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COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

Diversity of *Gyrinops versteegii* from Several Agarwood Plantation on Lombok Island as Raw Material of Gyrinops Tea

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Biodiversitas: Journal of Biological Diversity

Novelty:

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Intrasepsific study of *G. versteegii* from Lombok Island using combination of morphology character, phytochemical character, and molecular character. Diversity of *G. versteegii* based on those combination characters provides essential information for development of Gyrinops Agarwood Tea Product.

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Sincerely yours,

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I Gde Adi Suryawan Wangiyana

Diversity of *Gyrinops versteegii* from Several Agarwood Plantation on Lombok Island as Raw Material of Gyrinops Tea

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Abstract. This research aims to examine the diversity of *Gyrinops versteegii* as a raw material of agarwood Gyrinops tea based on morphology, phytochemical, and molecular characters. *G. versteegii* samples were taken from 5 agarwood plantation on Lombok Island: Lingsar, Rarung, Mataram, Kekait, and Pejaring. Observation of *G. versteegii* stem, leaves, and fruit was used as morphology character. Qualitative phytochemical screening, Quantitative tannin concentration, and the hedonic score of Gyrinops tea were used as a phytochemical character. DNA fingerprinting by RAPD analysis was used as a molecular character. Morphological, chemical, and molecular characters were used for numeric-phenetics analysis. Dendrograms were constructed using the MVSP program with UPGMA algorithm as clustering method and Simple Matching Coefficient for similarity analysis. The result has shown variation topology of morphology character dendrogram, chemical character dendrogram, molecular character dendrogram, and combination character dendrogram. However, all dendrograms had the same number of clusters and the member of that cluster. *G. versteegii* kekait and *G. versteegii* Pejaring were grouped to cluster 1 while *G. versteegii* Rarung and *G. versteegii* Mataram were grouped to cluster 2. *G. versteegii* Lingsar was grouped later on the node after cluster 1 or cluster 2. It could be concluded that *G. versteegii* from Lombok Island Plantation could be divided into three different cluster groups based on the variation of morphological, chemical, and molecular characters.

Key words: Diversity, Gyrinops tea, Lombok Island.

Abbreviations RAPD (Randomized Amplified DNA Polymorphism), .PCR (Polymerase Chain Reaction), UPGMA (Unweighted Pair group Method with Arithmetic mean)

Running title: Diversity *Gyrinops versteegii* Lombok Island

INTRODUCTION

Gyrinops tea is an agarwood tea product from Lombok Island that is made from *Gyrinops versteegii* leaves. This product has emerged as a new type of agarwood tea product in Indonesia (Wangiyana et al. 2018). Formerly, Agarwood tea products from Indonesia were dominated by *Aquilaria malaccensis* as raw material (Adam et al. 2017). Aquilaria agarwood tea product has been well developed in Sumatera Island (Batubara et al. 2020; Surjanto et al. 2019a). Gyrinops agarwood tea from Lombok Island could give a variation of agarwood tea products in Indonesia.

G. versteegii, as a raw material of Gyrinops tea, was a native agarwood species of Lombok Island. This species has a wide distribution in almost all regions of Lombok Island, including North Lombok, West Lombok, Center Lombok, and East Lombok (Sutomo & Oktaviani, 2019). The distribution of *G. versteegii* on a different region of Lombok Island has resulted in intraspecific diversity of this species (Mulyaningsih et al. 2017). Intraspecific diversity study of *G. versteegii* from Lombok Island is essential for the standardization of Gyrinops tea for further development of this product.

Intraspecific diversity of *G. versteegii* was intensively conducted on the west region of Lombok Island. The morphology and anatomy of the *G. versteegii* organ were the primary data of intraspecific diversity study on this region (Mulyaningsih et al. 2017). Molecular character in the form of Karyomorphology and Chromosome number analysis was also a different basis of this *G. versteegii* intraspecific diversity study (Iswantari et al. 2017). The result has shown that *G. versteegii* from the west region of Lombok Island could be divided into five main groups: Beringin, Buaya, Madu, Pantai, and Soyun (Mulyaningsih et al. 2017). Intraspecific diversity of *G. versteegii* from the West Lombok Region should be further expanded to cover other regions of *G. versteegii* habitat on Lombok Island (Wangiyana et al. 2021a).

Intraspecific diversity study of *G. versteegii* from other regions on Lombok Island should be focused on characters that could support a better quality of Gyrinops tea product. The former intraspecific diversity study of *G. versteegii* from the west region of Lombok Island only focuses on morphology character (Mulyaningsih et al. 2017). This Morphology character should be added with characters that could be contributed to the diversity of Gyrinops agarwood tea product. Phytochemical character (Parwata et al. 2018) and molecular character in the form of DNA fingerprinting (Siburian et al. 2017) are two potential additional basis data that could fulfill this requirement. The phytochemical profile had an essential role in determining the variation of agarwood tea products from *Aquilaria malaccensis* (Batubara et al. 2020; Surjanto et al. 2019) and *Gyrinops versteegii* (Wangiyana et al. 2021a). DNA fingerprinting was a part of the DNA barcoding project that has become a quality standard for agarwood products on the market (Lee et al. 2016; Pern et al. 2020). However, no research combines all these characters for intraspecific diversity study of agarwood species, especially *G. versteegii*. The combination of morphology, phytochemical, and DNA fingerprinting characteristics could lead to a comprehensive intraspecific diversity study of *G. versteegii*.

This research aims to examine the diversity of Gyrinops versteegii as a raw material of agarwood Gyrinops tea based on morphology, phytochemical, and DNA fingerprinting characters. It is expected that the correlation of *G. versteegii* diversity and the quality of Gyrinops tea products could be revealed by this research. This information is essential for the further development of Gyrinops tea products from Lombok Island.

MATERIALS AND METHODS

Study area

The study area on this research was five regions on Lombok Island where *G. versteegii* samples were taken. These regions are: Lingsar (North Lombok), Rarung (Central Lombok), Mataram (Mataram City), Kekait (North Lombok), and Pejaring (East Lombok). More detail about the map and coordinate of each location is shown in figure 1.

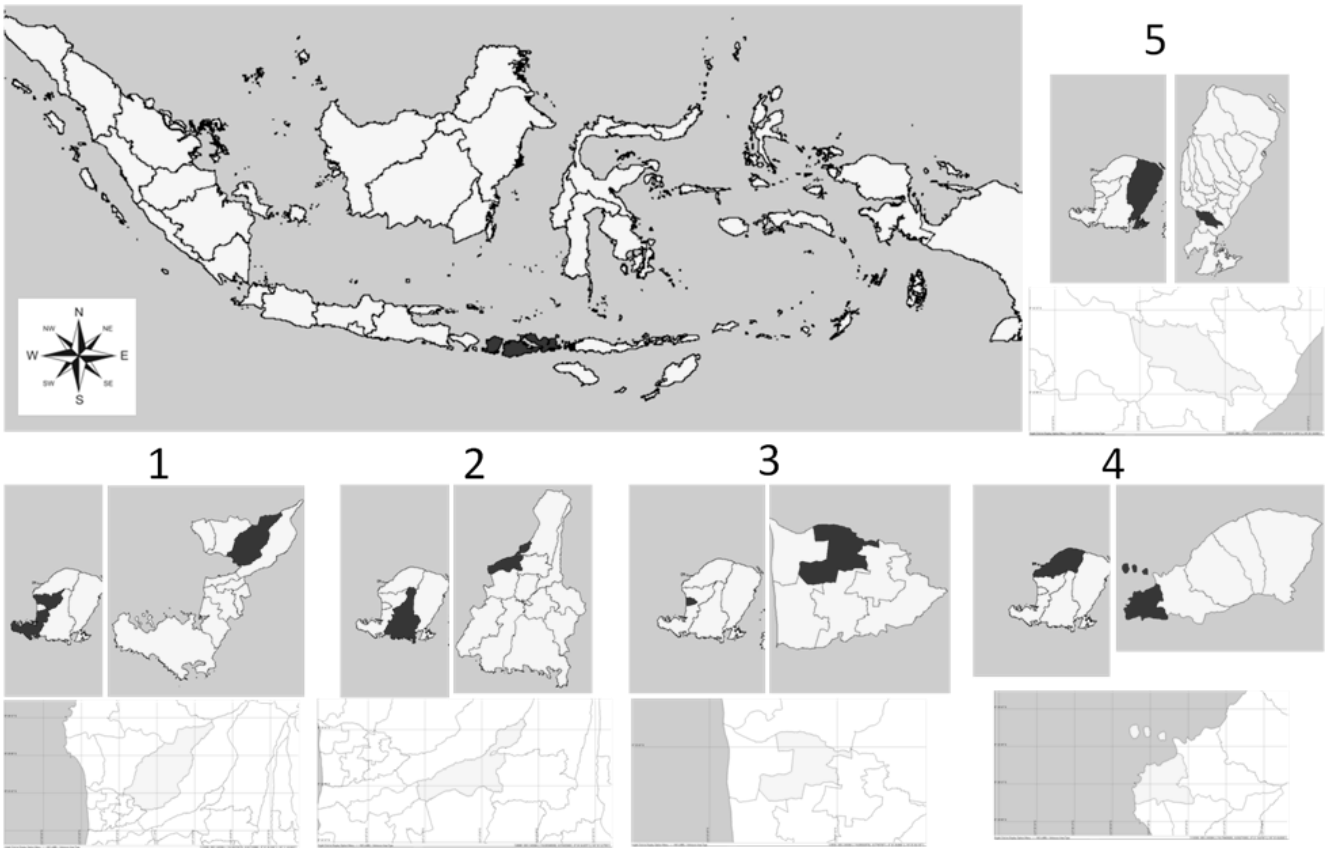

Figure 1. Sampling location of *G. versteegii* on Lombok Island agarwood plantation. Number 1 is Lingsar (8º33'32'' S 116º09'25'' E), Number 2 is Rarung (8º33'26''S 116º17'38''E), Number 3 is Mataram (8º33'58''S 116º07'47'' E), Number 4 is Kekait (8º31'26''S 116º07'03'' E), and Number 5 is Pejaring (8º42'28'' S 116º27'11'' E)

Procedure for Obtaining Morphology Character

Morphology characters of *G. versteegii* from 5 agarwood plantations were obtained by observing stem, leaves, and fruit. Key characters of *Gyrinops versteegii* that first described by Ding Hou (1960) were used as source data of

morphology characters. Supplement characters also were used to accommodate morphology character variation of *G. versteegii* from Lombok Island (Mulyaningsih et al. 2017).

