

Comparison of the Morphological Structure of Pollen in Five Provenances of *Gyrinops versteegii* (Thymelaeaceae) in Lombok

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ABSTRACT

Gyrinops versteegii (Thymelaeaceae) is one of the agarwood-producing trees originating from Lombok, there are five provenances, namely Pantai, Madu, Buaya, Soyun, and Beringin. This study aims to compare the morphological structure of pollen in five provenances of *G. versteegii* in Lombok. The method of sampling and data analyzes were used random sampling and descriptive. Samples were taken from West Lombok: Sidemen Village (Pantai), North Lombok: Menggala Village (Buaya), Gangga Village (Beringin), Central Lombok: Taman Baru Village (Madu), and Soyun from Karang Pule Village, Mataram. The study parameters were pollen diameter and pollen morphology: shape, aperture, and sculpture. The results showed that the size of pollen from Buaya was wider than that from Madu, Beringin and Pantai. Based on the length of the polar axis/equatorial diameter, Buaya Provenance, Beringin and Pantai pollen are round in shape, and Madu Provenance is subprolate in shape. The Buaya, Madu and Beringin provenances have a monocolpate opening type, and the Periporate type for the Pantai provenance.

Keywords: Morphology, pollen, gaharu, *Gyrinops versteegii*, Lombok

INTRODUCTION

The Thymelaeaceae family or better known as agarwood-producing trees are forestry commodities that important functions as herbs, export commodities, and others. Indonesia is the largest producer of agarwood in the world (Abrori, 2018). The family Thymelaeaceae has been identified in 8 genera, *Aquilaria*, *Wikstroemia*, *Gonyistylus*, *Gyrinops*, *Dalbergia*, *Enkleia*, *Excoccaria*, and *Aetoxylon* (Hou, 1960; Triadiati et al., 2016). *Aquilaria* and *Gyrinops* are two genera of agarwood trees known as producers of high-quality agarwood commodities (Lopez-Sampson & Page, 2018).

Gyrinops versteegii is a species of agarwood plant endemic from Lombok Island (Wang et al., 2018). *G. versteegii* has a shrub-tree appearance, the fruit is yellow-orange. The shape of the fruit is elliptical, rectangular, or rounded,

depending on the provenance of the agarwood tree (Iswantari et al., 2017; Mulyaningsih, et al, 2017). Delta seeds, round-flat, and elliptical-elliptic (lanceolate) leaves (Hou, 1960). *G. versteegii* agarwood trees on Lombok Island, there are 5 provenances/groups, namely Madu, Pantai, Buaya, Beringin, and Soyun which are differentiated based on phenotype (morphology, anatomy, and phytochemistry) as well as the geographical location between each provenance (Mulyaningsih et al., 2014). The natural distribution of this agarwood tree is in the Muteran mountain, Tanjung to Gangga forest area, at the foot of Mount Pelola, West Lombok, behind the mountains along the coast of Krandangan, Senggigi, and Pandanan which is part of Batulayar District, West Lombok; Around Mount Krujuk,

Pemenag District; and in the Pringgarata area of Central Lombok.

Palynology is the science that studies pollen and spores which are used in taxonomy and morphology (Kumaladita, 2014). Pollen is a male genital organ that is produced in the anthers. The anthers will release pollen when the fruit ripens, and can be carried by the wind up to hundreds of miles from their place of origin. The spread of pollen is influenced by several factors such as air turbulence, wind direction and speed, altitude, strength, weight and shape of the pollen. The type, size, shape and ornamentation of the outer surface of the pollen wall can be used to identify the source plant (Fakhrizal, 2015). Pollen morphology can be used to identify taxa at the tribe, genus, type and below type level, placement of doubtful taxa, rearrangements, combinations and separations, as well as being used to strengthen evidence for plant taxonomy. (Radford et al., 1974). The variations shown by pollen include the number, location of grooves and holes (apertures) on its surface, as well as the shape and size of its exine which can be an important source of taxonomic evidence (Des et al., 2013; Pudjoarinto & Hasanudin, 1996.). Research on the anatomical characteristics of the leaves and chromosomal characteristics of the gaharu-producing tree *G. versteegii* has been carried out, however information about pollen is still very limited because no one has carried out research. Therefore, this research is very important to find out the morphological characteristics of pollen that can be used for infraspecific identification of *G. versteegii*.

