male flowers with a crown forming a small tube that ends with small five-sectioned strands that are usually short-stemmed. The female dioecious displayed the presence of slightly elongated, round, bell-shaped female flowers, whose crown ended with large strands with five parts, which are usually short-stemmed. These flowers resulted in various individuals, thus, labeling *V. pubescens* as a dioecious plant.

Male and female flowers also appeared in the same individual but usually had long-stemmed, indicating that *V. pubescens* is a monoecious plant. However, remarkable differences in both sexes emerged, with the stamens in male flowers more deeply submerged in dioecious plants. The petals of male and female flowers are more curvature in dioecious plants (Figure 2c). The emergence of flowers with clear morphological differences between male, female, and monoecious plants of *V. pubescens* occurred about two years after planting. Pistils on female dioecious and monoecious plants had pollen fertilization from the male parts of both plants.

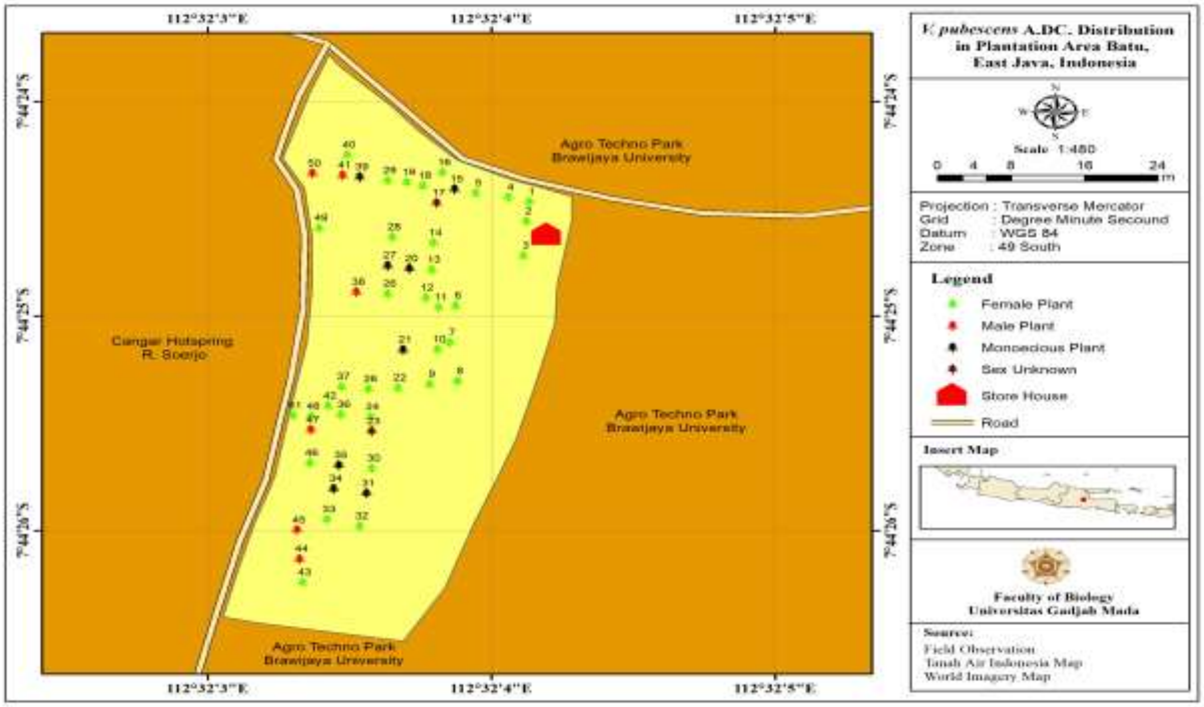

Figure 3. *V. pubescens* distribution in Batu, East Java, Indonesia, based on sex types.

Both female dioecious and monoecious plants produced the *V. pubescens* fruit. However, the significant differences between them were the presence of a long stalk and a slimmer fruit in monoecious plants. The fruits on female plants appeared to be attached to the stem, with the trunk barely visible, rounder, and tighter (Figures 2d, 2e).