Procedure for Obtaining Phytochemical Character

G. versteegii leaves processing

G. versteegii leaves were the primary material for phytochemical analysis. Leaves were cleaned by washing with distilled water then dried at temperature 30ºC until leaves lost 10% of water content. Dried leaves were then chopped using a grinding machine to form 1 – 2 mm particle size (Wangiyana et al. 2021a). Leaves particles were stored in the oxidation chamber for 14 days until the color of leaves particles became reddish-green or light green. These leaves particles were then stored at 4ºC for further phytochemical analysis.

Gyrinops tea extraction

G. versteegii leaves particle were raw material for Gyrinops tea product. These leaves particles were extracted using distilled water with a concentration of 0.02 gr/L and temperature 70ºC for 5 minutes. Filtrations using qualitative filter paper were carried to separate filtrate and residue (Wangiyana et al. 2021a). Filtrates from this process (Gyrinops tea) were stored at 4ºC for further analysis.

Qualitative phytochemical screening

Four compounds were analyzed from Gyrinops tea for qualitative phytochemical screening, including tannin, flavonoid, alkaloid, and saponins. $FeCl_3$ reagents were used for tannin assay with the positive result of changing Gyrinops tea solution into blackish brown (Ezeonu and Ejikeme 2016). $PbCH_3COO$ reagents were used for flavonoid assay with the positive result of white precipitates formation on Gyrinops tea solution (Geoffrey et al. 2014). Wagner reagent, Dragendroff reagent, and Mayer reagent were used for alkaloid assay with the positive result of precipitate formation on Gyrinops tea solution (Inamdar et al. 2014). HCl reagent was used for the saponins test with the positive result of foam formation on Gyrinops tea solution (Gul et al. 2017).

Quantitative tannin assay

A quantitative tannin concentration assay was performed by titrating Gyrinops tea with $KMnO_4$ solution that was previously standardized based on procedure (Khasnabis et al. 2015). Twenty-five ml of Gyrinops tea were mixed with 25 ml indigo carmine solution. The mixtures were diluted with 750 ml distilled water. Titration of the mixture with $KMnO_4$ was carried until the blue color of the mixture changed into green color. Few drops of $KMnO_4$ were added until the color of the mixture became golden yellow. Titration of indigo carmine solution without Gyrinops tea was carried for the blank test. Tannin concentration (%T) was calculated based on the equation (Wangiyana et al. 2019):

$$T(\%) = \frac{(V - V_0) \times 0.004157 \times 50}{g \times 25} \times 100\%$$

V is the volume of 0.1 N KMnO4 for Gyrinops tea solution (ml), V0 is the volume of 0.1 N KMnO4 for titration of blank test (ml), 0.004157 is tannins equivalent in 1 ml of 0.1 N KMnO4, g is mass of the sample taken for analysis (gram), 25 is the volume of sample, 50 is the volume of extraction solvent for sample.

Antioxidant activity assay

DPPH free radical scavenging method was used for antioxidant activity assay of Gyrinops tea (Tay et al. 2014). Six serial dilutions with concentration 150µl/ml, 125 µl/ml, 100 µl/ml, 75 µl/ml, and 50 µl/ml. Ascorbic acid was used as a positive control. Measurement of each dilution was performed by UV-Vis spectrophotometer at 516 nm wavelength. Scavenging activity (%) was measured using an equation: (Prihantini dan Rizqiani 2019)

$$\%\,\text{Scavenging Activity} = \left(\frac{A_{blanko} - A_{sample}}{A_{sample}} \right) \times 100\%$$

IC50 was calculated based on scavenging activity percentage data using linear regression interpolation approaches. The IC50 value is the concentration of Gyrinops tea that gives 50% scavenging activity inhibition. IC50 was the standard for antioxidant power of Gyrinops tea samples with scoring category: very strong antioxidant power (IC50 value < 50µg/ml), strong antioxidant power (IC50 value 50 µg/ml - 100 µg/ml), moderate antioxidant power (IC50 value 101 µg/ml - 150 µg/ml), weak antioxidant power (IC50 value 151 µg/ml - 200 µg/ml) (Surjanto et al. 2019b)

Hedonic assay of Gyrinops tea

The hedonic assay was conducted as a preference test to measure the evaluation score of Gyrinops tea by the panelist. Thirty panelists with an age range from 20 years old to 50 years old were given their evaluation of Gyrinops tea based on color, aroma, and flavor parameters. Five hedonic scales were used for scoring the evaluation from panelists based on category: 1=dislike very much, 2= dislike moderately, 3= neither like nor dislike, 4=like moderately, 5=like very much (Batubara et al. 2018). The mean score of the hedonic scale from 30 panelists was used as the phytochemical character of *G. versteegii* from different agarwood plantations.

Procedure for Obtaining Molecular Character

G. versteegii DNA extraction

G. versteegii leaves were used as a sample for DNA extraction. According to the manufacturer's recommendations, the Blood Animal Plant DNA Preparation Kit (Jena Bioscience) was used for genomic DNA extraction (Simon-Oke et al. 2018). Leaves were stored at -70ºC overnight as preparation for DNA extraction. Leaves were then grinded using liquid nitrogen into frozen powder. Eighty milligrams of frozen powder samples were transferred into a 1.5 microcentrifuge tube for cell lysis. Addition of 300 µl Lysis buffer and 2 µl RNase subjected to sample followed by homogenizing for 30 seconds. Proteinase K (8 µl) was added to the mixture with incubation at 60ºC for 20 minutes followed by centrifugation at 10,000 g for 5 minutes. The supernatant was injected into the activated column followed by centrifugation at 10,000 g for 30 seconds. The columns were washed two times with 500 µl washing buffer. Samples were placed in the elution tube with the addition of 50 µl elution buffer followed by centrifugation at 10,000 g for 2 minutes.

Genomic DNA concentration and purity analysis were carried using UV-1601PC Shimadzu by measuring absorbance at wavelength 260 nm, 280 nm, and 230 nm (Lucena-Aguilar et al. 2016). Visualization of isolated DNA was assessed by electrophoresis on a 0.8% agarose gel with ethidium bromide staining. Ladder 1000bp (Invitrogen) was used as a marker for molecular weight estimation of genomic DNA.

RAPD – PCR

Random OPA primers were used for RAPD analysis (Table 1). Those primers were arbitrarily selected from the OPA series commonly used for RAPD. PCR was carried out in total volume of 25 µl containing 12.5 µl 2 x KAPA 2G PCR mix (KAPA Biosystems), 8.5 µl ddH2O, 2 µl of each OPA primer (10 pmol/ µl), and 2 µl *G. versteegii* template DNA (40 ng/ µl). PCR Amplification was conducted on Labcycler thermocycler with the following profile: initial denaturation at 95ºC for 3 minutes, followed by 40 cycles of denaturation at 95ºC for 15 s, annealing at 37ºC for 1 minute, extension at 72ºC for 2 minutes, and final extension at 72ºC for 5 minutes (Wangiyana et al. 2021b). The amplified DNA fragments were visualized by electrophoresis on 1.2% agarose gels with ethidium bromide staining. Ladder 1000bp (Invitrogen) was used as a marker for molecular weight estimation of RAPD bands. Each RAPD band on all samples and Random OPA primer were considered molecular characters used for clustering analysis.

Table 1. Random OPA primer sequence for PCR amplification

Random Primer	Sequence
OPA-01	5′- CAGGCCCTTC-3′
OPA-02	5′- TGCCGAGCTG-3′
OPA-04	5′-AATCGGGCTG- 3′
OPA-08	5′- GTGACGTAGG -3′
OPA-09	5′-GGGTAACGCC-3′
OPA-18	5′-AGGTGACCGT-3′

Data analysis

G. versteegii samples from 5 agarwood plantations on Lombok Island were numeric- phenetic analysis's Organism Taxonomical Unit (OTU). Morphology, phytochemical, and molecular character were tabulated as the primary basis data for similarity analysis (presence or absence of several characters). The tabulation data Dendrograms of each morphology character, phytochemical character, molecular character, and combination of those three characters were constructed using the MVSP program. UPGMA algorithm was used as clustering method, and Simple Matching Coefficient was used for similarity analysis. Cophenetic – correlation analysis was conducted to observe distortion between sorted similarity matrix and unsorted similarity matrix (Saracli et al. 2013). The significant score of cophenetic - correlation analysis was examined using Co-Stat for the windows program.

RESULTS AND DISCUSSION

Numeric-phenetic based on morphology characters

G. versteegii from 5 agarwood plantations have shown morphology variation mostly on leaves and stems. Leaves were the raw material of Gyrinops agarwood tea products. Variation of *G. versteegii* leaves could support a better understanding of which characteristics of the leaves could produce Gyrinops tea with good quality. Thus, the development of Gyirnops tea products could be supported by these data. The stem was the other essential organ that affected agarwood tea production. There were two groups of *G. versteegii* based on stem characteristics observation: shrub *G. versteegii* group and tree *G. versteegii* group. Agarwood farmers from *Aquilaria malaccensis* plantation prefer agarwood shrub group to agarwood tree group. The agarwood shrub group was more accessible to harvest its leaves than the agarwood tree group. Thus, stem character could be an essential for diversity study of *G. versteegii* (Rindyastuti et al. 2019).