MATERIAL AND METHOD

Time and place

This research was carried out from April to June, at the Advanced

Biology Laboratory. Flower samples for Buaya provenance were obtained from Sidemen Village, West Lombok Regency with latitude 8030'34" S and longitude 116005'52" E, Soyun provenance from Pusuk Lestari Village, West Lombok Regency with latitude 8027'59" S and longitude 116005'15" E, Buaya provenance from Menggala Village, North Lombok Regency with latitude 8026'04" S and longitude 116005'17" E, Beringin provenance from Gangga Village, North Lombok Regency with latitude 8021'54" S and longitude 116014'09" E, and provenance of Madu from Taman Sari Village, Central Lombok Regency with latitude 8033'59" S and longitude 116017'06" E.

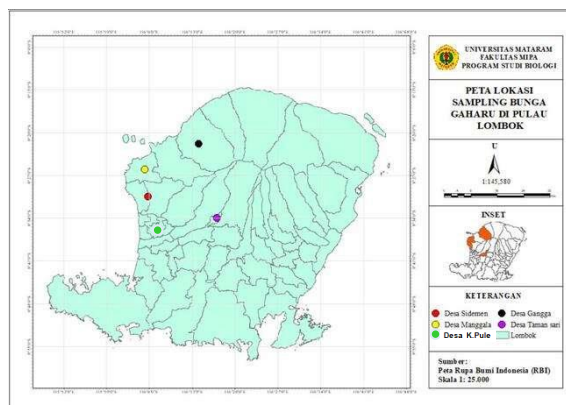


Figure 1. Map of sampling locations flowers of *G. versteegii* five Provenans

The tools used in the research were specimen bottles, object glasses, stirrers, cover glasses, Zeis Primo Star microscope, tweezers, dropper pipettes, centrifuges, vial tubes. The ingredients used in the research were glacial acetic acid (GAA), 50% alcohol, distilled water, entelan, FAA (10 mL of 37% formaldehyde, 10 mL of acetic acid, 100 mL of 95% alcohol, 80 mL of distilled water), glycerin jelly (5 gr gelatin, 30 mL distilled water, 35 mL glycerin, 5 gr phenol crystals), label paper, tissue paper,

5 *G. versteegii* infraspecific pollen, safranin 0.1%.

Results were presented in the form of photographs, dendrograms, and identification keys of *G. versteegii* provenances. Observations of pollen diameter and pollen morphology (pollen shape, aperture, and sculpura or exine ornamentation), descriptively.

RESULT AND DISCUSSION

Results

Results of the infraspecific equation of *G. versteegii* based on pollen characters

Table 1. Pollen characteristics of five provenances of *Gyrinops versteegii* in Lombok.

Prove-nance	Polar axis length (P) and equatorial diameter (E) (µm)		Index P/E x 100 (µm)	Form	Apertu-ra	Sculpura
	P	E				
Buaya	31,2±5,62	33,8±5,95	0,93±0,18	Oblate spheroidal	Monocolpate	Echinate
Madu	34±3,00	29±2,68	1,18±0,15	Subprolate	Monocolpate	Baculate
Beringin	28,2±3,01	29,2±2,55	0,96±0,14	Oblate spheroidal	Monocolpate	Echinate
Pantai	21,4±0,74	22,9±0,90	0,93±0,03	Oblate spheroidal	Periporate	Clavate
Soyun	26,3±1,92	28,7±3,04	0,91±0,09	Oblate spheroidal	Monocolpate	Baculate

Based on the observations, each provenance was similar in shape, namely for the Buaya, Banyan and Pantai provenances they had an oblate spheroidal shape which was based on the P/E index of each provenance, while for the Honey provenance they had a subprolate shape. Another similarity is found in the aperture, namely the monocolpate type is found in the Buaya, Madu and Beringin provenances, while the periporate type is found in the Pantai provenance. The exine or skulpur ornamentation of Buaya and banyans is echinate, while Madu provenance is baculate and the Pantai provenance is clavate (see table 1 and figure 2).