Based on the observation area in Batu, Indonesia, most of the *V. pubescens* plants grew by seed, with the population dominated by female dioecious plants. Consumers expected the *V. pubescens* fruit to derive from a female fruit, mostly easier to locate and with a slightly larger volume. Based on the distribution of plants with three sex variations, the study discovered that monoecious plants produced male and female flowers on the same inflorescence (Figure 3). In mature *V. pubescens*, the fruit presence is one of the markers that made it easier to identify the sex of the plant apart from the existence of flowers. The percentage presence of male dioecious and monoecious plants was very similar, with an average of 27.44% of the total number of individuals in the planted population (Table 1). However, in *V. pubescens*, the female plants accounted for 68% of the total population. The pattern of plant segregation based on sex in males (dioecious), females (dioecious), and monoecious was 1:5:1.

Table 1. Sex types in *V. pubescens* population in Batu, East Java, Indonesia.

Sex types	Number of plants	Percentage of plants (%)
Female dioecious	35	68.63
Male dioecious	6	11.76
Monoecious	8	15.68
Sex unknown (immature)	2	3.92
Total Plants	51	100

Pollen morphology based on sex

The *V. pubescens* had a single pollen unit (monad), isopolar polarity, and radial symmetry. The varied measurement results showed round pollen, with the exine ornament and pollen surface reticulate with a net-like pattern. In *V. pubescens* populations, all the observed pollen grains had three openings, with the stephanocolporate type/position and the aperture at the equator. The P/E value (0.70) was higher for males dioecious than monoecious (0.52). The results of the pollen morphology analysis are in Table 2. Images of the pollen morphology analysis using a light microscope and scanning electron microscopy (SEM) appear in Figure 4. Salvatierra-González and Jana-Ayala's research measured the