Table 2. *G. versteegii* morphology character for similarity analysis

No	Character	Pejaring	Kekait	Lingsar	Mataram	Rarung
1	Shrub up to 6 m	-	+	-	+	-
2	tree up to 21 m	+	-	+	-	+
3	young branchlets bark grayish	+	+	+	+	+
4	young branchlets pubescent	+	+	+	+	+
5	Leaves texture chartaceous	+	+	-	-	+
6	Leaves texture subcoriaceous	-	-	-	+	+
7	Leaves pubescent on beneath	+	+	+	+	+
8	leaves shapes elliptic-oblong	-	-	-	+	+
9	leaves shapes ovate-oblong	+	+	+	-	-
10	leaves surface dark green	+	-	-	-	+
11	leaves surface shining yellow-green	-	+	+	+	-
12	leaves base cuneate	+	+	+	+	+
13	leaves apex narrow-acuminate	+	+	+	+	+
14	leaves width 1 – 2,4 cm	+	+	+	-	-
15	leaves width 2,5 – 5 cm	-	-	-	+	+
16	leaves length 8 cm – 11,4 cm	-	-	-	+	+
17	leaves length 11,5 cm – 15 cm	+	+	-	-	-
18	Obelique-parallel of leaves nerves and veins	+	+	+	+	+
19	Fruit color yellow	+	+	-	-	-
20	Fruit color orange	-	-	-	+	+

Note: + = presence of character, - = absence of character

Dendrogram, based on morphology characters, was grouped *G. versteegii* into 3 clusters. The first cluster was the group of *G. versteegii* Rarung and *G. versteegii* Rarung, with the highest similarity among other clusters (80%). The second cluster was the group of *G. versteegii* Mataram and *G. versteegii* Lingsar with 75% similarity. *G. versteegii* Lingsar was not grouped with other *G. versteegii*. This OTU stands alone as a member of cluster 3. Intraspecific study of *G. versteegii* from the western part of Lombok Island based on morphology characters resulting dendrogram with cluster similarity value range from 75% - 78% (Mulyaningsih et al. 2017). Thus, morphology character dendrogram in this study could confirm morphology character dendrogram from Western Lombok. Morphology character dendrogram on this study also could provide intraspecific study data of *G. versteegii* other than Western Lombok.

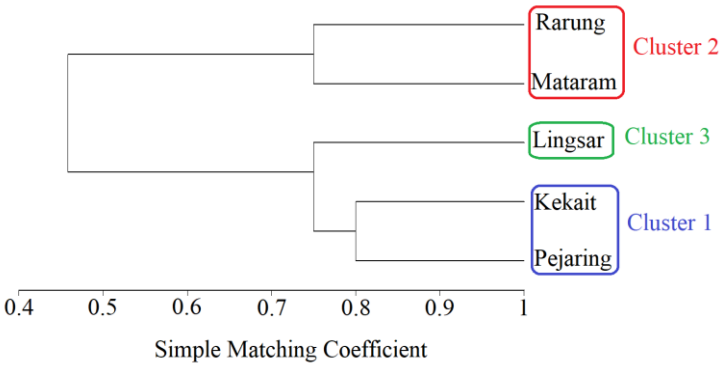

Figure 2. Dendrogram constructed based on morphology character

Numeric – phenetic based on Phytochemical character

The phytochemical character of *G. versteegii* is mainly affected by its leaves as the raw material of Gyrinops tea (Wangiyana et al. 2021). *G. versteegii* from 5 sampling locations have shown variation on leaves characters. Leaves variation has affected characteristics of Gyrinops tea, especially the color and turbidity (Figure 3). Based on this result, the correlation between the variation characteristic of *G. versteegii* leaves and its phytochemical profile variation was revealed. This result could also recommend *G. versteegii* leaves selection to produce Gyrinops tea with the particular phytochemical profile.

Figure 3. Variation characteristic of *G. versteegii* leaves and Gyrinops tea

Variation characteristic of Gyrinops tea from 5 different sampling locations was related to a variation on several phytochemical characters on the leaves of *G. versteegii*. Tannin concentration, IC50 value, and hedonic score were the characters primarily responsible for the phytochemical profile variation of *G. versteegii* (table 3). Tannin is the main compound that responsible for the quality of agarwood tea both from Aquilaria (Batubara et al. 2018) and Gyrinops (Wangiyana et al. 2018). The IC50 value is an antioxidant power measurement of agarwood tea that determined its quality as health beneficial herbal tea product (Parwata et al. 2016). The hedonic assay was the standard consumer preference test to determine the quality of agarwood tea products with different processing methods (Batubara et al. 2018). Thus, variation of tannin concentration, IC50 value, and hedonic assay on *G. versteegii* from different sampling locations was essential information for standardization of Gyrinops tea quality product from Lombok Island.

Table 3. *G. versteegii* phytochemical characters for similarity analysis

No	Character	Pejaring	Kekait	Lingsar	Mataram	Rarung
1	oxidation produce green-reddish chopped dried leaves	+	+	-	-	-
2	oxidation produce light green chopped dried leaves	-	-	+	+	+
3	brewing leaves contain saponin	-	-	-	-	-
4	brewing leaves contain flavonoid	+	+	+	+	+
5	brewing leaves contain alkaloid	-	-	-	-	-
6	Gyrinops tea product with high turbidity	-	+	-	-	-
7	Gyrinops tea product with medium turbidity	+	-	-	-	+
8	Gyrinops tea product with low turbidity	-	-	+	+	-
9	Precipitate from $FeCl_3$ reagent	+	+	+	+	+
10	Tannin concentration 2,01% - 3,00%	+	-	-	-	-
11	Tannin concentration 3,01% - 4,00%	-	-	+	-	-
12	Tannin concentration 4,01% - 5,00%	-	+	-	-	-
13	IC50 Gyrinops tea product less than 50 µg/ml	-	-	-	-	-
14	IC50 Gyrinops tea product 50 µg/ml - 100 µg/ml	-	-	-	+	-
15	IC50 Gyrinops tea product 101 µg/ml - 150 µg/ml	+	+	+	-	-
16	IC50 Gyrinops tea product 151 µg/ml - 200 µg/ml	-	-	-	-	+
17	Color parameter of hedonic score range 2,00 – 2,99	-	-	-	-	-
18	Color parameter of hedonic score range 3,00 – 3,99	+	+	-	-	-
19	Color parameter of hedonic score range 4,00 – 4,99	-	-	+	-	-
20	Aroma parameter of hedonic score range 2,00 – 2,99	-	-	+	-	-
21	Aroma parameter of hedonic score range 3,00 – 3,99	+	+	-	-	-
22	Aroma parameter of hedonic score range 4,00 – 4,99	-	-	-	-	-
23	Taste parameter of hedonic score range 2,00 – 2,99	-	-	-	-	-
24	Taste parameter of hedonic score range 3,00 – 3,99	+	+	+	-	-
25	Taste parameter of hedonic score range 4,00 – 4,99	-	-	-	-	-

Note: + = presence of character, - = absence of character

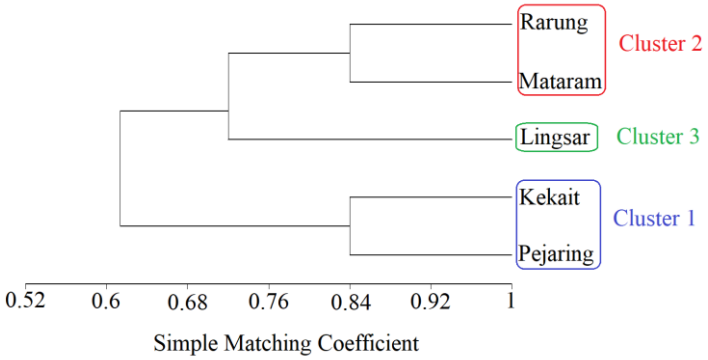

Figure 4. Dendrogram constructed based on phytochemical character

Dendrogram constructed based on phytochemical characters resulted in the 3 clusters with the same pair member like morphology character dendrogram. However, the similarity of cluster pair members on this dendrogram was slightly higher than the similarity of morphology dendrogram. *G. versteegii* Kekait and *G. versteegii* Pejaring were grouped in cluster 1 with 84% similarity values. *G. versteegii* Rarung and *G. versteegii* Mataram were grouped in cluster 2 with the same similarity value with cluster 1. *G. versteegii* Lingsar stands alone as a cluster 3, just like morphology character dendrogram. However, *G. versteegii* Lingsar joint the node with cluster 2 before joint with other clusters. On the morphology character dendrogram, this OTU joints the node with cluster 1 before joining other clusters.

Numeric phenetic based on molecular character

RAPD – PCR of *G. versteegii* samples from 5 agarwood plantations has resulted in various banding patterns from different OPA primers. The bands at a particular position represent RAPD loci which could be classified as monomorphic or polymorphic. A locus is monomorphic if the band is present in all OTU. On the other hand, the polymorphic locus is a band that is absent in at least one OTU (Wangiyana et al. 2021b). The number of polymorphic bands determined the random primer's ability to differentiate OTU based on the molecular character.