Description five provenances of *G. versteegii* pollen

1. Provenant Buaya

Provenant Buaya have a polar axis length (P): 31.2 ± 5.62 and an equatorial diameter (E) 33.8 ± 5.95 so the P/E index is 0.93 ± 0.18 and the shape is oblate spheroidal. The aperture monocolpate and echinate exine or sculpura ornamentation. The exine line of the Buaya provenance is thicker compared to the Madu provenance, the exine line curves downward.

2. Provenant Madu

Madu Provenance has a polar axis length (P) which has been averaged over three repetitions, namely 34 ± 3.00 and an equatorial diameter (E) of 29 ± 2.68 so that the P/E index is 1.18 ± 0.15 and the shape Subprolate, the aperture is monocolpate and the exine ornamentation or sculpura is baculate.

3. Provenant Beringin

Beringin provenance has a polar axis length (P) which has been averaged over three repetitions, namely 28.2 ± 3.01 and an equatorial diameter (E) of 29.2 ± 2.55 so that the P/E index is $0.96 \pm 0,14$ and includes an oblate spheroidal shape, the aperture is monocolpate and the ornamentation of the exine or sculpura is echinate.

4. Provenant Pantai

Pantai provenance has a polar axis length (P) which has been averaged over three repetitions, namely 21.4 ± 0.74 and an equator diameter (E) of 22.9 ± 0.90 so that the P/E index 0.93 ± 0.03 and the shape is oblate spheroidal, the aperture is periporate and the exine or sculpura ornamentation is baculate.

5. Provenant Soyun

Provenant Soyun has a polar axis length (P) which has been averaged over three repetitions, namely $26.3 \pm 1.92 \mu\text{m}$ and an equatorial diameter (E) of $28.7 \pm 3.04 \mu\text{m}$ so that the P/E index is $0.91 \pm 0.09 \mu\text{m}$ and the shape is oblate spheroidal, the aperture is monocolpate and the sculpura is baculate.

Table 2. Pollen character scoring instructions for four provenances of *Gyrinops versteegii* in Lombok.

Character	Scoring		
	1	2	3
Polar axis length (μm)	21-26	>26-31	>31
Equatorial diameter (μm)			
Polar axis length (μm)	22-26	>26-31	>31
Equatorial diameter (μm)			
Index P/E	<1	≥1	
Form	Oblate spheroidal	subprolate	
Apertura	monocolpate	periporate	
Sculpura	clavate	baculatee	echinate

Key to Identification of *G. versteegii* provenance based on pollen characters

- 1a. Length of polar axis 21-26 μm, equatorial diameter 21-26 μm, aperture periporate, exine ornamentation or clavate sculpura.....provenant Pantai
- b. Polar axis length >26 – 31 μm, equatorial diameter >26 – 31 μm, aperture monocolpate, exine ornamentation or baculate-echinate sculpura2
- 2a. P/E index ≥1, subprolate form3
- b. P/E index <1, oblate spheroidal shape.....4
- 3a. Polar axis length >31 μm, equatorial diameter >31 μmprovenant Beringin
- b. Polar axis length >26-31 μm, equatorial diameter >26- 31μmprovenant Soyun
- 4a. Sculpura echinate.....provenant Buaya
- b. Sculpura baculate.....provenant Madu

Discussion

Pollen is generally classified based on physical appearance (morphology). Pollen has main characteristics that can be used for determination and identification, namely unit, aperture, size, shape and ornamentation of the exine, so that the taxon level of the plant producing it can be known (Nugroho, 2014). *G. versteegii* is included in the pollen group

of the eurypalynous tribe, namely a group of plants whose pollen varies greatly, for example in exine ornamentation (sculpura) (Kapp, 1969). Most plants have a monad pollen unit form (Faegri & Iversen, 1989).