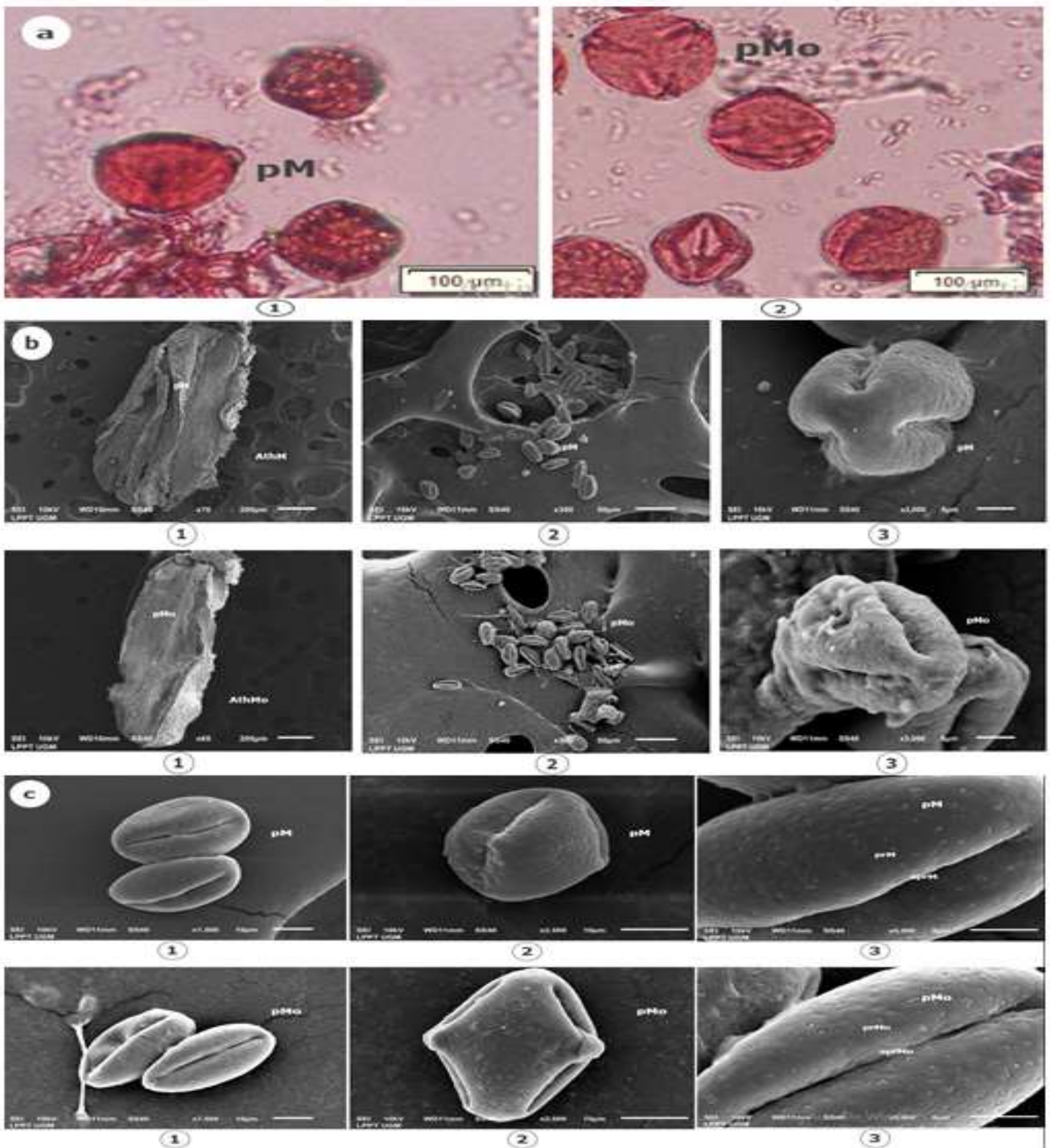

Figure 4. Pollen morphology by Olympus Microscope CX 51 (a): male plant (1), monoecious plant (2); Pollen morphology by SEM (b): anther morphology showing the release of pollen grain (1), pollen grain (2), pollen grain tip (above: male plant, below: monoecious plant) (3); Pollen morphology by SEM (c): pollen with a horizontal view (1), pollen with a vertical view (2), pollen surface showing pore (above: male plant, below: monoecious plant) (c). pM = male pollen, pMo = monoecious pollen, Ath = anther, pr = pore, apr = aperture.

Table 2. Pollen morphological characteristics based on sex types in *V. pubescens.*

Sex type	Unit	Polar (P) (μm)	Equatorial (E) (μm)	Index (P/E)	Shape	Symmetry	Aperture	Polarity	Ornamentation
Male Dioecious	Monad	24.76	35.13	0.70	Prolate spheroidal	Radial	Tricolporate	Isopolar	Reticulate
Male Monoecious	Monad	19.64	37.418	0.52	Prolate spheroidal	Radial	Tricolporate	Isopolar	Reticulate

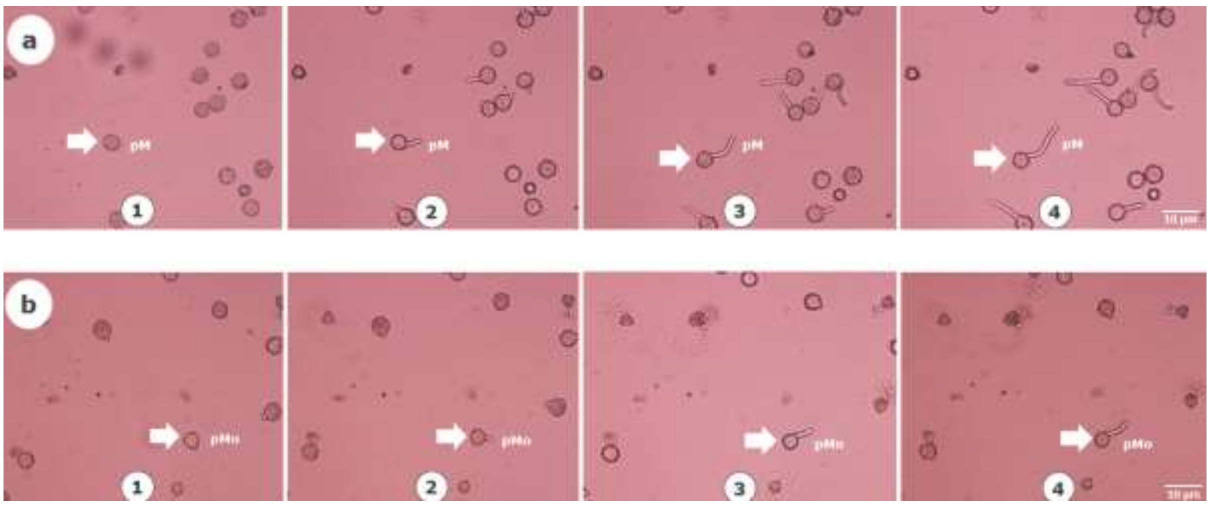

Figure 5. Pollen germination in male plant (a), monoecious plant (b): 15 minutes (1), 30 minutes (2), 45 minutes (3), 60 minutes (4), (pollen marked with white arrows is an example of the development of a pollen tube over time). pM = male pollen, pMo = monoecious pollen.