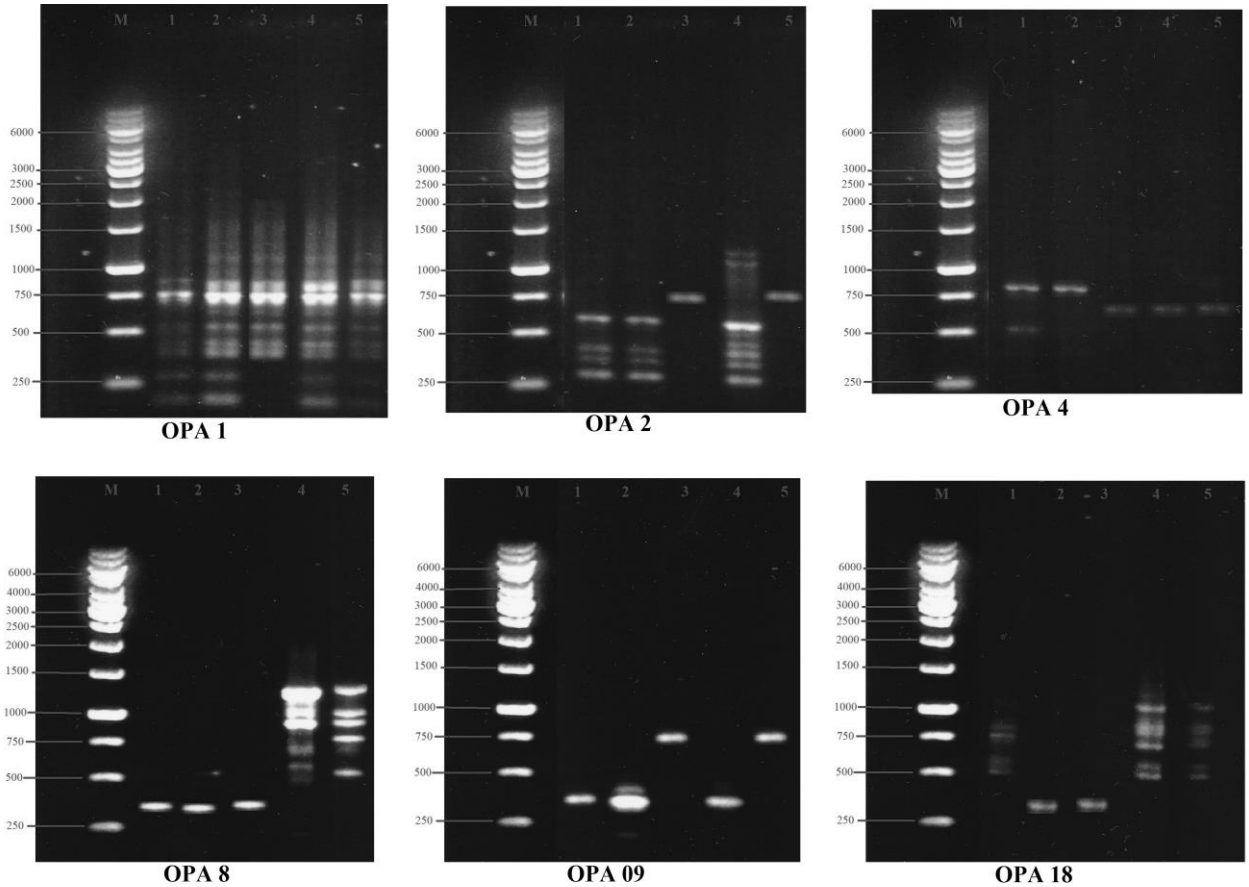

Figure 5. DNA fingerprinting based on RAPD – PCR. (M = marker, 1=Pejaring, 2=Kekait, 3=Lingsar, 4=Mataram, 5=Rarung)

The different number of bands produced by OPA primers could determine their efficiency for genetic variation study on *G. versteegii* (Siburian et al. 2017). OPA 1 primer produces the highest number of the band among other OPA primers. However, most bands on the OPA 1 primer were monomorphic and had no significant impact on OTU differentiation. OPA 4 and OPA 9 were two random primers that produced the least number of bands. OPA 2, OPA 8, and OPA 18 produce several numbers of polymorphic bands that were useful for similarity analysis. OPA 2 produces the highest number of polymorphic bands among other primers, which means that this primer was an ideal primer for the genetic variation study of *G. versteegii*.

Table 4. *G. versteegii* molecular character for similarity analysis

	Character	Pejaring	Kekait	Lingsar	Mataram	Rarung
1	1270 bp band	-	-	-	+	+
2	1150 bp band	-	-	-	+	-
3	1050 bp band	-	-	-	+	-
4	1000 bp band	-	-	-	+	+
5	990 bp band	-	-	-	+	+
6	910 bp band	-	-	-	+	+
7	860 bp band	-	-	-	+	+
8	850 bp band	-	+	-	+	+
9	830 bp band	+	-	-	+	+
10	820 bp band	+	+	-	-	-
11	760 bp band	-	-	+	-	+
12	750 bp band	+	+	+	+	+
13	730 bp band	-	-	-	+	+
14	670 bp band	+	-	-	+	+
15	650 bp band	-	+	-	+	-
16	580 bp band	-	-	-	+	-
17	560 bp band	+	+	-	+	-
18	550 bp band	+	+	+	+	+
19	540 bp band	+	-	-	+	+
20	530 bp band	-	+	-	-	+
21	500 bp band	+	-	-	-	-
22	480 bp band	+	-	-	+	+
23	470 bp band	+	+	+	+	+
24	430 bp band	-	-	-	+	-
25	410 bp band	-	+	-	-	-
26	380 bp band	+	+	-	+	-
27	360 bp band	+	+	-	+	-
28	330 bp band	+	+	-	+	-
29	310 bp band	-	+	-	+	-
30	290 bp band	+	+	-	+	-
31	270 bp band	+	+	-	+	-

Note: + = presence of character, - = absence of character

All bands produced by the random primers were tabulated and sorted based on their molecular weight (table 4). These bands were treated as characters of similarity analysis just the same as morphology character and phytochemical character. These bands also represent the DNA fingerprinting of each OTU for dendrogram construction. This tabulated band character shows the polymorphic band pattern of each OTU more clearly than the electrophoresis result in figure 5.

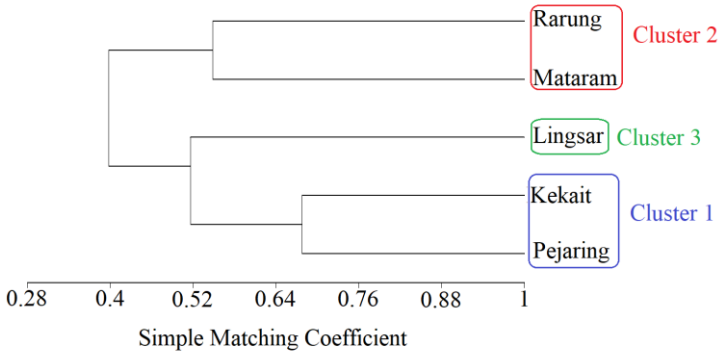

Figure 6. Dendrogram constructed based on molecular character

Dendrogram based on DNA fingerprinting molecular character has resulted in the same number of clusters and the same cluster member as morphology character dendrogram and phytochemical character dendrogram. However, the similarity value of cluster member on this dendrogram was lower than the similarity value of cluster member on morphology character dendrogram and phytochemical character dendrogram. Members of cluster 1 were *G. versteegii* Pejaring and *G. versteegii* Kekait with 67.7% similarity value. Members of cluster 2 were *G. versteegii* Rarung and *G. versteegii* Mataram, with a 54.8% similarity value. *G. versteegii* Lingsar joins the node after cluster 1 and forms cluster 3. Clustering analysis that uses RAPD profile as basis data commonly produces low similarity value among OTU. However, the DNA fingerprinting profile of RAPD could reveal variation that could not be observed based on morphological analysis or chemical analysis. RAPD also could provide genetic variation data that could support morphology and chemical variation data for diversity study (Irsyad et al. 2020).

Numeric phenetic analysis based on combination character

The diversity study of *G. versteegii* mostly takes primary data from morphology character, phytochemical character, or molecular character without combining all of those characters. A combination of morphology, phytochemical, and molecular character could provide better comprehensive data for variation analysis of *G. versteegii*. Combining these three characters as basis data also could be a useful to examine how the characters support each other to generate a better clustering analysis method.

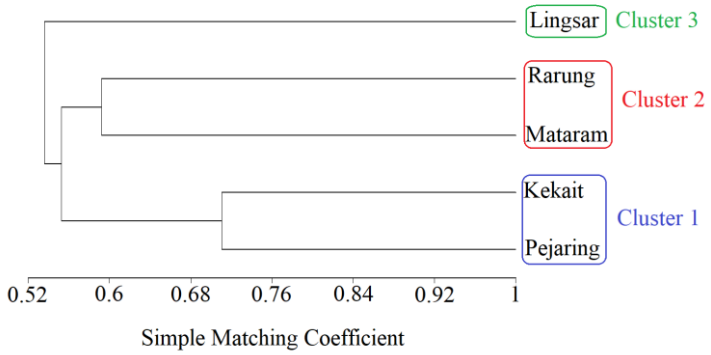

Figure 7. Dendrogram based on combination of morphology, phytochemical, and molecular character

Dendrogram constructed based on combination characters has resulted in the same number of clusters and cluster members with morphology, phytochemical, and molecular dendrogram. However, *G. versteegii* Lingsar was not directly clustered on the node with cluster 1 as it did on morphology character dendrogram and molecular dendrogram. This OTU was not directly clustered on the node with cluster 2 on the phytochemical character dendrogram. This OTU was clustered after cluster 1, and cluster 2 was clustered into a new node. This result confirmed that *G. versteegii* Lingsar has a minor similarity among others *G. versteegii* from different sampling locations. However, this result also implies that *G. versteegii* Lingsar is a unique variant of *G. versteegii* from Lombok Island that needs further exploration about its potency.

Table 5. Cophenetic – correlation analysis of each clustering method

character	Corr (r)	S.E. of r	P(r=0)	significant notation
Morphology	0.893	0.159	0.0005	**
phytochemical	0.951	0.109	0.00001	**
molecular	0.697	0.254	0.0251	*
combination	0.736	0.239	0.0153	*

Note: * = significant correlation, ** = very significant correlation

Cophenetic – correlation analysis of clustering method using different characters has resulted in a significant correlation on all characters that have been used. The morphology character and phytochemical character even have a very significant correlation with their clustering method. It means that there was no distortion between the unsorted similarity matrix as an input for clustering analysis and the sorted similarity matrix as an output of clustering analysis (Carvalho et al. 2019). Thus, dendrograms that were constructed based on this clustering method have high reliability.