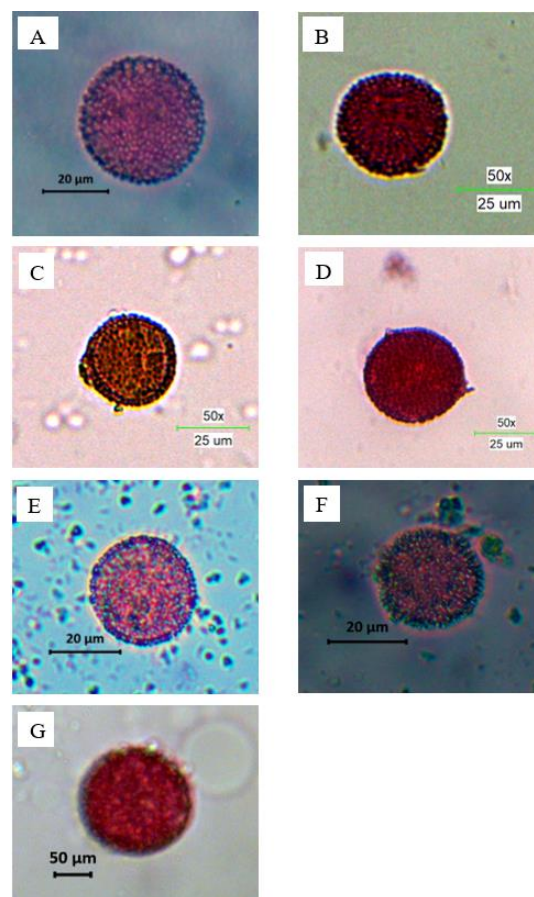


Figure 2. Pollen of five provenances of *Gyrinops versteegii*. Notes: A. Madu Provenance, B. Buaya Provenance, C. Beringin Provenance, D. Pantai Provenance, E. Soyun Provenance.

The observations of pollen units from five provenances of *G. versteegii* show that there are similar types, namely that they are all of the monad (single) type. The pollen grains are separate from each other and independent of each other. According to Hesse et al., (2009) monads are pollen grains that are scattered singly or one by one from their tetrad. The shape, size and type of pollen can vary according to the stage of maturity. In this

research, all pollen was taken from flowers that had already bloomed so it was hoped that the pollen would be in the optimum stage, namely the final stage and ready to fertilize (Aprianty and Kriswyanti, 2008).

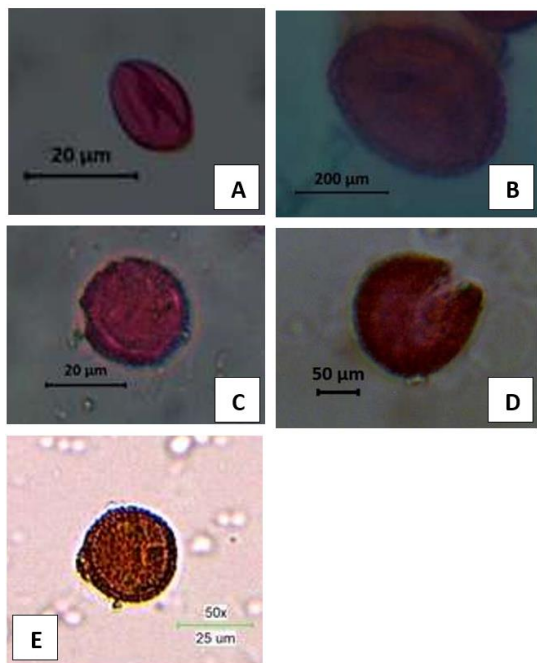


Figure 3. Pollen from five provenances of *Gyrinops versteegii* is monocolpate. Notes: A. Provenant Beringin and, B. Provenant Buaya seen from the front; C. Provenant Madu, and D. Provenant Soyun seen from the side.

Pollen shape is determined based on the P/E index which is a comparison of the length of the polar axis (P) and the equatorial diameter (E) (Erdtman, 1943; Zahrina et al., 2017). The shape of the pollen in the four provenances of *G. versteegii* is oblate spheroidal and subprolate. Oblate spheroidal is a form of pollen with P/E index criteria between 0.88 - 1.00 µm and subprolate is a pollen form with P/E index criteria of 1.14 - 1.33 µm. The Buaya provenance has a P/E index of 0.93 ± 0.18 µm so it is included in the oblate spheroidal shape. Provenant Madu has a P/E index of 1.18 ± 0.15 µm so it is included in the subprolate form. The Beringin

provenance has a P/E index of 0.96 ± 0.14 µm so it is included in the oblate spheroidal form, the Soyun provenance has a P/E index of 0.91 ± 0.09 µm so it is included in the oblate spheroidal form and the Pantai provenance has a P/E 0.93 ± 0.03 µm so it is included in the oblate spheroidal shape.