germination of pollen, while this study measured the pollen germination and the length of the pollen tube. The results indicated no significant differences in the pollens of male dioecious and monoecious plants based on optical microscopy observations, and both had three apertures. The SEM observations showed that the anthers and pollen grains of male flowers on male plants appeared more rounded, while those on monoecious plants were more elongated. Furthermore, no difference occurred in the appearance of pores and openings in the two plant sexes.

Pollen germination based on sex

The observations showed variations in the germination response of the different sexes of *V. pubescens*. The pollen tube served as the vehicle that delivered the male gametes (sperm cells) to the female gametophyte during double fertilization, eventually leading to seed formation, and was one of the fastest-elongating structures in plants (Adhikari *et al*., 2020). The results further revealed that the available time affected the germination development of *V. pubescens* (Figure 5). The pollen germination rate was better for pollen from male dioecious flowers, with a mean of 7.37 μm than for male monoecious, with a mean of 4.93 μm based on the t-test (Table 3). These observations also recognized that the pollen tube in the pollen grains of male dioecious flowers reached the ovary quickly to fertilize it.

Germination of dioecious male pollen was significantly higher than germination of monoecious male pollen in germination media for one hour. The germination percentage of dioecious male pollen was 57.57%, while for the monoecious male pollen was 28.12%. The growth rate of the pollen tube in male and

Table 3. T-test of male (dioecious) and monoecious pollen tube length in *V. pubescens.*

Observations	Male	Monoecious
Mean	7.375	4.93
Variance	4.168472	4.87788889
Observations	10	10
Hypothesized Mean Difference	0	
d.f.	18	
t Stat	2.570644	
P(T<=t) one-tail	0.009625	
t Critical one-tail	1.734064	
P(T<=t) two-tail	0.01925	
t Critical two-tail	2.100922	