In conclusion, *G. versteegii* from 5 sampling locations of agarwood plantation on Lombok Island have genetic diversity on the DNA fingerprinting as molecular characters. This genetic diversity has been expressed as diversity on morphology character, especially on leaves organ and phytochemical profile. These variations were the main basis data to divided *G. versteegii* samples from Pejaring, Kekait, Lingsar, Mataram, and Rarung into 3 cluster group: cluster 1 (*G. versteegii* Kekait and *G. versteegii* Pejaring), cluster 2 (*G. versteegii* Rarung and *G. versteegii* Mataram), and cluster 3 (*G. versteegii* Lingsar).

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Diversity of *Gyrinops versteegii* from several agarwood plantation on Lombok Island as raw material of Gyrinops tea

Abstract. The purpose of this research is to examine the diversity of *Gyrinops versteegii* as a raw material of agarwood Gyrinops tea based on morphology, phytochemical, and molecular characters. *G. versteegii* samples were taken from 5 agarwood plantation on Lombok Island: Lingsar, Rarung, Mataram, Kekait, and Pejaring. Variation of *G. versteegii* stem, leaves, and fruit was the morphology character observed in this study. Qualitative phytochemical screening, qQuantitative tannin concentration, and the hedonic score of Gyrinops tea were the essential phytochemical character in this study. DNA fingerprinting and RAPD analysis were the molecular characters of this study. All of those characters were used in numeric-phenetics analysis to construct a dendrogram. MVSP program with UPGMA algorithm as clustering method and Simple Matching Coefficient for similarity analysis was used to construct dendrogram. The result has shown variation topology of dendrogram based on morphology, phytochemical, molecular, and combination character. However, all dendrograms had the same number of clusters and cluster members. *G. versteegii* kekait and *G. versteegii* Pejaring were grouped to cluster 1 while *G. versteegii* Rarung and *G. versteegii* Mataram were grouped to cluster 2. *G. versteegii* Lingsar was grouped later on the node after cluster 1 or 2. It could be concluded that *G. versteegii* from Lombok Island Plantation could be divided into three different cluster groups based on the variation of morphology, chemical, and molecular characters.~~This research aims to examine the diversity of *Gyrinops versteegii* as a raw material of agarwood Gyrinops tea based on morphology, phytochemical, and molecular characters. *G. versteegii* samples were taken from 5 agarwood plantation on Lombok Island: Lingsar, Rarung, Mataram, Kekait, and Pejaring. Observation of *G. versteegii* stem, leaves, and fruit was used as morphology character. Qualitative phytochemical screening, Quantitative tannin concentration, and the hedonic score of Gyrinops tea were used as a phytochemical character. DNA fingerprinting by RAPD analysis was used as a molecular character. Morphological, chemical, and molecular characters were used for numeric-phenetics analysis. Dendrograms were constructed using the MVSP program with UPGMA algorithm as clustering method and Simple Matching Coefficient for similarity analysis. The result has shown variation topology of morphology character dendrogram, chemical character dendrogram, molecular character dendrogram, and combination character dendrogram. However, all dendrograms had the same number of clusters and the member of that cluster. *G. versteegii* kekait and *G. versteegii* Pejaring were grouped to cluster 1 while *G. versteegii* Rarung and *G. versteegii* Mataram were grouped to cluster 2. *G. versteegii* Lingsar was grouped later on the node after cluster 1 or cluster 2. It could be concluded that *G. versteegii* from Lombok Island Plantation could be divided into three different cluster groups based on the variation of morphological, chemical, and molecular characters.~~

Keywords: Diversity, Gyrinops tea, Lombok Island

Abbreviations RAPD: Randomized Amplified DNA Polymorphism; PCR: Polymerase Chain Reaction; UPGMA: Unweighted Pair group Method with Arithmetic mean

Running title: Diversity *Gyrinops versteegii* Lombok Island

INTRODUCTION

Gyrinops tea is an agarwood tea product from Lombok Island that is made from *Gyrinops versteegii* leaves. This product has emerged as a new type of agarwood tea product in Indonesia (Wangiyana et al. 2018). Formerly, ~~Agarwood~~ agarwood tea products from Indonesia were dominated by *Aquilaria malaccensis* as raw material (Adam et al. 2017). Tea product based on Aquilaria species~~Aquilaria agarwood tea product~~ has been well developed in Sumatera Island (Batubara et al. 2020; Surjanto et al. 2019a). Gyrinops ~~agarwood~~ tea from Lombok Island could give a variation of agarwood tea products in Indonesia.

G. versteegii, as a raw material of Gyrinops tea, was a native agarwood species of Lombok Island. This species has a wide distribution in almost all regions of Lombok Island, including North Lombok, West Lombok, Center Lombok, and East Lombok (Sutomo & Oktaviani, 2019). The distribution of *G. versteegii* on a different region of Lombok Island has

resulted in intraspecific diversity of this species (Mulyaningsih et al. 2017). Intraspecific diversity study of *G. versteegii* from Lombok Island is essential for the standardization of Gyrinops tea for further development of this product.

Intraspecific diversity of *G. versteegii* was intensively foundconducted on the west region of Lombok Island. Intraspecific study of *G. versteegii* on this region used this species's morphology and anatomy as the primary data.The morphology and anatomy of the *G. versteegii* organ were the primary data of intraspecific diversity study on this region (Mulyaningsih et al. 2017). Molecular character in the form of Kkaryomorphology and Cchromosome number analysis was also a different basis of this study of *G. versteegii* intraspecific diversity study (Iswantari et al. 2017). The result has shown that *G. versteegii* from the west region of Lombok Island could be divided into five main groups: Beringin, Buaya, Madu, Pantai, and Soyun (Mulyaningsih et al. 2017). Intraspecific diversity of *G. versteegii* from the West Lombok Region should be further expanded to cover other regions of *G. versteegii* habitat on Lombok Island (Wangiyana et al. 2021a).

Intraspecific diversity study of *G. versteegii* from other regions on Lombok Island should be focused on characters that could support a better quality of Gyrinops tea product. The former intraspecific diversity study of *G. versteegii* from the west region of Lombok Island only focuses on morphology character (Mulyaningsih et al. 2017). This Mmorphology character should be added with characters that could be contributed to the diversity of Gyrinops agarwood tea product. Phytochemical character (Parwata et al. 2018) and molecular character in the form of DNA fingerprinting (Siburian et al. 2017) are two potential additional basis data that could fulfill this requirement. The phytochemical profile had an essential role in determining the variation of agarwood tea products from *Aquilaria malaccensis* (Batubara et al. 2020; Surjanto et al. 2019) and *Gyrinops versteegii* (Wangiyana et al. 2021a). DNA fingerprinting was a part of the DNA barcoding project that has become a quality standard for agarwood products on the market (Lee et al. 2016; Pern et al. 2020). However, no research combines all these characters for intraspecific diversity study of agarwood species, especially *G. versteegii*. The combination of morphology, phytochemical, and DNA fingerprinting characteristics could lead to a comprehensive intraspecific diversity study of *G. versteegii*.

This research aims to examine the diversity of *Gyrinops versteegii* as a raw material of agarwood Gyrinops tea based on morphology, phytochemical, and DNA fingerprinting characters. It is expected that the correlation of *G. versteegii* diversity and the quality of Gyrinops tea products could be revealed by this research. This information is essential for the further development of Gyrinops tea products from Lombok Island.

MATERIALS AND METHODS

Study area

The study area on this research was five regions on Lombok Island where *G. versteegii* samples were taken. These regions are: Lingsar (North Lombok), Rarung (Central Lombok), Mataram (Mataram City), Kekait (North Lombok), and Pejaring (East Lombok). More detail about the map and coordinate of each location is shown in Figure 1.

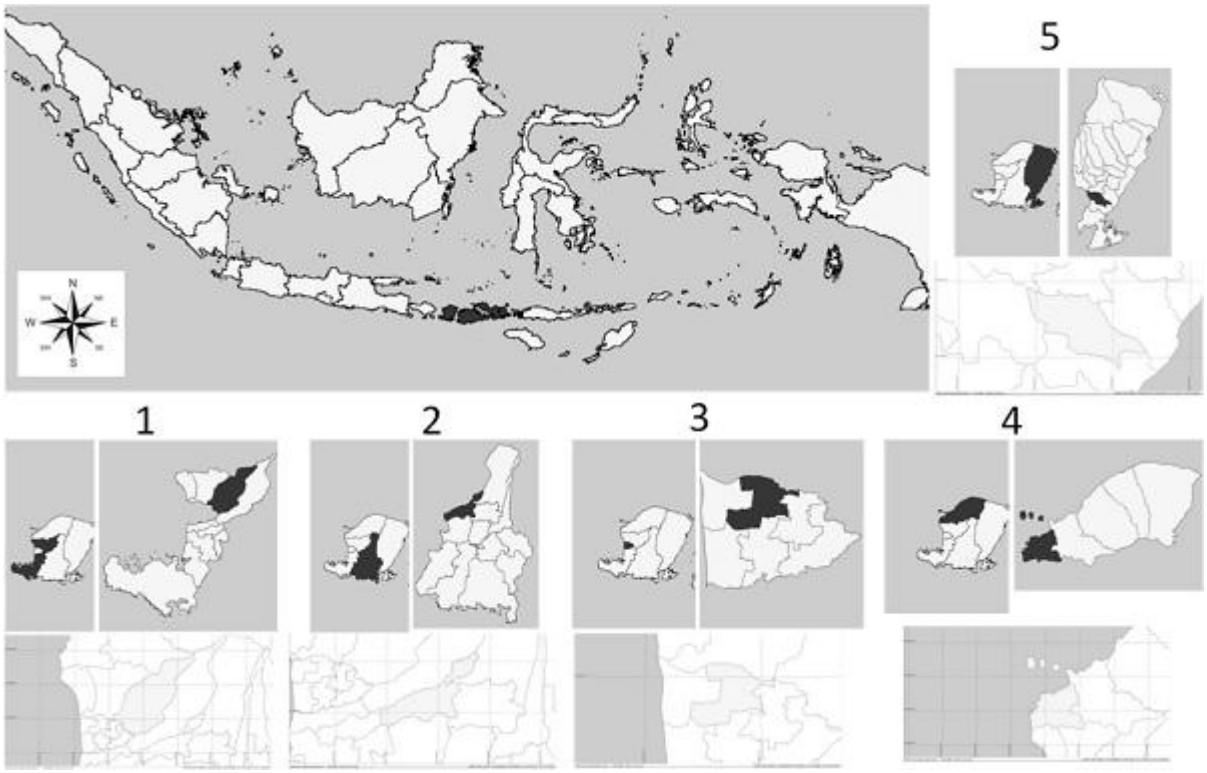

Figure 1. Sampling location of *G. versteegii* on Lombok Island agarwood plantation. Number 1 is Lingsar (8°33'32'' S 116°09'25'' E), Number 2 is Rarung (8°33'26''S 116°17'38''E), Number 3 is Mataram (8°33'58''S 116°07'47'' E), Number 4 is Kekait (8°31'26''S 116°07'03'' E), and Number 5 is Pejaring (8°42'28'' S 116°27'11'' E)

Procedure for obtaining morphology character

Morphology characters of *G. versteegii* from 5 agarwood plantations were obtained by observing stem, leaves, and fruit. Key characters of *Gyrinops versteegii* that first described by Ding Hou (1960) were used as source data of morphology characters. Supplement characters also were used to accommodate morphology character variation of *G. versteegii* from Lombok Island (Mulyaningsih et al. 2017).