Pollen size varies greatly, ranging from $<10.0 - >100$ µm. Determination of pollen size is based on the length of the longest axis (Zahrina, et al., 2017). The pollen sizes of the four provenances of *G. versteegii* were medium and minuta +. Media is a measure of pollen with the criteria for a polar axis length of 25.1 - <50 µm. The medium size was found in the Buaya, Madu, Beringin and Soyun provenances. Minuta + is a pollen size with the criteria for a polar axis length of 17.6 - <25.0 µm and the minuta + size is found in the Pantai provenance. Variations in pollen size are influenced by external and internal factors. Internal factors include the number of chromosomes, flower characteristics and water conditions. Chemical treatment and covering media also affect the size. External factors include temperature, mineral elements, and water supply in the soil where the plant is located (Fakhrizal, 2015).

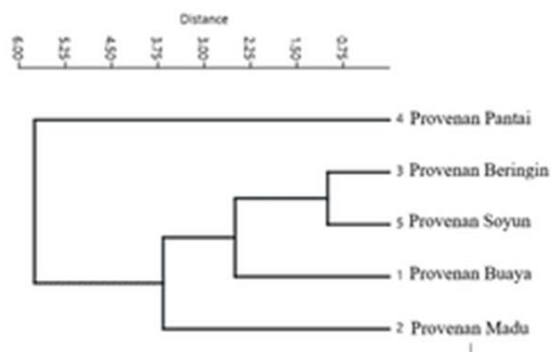


Figure 4. Relationship between five provenances of *Gyrinops versteegii* based on pollen characters.

The aperture is the opening or thin part of the exine and the intine layer is

usually thick. The aperture functions as protection, changing ions, and as a place for pollen sprouts to emerge. Five provenances of *G. versteegii* have monocolpate and periporate apertures. Monocolpate is a type of aperture that has one colpus. Monocolpate aperture is found in the Buaya, Madu, Beringin and Soyun provenances. The periporate aperture type has more than three pores that are spread apart. The periporate aperture is found in the coastal provenance. Exine ornamentation or pollen sculpura are carvings found on the outer walls of pollen and are characteristic of each type of plant. Sculpura can be determined by observing the outer surface of the exine (Erdtman, 1943). The sculpura in the five provenances of *G. versteegii* are echinate, baculate, and clavate. Sculpura echinate is a pollen ornamentation that is shaped like a thorn, found in Buaya and Beringin provenance. Sculpura baculate pollen ornamentation in the shape of a tall and slender cylinder, found in the Madu and Soyun provenances. Sculpura clavate pollen ornamentation which is like a stalk with narrow elements and the height is greater than the width, is found in Pantai provenance. The kinship between the *G. versteegii* provenance is depicted in the dendrogram (Figure 4), illustrating that the Pantai provenance is the ancestor of the other four provenances with a distance of (5.75) due to the presence of four characters. Provenan Beringin is closely related (with a distance of 100), only differing by one character. The smaller the index score, the closer the kinship relationship and conversely the larger the index score, the further away the kinship relationship (Quicke, 1993).

CONCLUSION

Based on the research that has been carried out, it can be concluded that: the longest polar axis length is found in the

Madu provenance and the shortest in the Pantai provenance. The widest equatorial diameter is found in the Buaya provenance and the narrowest is found in the Pantai provenance. The aperture of the Buaya, Madu, Beringin and Soyun provenances is of the monocolpate type and the Pantai provenance is periporate. The Sculpura provenances of Buaya and Beringin are echinate, while the provenances of Madu and Soyun are baculate, and Pantai is clavate. The pollen size of the Buaya, Madu, Beringin and Soyun provenances is in the medium category, while the Pantai provenance is in the minuta (+) category. The differences in pollen form in the Buaya, Beringin, Soyun and Pantai provenances are oblate spheroidal and Madu subprolate. Pollen characters such as polar axis length, equatorial diameter, P/E index, shape, aperture and sculpura are distinguishing markers that can be used for infraspecific identification of *G. versteegii*.

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