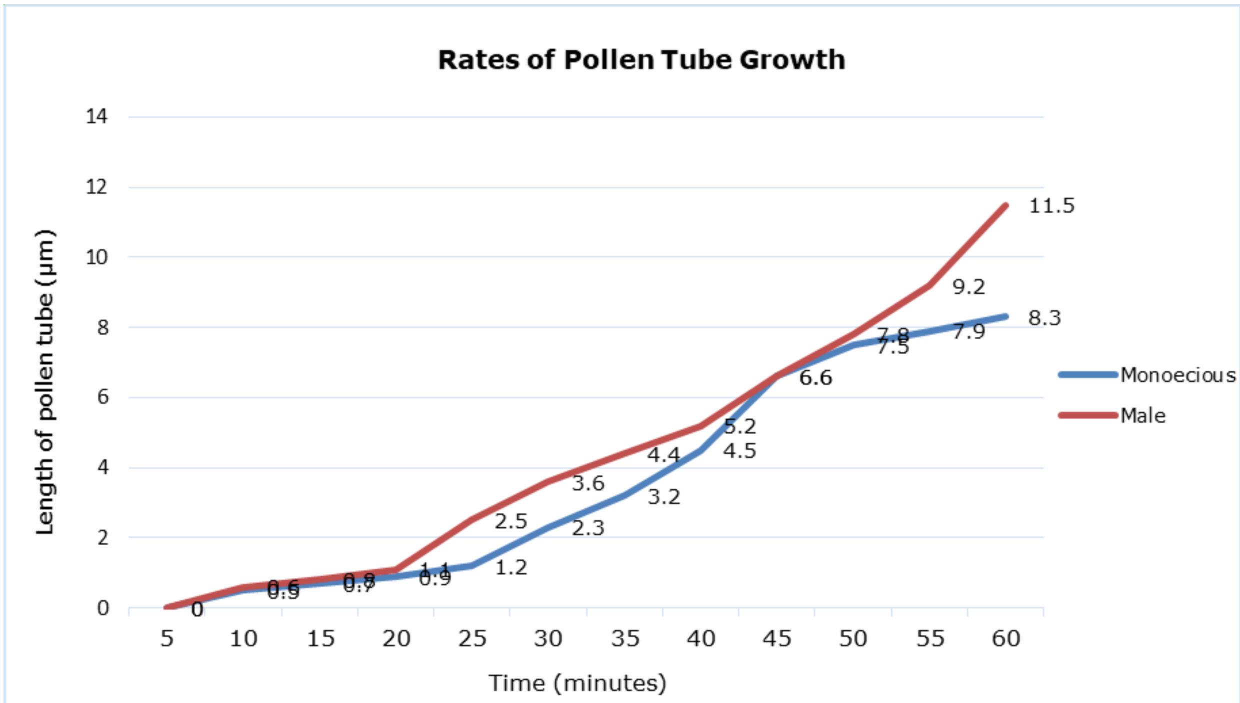

Figure 6. Comparison between rates of pollen tube growth of *V. pubescens* in male and monoecious plants.

monoecious plants shows in Figure 6. Based on the time needed for pollen tube formation, when the female pilot flower had a length of 18 mm, the ratio of the speed at which plant sperm cells reached the ovule was 25.14 days faster in pollen originating from male dioecious plants. Results further revealed that in *V. pubescens,* pollens of male dioecious flowers require 65.23 days for pollen tube formation, while in male monoecious, the said time was 90.36 days (Figure 7).

DISCUSSION

Previous studies on varying sexes of *V. pubescens* described trioecious in its central origin in Ecuador, Colombia, and Peru. However, under cultivation conditions of La Serena, Chile (30° S, 70° W), plants exhibited both dioecious and monoecious (Salvatierra-González and Jana-Ayala, 2016). *V. pubescens* was a model for determining sex evolution, as it possessed a primitive sex chromosome (Na

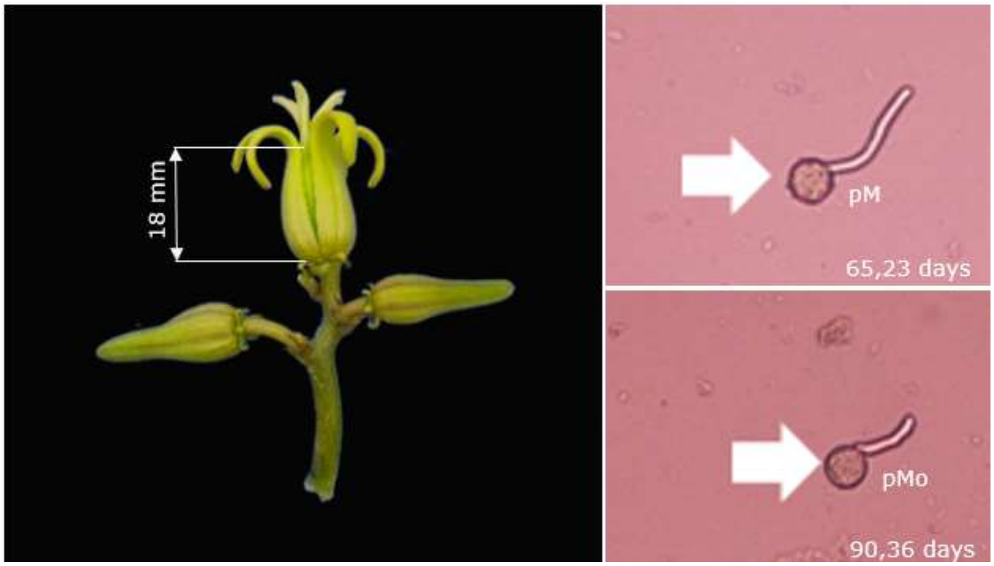

Figure 7. The length of the stylus reed through which the pollen tube of male plants and monoecious plants may pass to reach the ovule in *V. pubescens* flower.
pM = male pollen, pMo = monoecious pollen

et al., 2014). According to Laily *et al*. (2021), *V. pubescens* had three variations, i.e., male and female two-house plants and monoecious plants. The study found a wide variation in sex types in *Carica papaya*, with some plants being dioecious (having separate male and female flowers) and others being hermaphroditic (having both male and female flowers) (Suresh and Kumar, 2014). Similar findings occurred in *Jatropha curcas*, which exhibited three sexes: monoecious with male and female flowers, andromonoecious with male and monoecious flowers, and trimonoecious with male, female, and monoecious flowers (Dasumiati *et al*., 2017).