Procedure for obtaining phytochemical character

G. versteegii leaves processing

G. versteegii leaves were the primary material for phytochemical analysis. Leaves were cleaned by washing with distilled water then dried at ~~temperature~~ 30°C until the leaves lost 10% of water content. Dried leaves were then chopped using ~~a grinding machine~~ grinder into ~~form~~ 1 – 2 mm particle size (Wangiyana et al. 2021a~~a~~). Leaves particles were stored in ~~the~~ oxidation chamber for 14 days until the color of leaves particles became reddish-green or light green. These leaves particles were then stored at 4°C for further phytochemical analysis.

Gyrinops tea extraction

G. versteegii leaves particle were raw material for Gyrinops tea product. These leaves particles were extracted using distilled water with a concentration of 0.02 gr/L ~~and temperature~~ at 70°C for 5 minutes. Filtrations using qualitative filter paper were carried to separate the filtrate and residue (Wangiyana et al. 2021b~~a~~). The Filtrate~~s~~ produced from this process (Gyrinops tea) were stored at 4°C for further analysis.

Qualitative phytochemical screening

Four compounds were analyzed from Gyrinops tea for qualitative phytochemical screening, including tannin, flavonoid, alkaloid, and saponins. $FeCl_3$ reagents were used for tannin assay ~~with the positive result of changing Gyrinops tea solution into blackish brown~~ (Ezeonu and Ejikeme 2016). $PbCH_3COO$ reagents were used for flavonoid assay ~~with the positive result of white precipitates formation on Gyrinops tea solution~~ (Geoffrey et al. 2014). Wagner reagent, Dragendroff reagent, and Mayer reagent were used for alkaloid assay ~~with the positive result of precipitate formation on Gyrinops tea solution~~ (Inamdar et al. 2014). HCl reagent was used for the saponins assay~~test~~ ~~with the positive result of foam formation on Gyrinops tea solution~~ (Gul et al. 2017).

Quantitative tannin assay

A quantitative tannin concentration assay was performed by titrating Gyrinops tea with $KMnO_4$ solution that was previously standardized based on procedure (Khasnabis et al. 2015). Twenty-five ml of Gyrinops tea were mixed with 25 ml indigo carmine solution~~. The mixtures were diluted with 750 ml distilled water.~~ Titration of the mixture with $KMnO_4$ was carried until the blue color of the mixture changed into green color and continued to form golden yellow.~~ Few drops of $KMnO_4$ were added until the color of the mixture became golden yellow~~. Titration of indigo carmine solution without Gyrinops tea was carried for the blank test. Tannin concentration (%T) was calculated based on the equation (Wangiyana et al. 2021b~~19~~):

$$T(\%) = \frac{(V - V_0) \times 0.004157 \times 50}{g \times 25} \times 100\%$$

V is the volume of 0.1 N KMnO4 for Gyrinops tea solution (ml), V0 is the volume of 0.1 N KMnO4 for titration of blank test (ml), 0.004157 is tannins equivalent in 1 ml of 0.1 N KMnO4, g is mass of the sample taken for analysis (gram), 25 is the volume of sample, 50 is the volume of extraction solvent for sample.

Antioxidant activity assay

DPPH free radical scavenging method was used for antioxidant activity assay of Gyrinops tea (Tay et al. 2014). The absorbance of Six serial dilutions of Gyrinops tea samples with ascorbic acid as a positive control was measured at 516 nm wavelength using a UV-Vis spectrophotometer.~~Six serial dilutions with concentration 150µl/ml, 125 µl/ml, 100 µl/ml, 75 µl/ml, and 50 µl/ml. Ascorbic acid was used as a positive control. Measurement of each dilution was performed by UV-Vis spectrophotometer at 516 nm wavelength~~. Scavenging activity (%) was measured using an equation: (Prihantini dan Rizqiani 2019)

$$\%\ \text{Scavenging Activity} = \left(\frac{A_{blanko} - A_{sample}}{A_{sample}} \right) \times 100\%$$

IC50 was calculated based on scavenging activity percentage data using linear regression interpolation approaches. The IC50 value is the concentration of Gyrinops tea that gives 50% scavenging activity inhibition. IC50 was the standard for antioxidant power of Gyrinops tea samples with scoring category: very strong antioxidant power (IC50 value < 50µg/ml), strong antioxidant power (IC50 value 50 µg/ml - 100 µg/ml), moderate antioxidant power (IC50 value 101 µg/ml - 150 µg/ml), weak antioxidant power (IC50 value 151 µg/ml - 200 µg/ml) (Surjanto et al. 2019b)

Hedonic assay of Gyrinops tea

The hedonic assay was conducted as a preference test to measure the evaluation score of Gyrinops tea by the panelist. Thirty panelists with an age range from 20 years old to 50 years old were given their evaluation of Gyrinops tea based on color, aroma, and flavor parameters. Five hedonic scales were used for scoring the evaluation from panelists based on category: 1=dislike very much, 2= dislike moderately, 3= neither like nor dislike, 4=like moderately, 5=like very much (Batubara et al. 2018). The mean score of the hedonic scale from 30 panelists was used as the phytochemical character of *G. versteegii* from different agarwood plantations.

Procedure for obtaining molecular character

G. versteegii DNA extraction

G. versteegii leaves were used as a sample for DNA extraction. According to the manufacturer's recommendations, the Blood Animal Plant DNA Preparation Kit (Jena Bioscience) was used for genomic DNA extraction (Simon-Oke et al. 2018). *G. versteegii* Leaves were grinded using liquid nitrogen into frozen powder. Eighty milligrams of frozen powder samples were transferred to the extraction kit column, containing all necessary materials and reagents for extraction. Proteinase K and RNase were added to the mixture during extraction to degrade all RNA and Protein in the sample.~~Leaves were stored at -70°C overnight as preparation for DNA extraction. Leaves were then grinded using liquid nitrogen into frozen powder. Eighty milligrams of frozen powder samples were transferred into a 1.5 microcentrifuge tube for cell lysis. Addition of 300 µl Lysis buffer and 2 µl RNase subjected to sample followed by homogenizing for 30 seconds. Proteinase K (8 µl) was added to the mixture with incubation at 60°C for 20 minutes followed by centrifugation at 10,000 g for 5 minutes. The supernatant was injected into the activated column followed by centrifugation at 10,000 g for 30 seconds. The columns were washed two times with 500 µl washing buffer. Samples were placed in the elution tube with the addition of 50 µl elution buffer followed by centrifugation at 10,000 g for 2 minutes~~.

Genomic DNA concentration and purity analysis were carried using UV-1601PC Shimadzu by measuring absorbance at wavelength 260 nm, 280 nm, and 230 nm (Lucena-Aguilar et al. 2016). Visualization of isolated DNA was assessed by electrophoresis on a 0.8% agarose gel with ethidium bromide staining. Ladder 1000bp (Invitrogen) was used as a marker for molecular weight estimation of genomic DNA.

RAPD – PCR

Random OPA primers were used for RAPD analysis (Table 1). Those primers were arbitrarily selected from the OPA series commonly used for RAPD. PCR was carried out in total volume of 25 µl containing 12.5 µl 2 x KAPA 2G PCR mix (KAPA Biosystems), 8.5 µl ddH2O, 2 µl of each OPA primer (10 pmol/ µl), and 2 µl *G. versteegii* template DNA (40 ng/ µl). PCR ~~A~~amplification was conducted on ~~L~~labcycler thermocycler with the following profile: initial denaturation at 95ºC for 3 minutes, followed by 40 cycles of denaturation at 95ºC for 15 s, annealing at 37ºC for 1 minute, extension at 72ºC for 2 minutes, and final extension at 72ºC for 5 minutes (Wangiyana et al. 2021c~~1b~~). The amplified DNA fragments were visualized by electrophoresis on 1.2% agarose gels with ethidium bromide staining. Ladder 1000bp (~~I~~invitrogen) was used as a marker for molecular weight estimation of RAPD bands. Each RAPD band on all samples and ~~R~~random OPA primer were considered as molecular characters used for clustering analysis.

Table 1. Random OPA primer sequence for PCR amplification

Random Primer	Sequence
OPA-01	5′- CAGGCCCTTC-3′
OPA-02	5′- TGCCGAGCTG-3′
OPA-04	5′-AATCGGGCTG- 3′
OPA-08	5′- GTGACGTAGG -3′
OPA-09	5′-GGGTAACGCC-3′
OPA-18	5′-AGGTGACCGT-3′

Data analysis

G. versteegii samples from 5 agarwood plantations on Lombok Island were the Organism Taxonomical Unit (OTU) for numeric-phenetic analysis ~~numeric- phenetic analysis's Organism Taxonomical Unit (OTU)~~. Morphology, phytochemical, and molecular character were tabulated as the primary basis data for similarity analysis (presence or absence of several characters). The tabulation data ~~D~~dendrograms of each morphology character, phytochemical character, molecular character, and combination of those three characters were constructed using the MVSP program. UPGMA algorithm was used as clustering method, and Simple Matching Coefficient was used for similarity analysis. Cophenetic – correlation analysis was conducted to observe distortion between sorted similarity matrix and unsorted similarity matrix (Saracli et al. 2013). The significant score of cophenetic - correlation analysis was examined using Co-Stat for ~~the w~~Windows program.