In this study of *V. pubescens* populations, the pattern of segregation of male dioecious, female dioecious, and monoecious through seeds was 1:5:1., It is crucial to select the appropriate seed and determine their sex to ensure the development of productive plants. Therefore, various techniques for identifying the sex of plants at the seed stage and germinated seeds need careful research. According to local knowledge, Wahyuni and Bermawie (2015) reported that in nutmeg plants, the pits with smooth characteristics on both sides and no protruding parts germinated into female nutmeg. It highlights the importance of investigating the potential for sex change during the growth of *V. pubescens.* Salvatierra-González and Jana-Ayala's research measured the pollen germination, but this study measured the pollen germination and the germination of pollen length of the pollen tube. Similarly, Salvatierra-González and Jana-Ayala (2016) also stated that the existence of flowers of different sexes in *V. pubescens* appeared to depend on the seasons.

Some factors cause plant segregation based on sex in males (dioecious), females (dioecious), and monoecious. Genes determine the sex of a plant, with the environment playing a role in some species (Guttman and Charlesworth, 2017). The Temperature-sensitive Sex Determination (TSD) system in cucumbers is a genetic mechanism that determines the sex of flowers based on temperature, with the FT gene playing a key role by activating the AGL29 transcription factor (Wang *et al.*, 2015). In some plants, light exposure controls sex, with longer days promoting male development and shorter days promoting female development (Bhatt and Song, 2014). Hormones can also play a role in regulating the sex of a plant. Gibberellin treatment promoted the development of male flowers in cucumber plants, increasing the likelihood of male flower production (Guo *et al*. 2019). Plant segregation based on sex has evolution also shaped this. Plants can benefit from having separate male and female individuals or both male and female flowers on the same plant, depending on the environment and the plant's reproductive strategy (VanderWeele and Charlesworth, 2018).

Based on observations using light microscopy and SEM, *V. pubescens* had one unit of pollen scatter monad, with the mature pollen grains distributed singly as a monad. Zubkova *et al*. (2022) also investigated the morphological characteristics of the spring rapeseed (*Brassica napus* L.) pollen grains in detail using SEM, i.e., polar axis, equatorial diameter, shape index, the pattern of perforation of exines, and perimeter of perforation zones. The *V. pubescens* was isopolar, with the same polar polarity in both distal and proximal parts. Radial symmetry can also result when dividing an object vertically in any area to produce two symmetrical parts.

In the relevant study, *V. pubescens* pollens resulted in only one form, prolate spheroidal. The observations further revealed that the exine ornament of *V. pubescens* was forming a net-like reticulated. Phuangrat *et al*. (2013) also detected exine reticulate ornamentation at 2500× in *C. papaya,* indicating that an accurate description of the exine pollen ornament depended on magnification (Halbritter *et al*., 2018). After observing the openings of *V. pubescens*, it showed three colporates in the Stephano position. These findings followed previous studies on *V. pubescens* pollen, providing a similar statement (Ferreira and Oliveira, 2018). Meanwhile, no significant differences surfaced in the pollen morphology of the two pollen-producing sexes examined in this study. Furthermore, the morphology of the two sexes only differed in size, which was rounded or elongated shape.

Pollen morphology can also have effects from various factors, including genetics, environment, and pollination. The genes that control pollen morphology are often the same in dioecious and monoecious plants because these genes are often on sex chromosomes, which are the same in both male and female plants. The environment can also be crucial in pollen morphology, such as, temperature and humidity affecting pollen grains' size, shape, and structure. Likewise, pollination can affect pollen morphology; for example, if pollen transfer is from a male flower to a female flower of the same species, the pollen grains will be more likely to germinate and produce seeds (Zhu *et al*., 2019).