RESULTS AND DISCUSSION

Numeric-phenetic based on morphology characters

Table 2 was the tabulation result of *G. versteegii* morphology character from 5 sampling locations. This table has shown a variation of *G. versteegii* stem, branch, leaves, and fruit. This tabulation data were essential for dendrogram construction.

G. versteegii from 5 agarwood plantations have shown morphology variation mostly on leaves and stems. Leaves were the raw material of Gyrinops agarwood tea products. Variation of *G. versteegii* leaves could support a better understanding of which characteristics of the leaves could produce Gyrinops tea with good quality. Thus, the development of Gy~~i~~rinops tea products could be supported by these data. ~~The s~~Stem was the other essential organ that affected agarwood tea production. There were two groups of *G. versteegii* based on stem characteristics observation: ~~shrub~~ *G. versteegii* shrub group and ~~tree~~ *G. versteegii* tree group. Differentiated *G. versteegii* into shrubs and trees group could be essential for diversity study of this species since agarwood farmers from *Aquilaria malaccensis* plantation applied this differentiation as an essential character for agarwood tea raw material selection. ~~Agarwood farmers from *Aquilaria malaccensis* plantation prefer agarwood shrub group to agarwood tree group. The agarwood shrub group was more accessible to harvest its leaves than the agarwood tree group. Thus, stem character could be an essential for diversity study of *G. versteegii*~~ (Rindyastuti et al. 2019).

Table 2. *G. versteegii* morphology character for similarity analysis

No	Character	Pejaring	Kekait	Lingsar	Mataram	Rarung
1	Shrub up to 6 m	-	+	-	+	-
2	tree up to 21 m	+	-	+	-	+
3	young branchlets bark grayish	+	+	+	+	+
4	young branchlets pubescent	+	+	+	+	+
5	Leaves texture chartaceous	+	+	-	-	+

6	Leaves texture subcoriaceous	-	-	-	+	+
7	Leaves pubescent on beneath	+	+	+	+	+
8	leaves shapes elliptic-oblong	-	-	-	+	+
9	leaves shapes ovate-oblong	+	+	+	-	-
10	leaves surface dark green	+	-	-	-	+
11	leaves surface shining yellow-green	-	+	+	+	-
12	leaves base cuneate	+	+	+	+	+
13	leaves apex narrow-acuminate	+	+	+	+	+
14	leaves width 1 – 2,4 cm	+	+	+	-	-
15	leaves width 2,5 – 5 cm	-	-	-	+	+
16	leaves length 8 cm – 11,4 cm	-	-	-	+	+
17	leaves length 11,5 cm – 15 cm	+	+	-	-	-
18	Obelique-parallel of leaves nerves and veins	+	+	+	+	+
19	Fruit color yellow	+	+	-	-	-
20	Fruit color orange	-	-	-	+	+

Note: + = presence of character, - = absence of character

~~Dendrogram,~~ Figure 2 has shown dendrogram topology based on morphology character. This dendrogram has 3 clusters that were grouped based on their morphological similarity.. ~~based on morphology characters, *G. versteegii* was grouped *G. versteegii* into 3 clusters.~~ The first cluster wereas ~~the group of~~ *G. versteegii* Rarung and *G. versteegii* Rarung, with the highest similarity among other clusters (80%). The second cluster wereas ~~the group of~~ *G. versteegii* Mataram and *G. versteegii* Lingsar with 75% similarity. *G. versteegii* Lingsar was not grouped with other *G. versteegii*. This OTU stands alone as a member of cluster 3.

Intraspecific study of *G. versteegii* from the western part of Lombok Island based on morphology characters resulting dendrogram with cluster similarity value range from 75% - 78% (Mulyaningsih et al. 2017). This value range was observed in this study. Thus, morphology character dendrogram in this study could confirm morphology character dendrogram from Western Lombok. This character could provide intraspecific study data of *G. versteegii* from Western Lombok and other regions of Lombok Island.~~Morphology character dendrogram on this study also could provide intraspecific study data of *G. versteegii* other than Western Lombok~~.

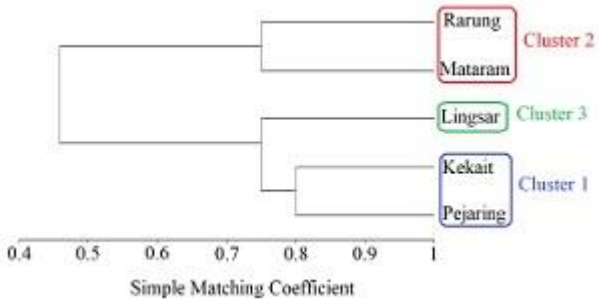

Figure 2. Dendrogram constructed based on morphology character

Numeric – phenetic based on phytochemical character

The phytochemical character of *G. versteegii* is mainly affected by its leaves as the raw material of Gyrinops tea (Wangiyana et al. 2021a). *G. versteegii* from 5 sampling locations have shown variation on leaves characters. Leaves variation has affected characteristics of Gyrinops tea, especially the color and turbidity (Figure 3). Based on this result, the correlation between the variation characteristic of *G. versteegii* leaves and its phytochemical profile variation was revealed. This result could also recommend *G. versteegii* leaves selection to produce Gyrinops tea with the particular phytochemical profile.

Figure 3. Variation characteristic of *G. versteegii* leaves and Gyrinops tea

Variation characteristic of Gyrinops tea from 5 different sampling locations was related to a variation on several phytochemical characters on the leaves of *G. versteegii*. Tannin concentration, IC50 value, and hedonic score were the characters primarily responsible for the phytochemical profile variation of *G. versteegii* (tTable 3). Tannin is the main compound that responsible for the quality of agarwood tea both from Aquilaria (Batubara et al. 2018) and Gyrinops (Wangiyana et al. 20198). The IC50 value is an antioxidant power measurement of agarwood tea that determined its quality as health beneficial herbal tea product (Parwata et al. 2016). The hedonic assay was the standard consumer preference test to determine the quality of agarwood tea products with different processing methods (Batubara et al. 2018). Thus, variation of tannin concentration, IC50 value, and hedonic assay on *G. versteegii* from different sampling locations was essential information for standardization of Gyrinops tea quality product from Lombok Island.

Table 3. *G. versteegii* phytochemical characters for similarity analysis

No	Character	Pejaring	Kekait	Lingsar	Mataram	Rarung
1	oOxidation produce green-reddish chopped dried leaves	+	+	-	-	-
2	oOxidation produce light green chopped dried leaves	-	-	+	+	+
3	bBrewing leaves contain saponin	-	-	-	-	-
4	bBrewing leaves contain flavonoid	+	+	+	+	+
5	bBrewing leaves contain alkaloid	-	-	-	-	-
6	Gyrinops tea product with high turbidity	-	+	-	-	-
7	Gyrinops tea product with medium turbidity	+	-	-	-	+
8	Gyrinops tea product with low turbidity	-	-	+	+	-
9	Precipitate from $FeCl_3$ reagent	+	+	+	+	+
10	Tannin concentration 2,01% - 3,00%	+	-	-	-	-
11	Tannin concentration 3,01% - 4,00%	-	-	+	-	-
12	Tannin concentration 4,01% - 5,00%	-	+	-	-	-
13	IC50 Gyrinops tea product less than 50 µg/ml	-	-	-	-	-
14	IC50 Gyrinops tea product 50 µg/ml - 100 µg/ml	-	-	-	+	-
15	IC50 Gyrinops tea product 101 µg/ml - 150 µg/ml	+	+	+	-	-
16	IC50 Gyrinops tea product 151 µg/ml - 200 µg/ml	-	-	-	-	+
17	Color parameter of hedonic score range 2,00 – 2,99	-	-	-	-	-
18	Color parameter of hedonic score range 3,00 – 3,99	+	+	-	-	-
19	Color parameter of hedonic score range 4,00 – 4,99	-	-	+	-	-
20	Aroma parameter of hedonic score range 2,00 – 2,99	-	-	+	-	-
21	Aroma parameter of hedonic score range 3,00 – 3,99	+	+	-	-	-
22	Aroma parameter of hedonic score range 4,00 – 4,99	-	-	-	-	-
23	Taste parameter of hedonic score range 2,00 – 2,99	-	-	-	-	-
24	Taste parameter of hedonic score range 3,00 – 3,99	+	+	+	-	-
25	Taste parameter of hedonic score range 4,00 – 4,99	-	-	-	-	-

Note: + = presence of character, - = absence of character

Dendrogram constructed based on phytochemical characters resulted in the 3 clusters group (Figure 4). This dendrogram has the same pair member like morphology character dendrogram. However, the similarity of cluster pair members on this dendrogram was slightly higher than the similarity of morphology dendrogram. *G. versteegii* Kekait and *G. versteegii* Pejaring were grouped in cluster 1 with 84% similarity values. *G. versteegii* Rarung and *G. versteegii* Mataram were grouped in cluster 2 with the same similarity value with cluster 1. *G. versteegii* Lingsar stands alone as a cluster 3, just like morphology character dendrogram. However, *G. versteegii* Lingsar join the node with cluster 2 before

join with other clusters. On the morphology character dendrogram, this OTU joints the node with cluster 1 before joining other clusters.