Pollen germination indicated pollen viability, while pollen tube length showed vigor (Sulusoglu and Cavusoglu, 2014). Pollen germination is one of the methods used to assess pollen fertility, previously carried out on 142 accessions of *Solanum tuberosum* L. (Gins *et al*., 2022). For male dioecious flowers of *V. pubescens,* the pollen germination was faster than in monoecious plants, indicating the need for male plants in the cultivation. According to Salvatierra-González and Jana-Ayala (2016), pollen germination of *V. pubescens* in Chile depends upon the season and not on the sex of the plants and the location. The results of this study cannot compare fully with the results of Salvatierra-González and Jana-Ayala's (2016) research because they measured different things. Salvatierra-González and Jana-Ayala's research measured the germination of pollen, while this study measured the germination of pollen and the length of the pollen tube. Furthermore, the study environments were different. In Chile, the monoecious plant has male flowers in early spring and female flowers in early summer. In Indonesia, there are only two seasons: dry and rainy. The pollen tube length of *V. pubescens* in Indonesia differed and was longer in male dioecious plants than in monoecious plants.

Some factors that cause differences in germination rates in dioecious pollen compared with monoecious pollen are pollen quality, quantity, dispersal, and stigma receptivity. Dioecious plants typically produce higher-quality pollen than monoecious plants. It is because dioecious plants can focus all their resources on producing pollen, while monoecious plants must also create flowers and fruits (Akagi *et al*., 2014). Dioecious plants typically produce more pollen than monoecious plants. It explains dioecious plants need to make enough pollen to ensure that some of it reaches a female flower (Zhu *et al*., 2019). Dioecious plants typically have better pollen dispersal mechanisms than monoecious plants. It is due to dioecious plants relying on wind or animals to disperse their pollen, while monoecious plants must depend on self-pollination (Lamont and Harder, 2018). The stigma of a female flower is more receptive to pollen from a different plant than it is from the same plant. It refers to self-pollination leading to genetic defects in the offspring (Pavlova and Ennos, 2017). As a result of these factors, dioecious pollen typically has a higher germination rate than monoecious pollen. Thus, dioecious plants are often more successful at reproducing than monoecious plants.

Therefore, the significant study revealed that pollen produced by male *V. pubescens* has better quality than monoecious plants. It was because monoecious plants allocated most of their energy to form simultaneously pollen grains and ovules. However, male and female dioecious plants only used their strength to produce pollens and ovules, respectively. The results indicated vitally maintaining male plants in a *V. pubescens* plantation, considering that some places often cut the male dioecious plants down due to their failure to produce fruits.

The study of plant segregation and pollen characteristics of *V. pubescens* based on sex types has several contributions to science and technology. It provides a better understanding of the genetic basis of plant sex determination. This knowledge could help develop new breeding strategies for improving crop yields and quality. The study identified several factors that affect pollen germination, such as, temperature and plant sex. This information could be useful to improve the pollination efficiency of *V. pubescens* orchards. The study also found a significant difference in the pollen characteristics of male and female flowers. This information could further aid in enhancing breeding strategies for advancing the fruit set of *V. pubescens* trees.

Some specific examples of how the study could benefit breeders, geneticists, and other scientists are using the information on plant sex determination to develop new varieties of *V. pubescens* that are more productive and with better fruit quality. Geneticists could use the information on pollen germination to acquire new methods of pollinating *V. pubescens* trees, which could improve fruit set and yield. Similarly, other scientists, such as, plant pathologists and entomologists, could use the information on pollen characteristics to develop new strategies for controlling pests and diseases affecting *V. pubescens* trees. Overall, studying plant segregation and pollen characteristics of *V. pubescens* is a valuable contribution to science and technology. The information generated by this study could better improve the breeding, cultivation, and management of this essential crop.

CONCLUSIONS

Research of the *V. pubescens* population revealed that the highest ratio of plants is dioecious females, followed by dioecious males and monoecious males. Male dioecious pollen exhibited characteristics, i.e., monads, prolate spheroidal shape, radial symmetry, tricolporate structure, isopolar distribution, and reticulate surface. In contrast, monoecious pollen showed monads. The anthers and pollen of male plants exhibited a more rounded shape than the elongated shape observed in monoecious plants. Furthermore, no significant variations resulted in pollen grains among the different sexes. The pollen from male flowers on male dioecious plants exhibited faster germination versus monoecious plants. The study is useful for breeders, geneticists, and other scientists. First, it provides a valuable resource for understanding the genetic basis of plant sex determination. Second, it identifies several factors that affect pollen germination, which could help improve pollination efficiency. Third, it finds a significant difference in the pollen characteristics of male and female flowers, which could aid in developing new breeding strategies for improving fruit sets.

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