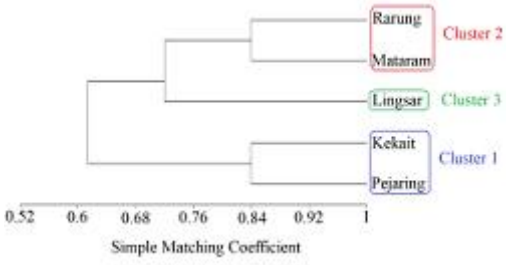

Figure 4. Dendrogram constructed based on phytochemical character

~~Dendrogram constructed based on phytochemical characters resulted in the 3 clusters with the same pair member like morphology character dendrogram. However, the similarity of cluster pair members on this dendrogram was slightly higher than the similarity of morphology dendrogram. *G. versteegii* Kekait and *G. versteegii* Pejaring were grouped in cluster 1 with 84% similarity values. *G. versteegii* Rarung and *G. versteegii* Mataram were grouped in cluster 2 with the same similarity value with cluster 1. *G. versteegii* Lingsar stands alone as a cluster 3, just like morphology character dendrogram. However, *G. versteegii* Lingsar joint the node with cluster 2 before joint with other clusters. On the morphology character dendrogram, this OTU joints the node with cluster 1 before joining other clusters.~~

Numeric phenetic based on molecular character

RAPD - PCR result of *G. versteegii* samples from 5 agarwood plantations has ~~resulted in~~shown various banding patterns from different OPA primers (Figure 5). The bands at a particular position represent RAPD loci which could be classified as monomorphic or polymorphic. A locus is monomorphic if the band is present in all OTU. On the other hand, the polymorphic locus is a band that is absent in at least one OTU (Wangiyana et al. 2021c~~b~~). The number of polymorphic bands determined the random primer's ability to differentiate OTU based on the molecular character.

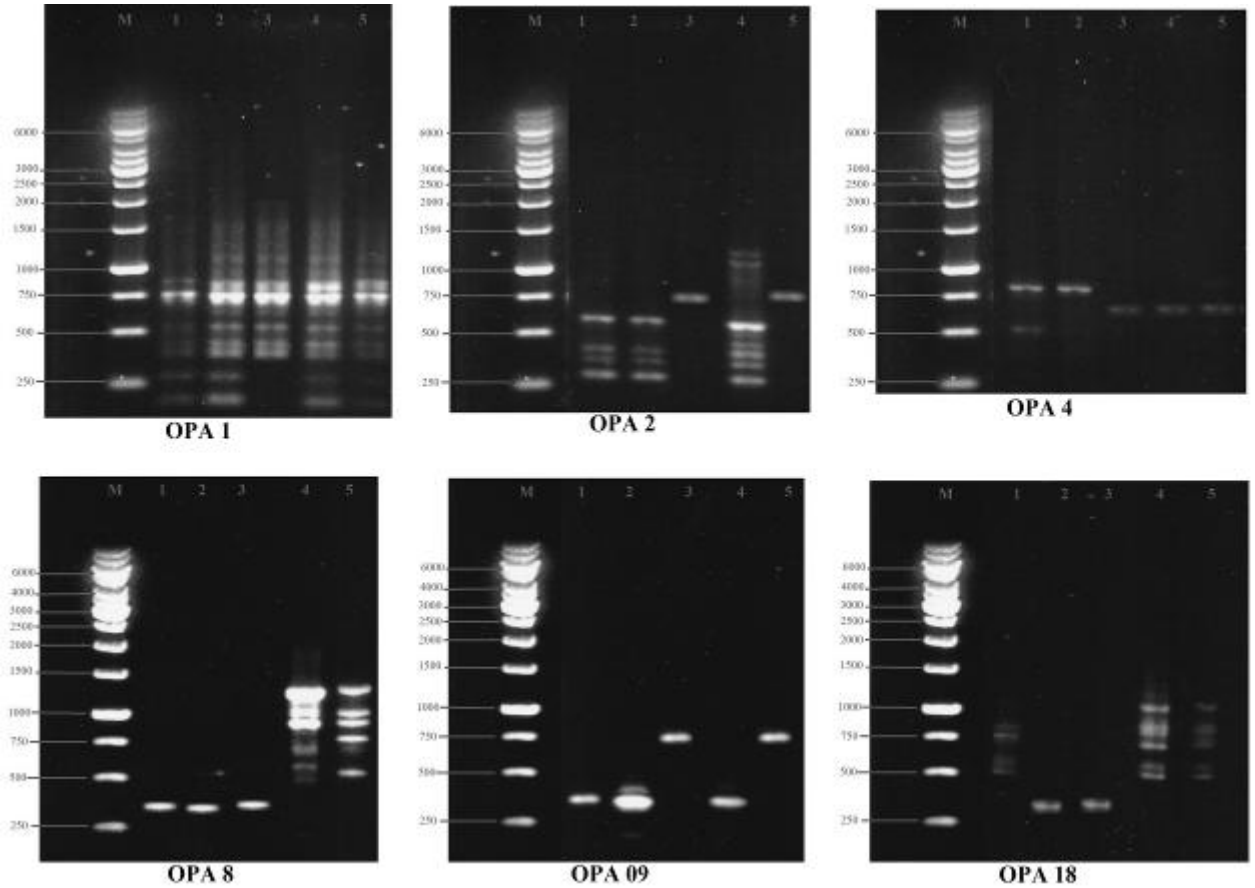

Figure 5. DNA fingerprinting based on RAPD – PCR. (M = marker, 1=Pejaring, 2=Kekait, 3=Lingsar, 4=Mataram, 5=Rarung)

The different number of bands produced by OPA primers could determine their efficiency for genetic variation study on *G. versteegii* (Siburian et al. 2017). OPA 1 primer produces the highest number of the band among other OPA primers. However, most bands on the OPA 1 primer were monomorphic and had no significant impact on OTU differentiation. OPA 4 and OPA 9 were two random primers that produced the least number of bands. OPA 2, OPA 8, and OPA 18 produce several numbers of polymorphic bands that were useful for similarity analysis. OPA 2 produces the highest number of polymorphic bands among other primers, which means that this primer was an ideal primer for the genetic variation study of G. versteegii.

The various banding patterns of OPA primer were tabulated in table 4. These bands were sorted based on their highest to the lowest molecular weight. These bands were treated as characters of similarity analysis just the same as morphology character and phytochemical character. The presence or absence of band on specific molecular weight determines the DNA fingerprinting variation of *G. versteegii* from different sampling locations. This tabulated band character shows the polymorphic band pattern of each OTU more clearly than the electrophoresis result in Figure 5.

Table 4. *G. versteegii* molecular character for similarity analysis

	Character	Pejaring	Kekait	Lingsar	Mataram	Rarung
1	1270 bp band	-	-	-	+	+
2	1150 bp band	-	-	-	+	-
3	1050 bp band	-	-	-	+	-
4	1000 bp band	-	-	-	+	+
5	990 bp band	-	-	-	+	+
6	910 bp band	-	-	-	+	+
7	860 bp band	-	-	-	+	+
8	850 bp band	-	+	-	+	+
9	830 bp band	+	-	-	+	+
10	820 bp band	+	+	-	-	-
11	760 bp band	-	-	+	-	+
12	750 bp band	+	+	+	+	+
13	730 bp band	-	-	-	+	+
14	670 bp band	+	-	-	+	+
15	650 bp band	-	+	-	+	-
16	580 bp band	-	-	-	+	-
17	560 bp band	+	+	-	+	-
18	550 bp band	+	+	+	+	+
19	540 bp band	+	-	-	+	+
20	530 bp band	-	+	-	-	+
21	500 bp band	+	-	-	-	-
22	480 bp band	+	-	-	+	+
23	470 bp band	+	+	+	+	+
24	430 bp band	-	-	-	+	-
25	410 bp band	-	+	-	-	-
26	380 bp band	+	+	-	+	-
27	360 bp band	+	+	-	+	-
28	330 bp band	+	+	-	+	-
29	310 bp band	-	+	-	+	-
30	290 bp band	+	+	-	+	-
31	270 bp band	+	+	-	+	-

Note: + = presence of character, - = absence of character

Figure 6 shown dendrogram based on DNA fingerprinting molecular character. This dendrogram has resulted in the same number of clusters and the same cluster member as morphology character dendrogram and phytochemical character dendrogram. However, the similarity value of cluster member on this dendrogram was lower than the similarity value of cluster member on morphology character dendrogram and phytochemical character dendrogram. Members of cluster 1 were *G. versteegii* Pejaring and *G. versteegii* Kekait with 67.7% similarity value. Members of cluster 2 were *G. versteegii* Rarung and *G. versteegii* Mataram, with a 54.8% similarity value. *G. versteegii* Lingsar joins the node after cluster 1 and forms cluster 3.~~All bands produced by the random primers were tabulated and sorted based on their molecular weight (Ttable 4). These bands were treated as characters of similarity analysis just the same as morphology character and phytochemical character. These bands also represent the DNA fingerprinting of each OTU for dendrogram construction. This tabulated band character shows the polymorphic band pattern of each OTU more clearly than the electrophoresis result in fFigure 5.~~

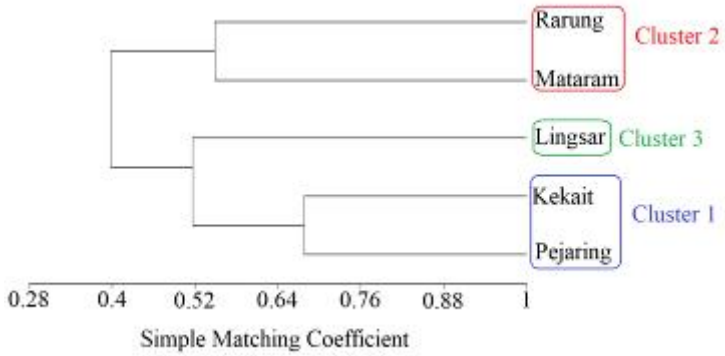

Figure 6. Dendrogram constructed based on molecular character

~~Dendrogram based on DNA fingerprinting molecular character has resulted in the same number of clusters and the same cluster member as morphology character dendrogram and phytochemical character dendrogram. However, the similarity value of cluster member on this dendrogram was lower than the similarity value of cluster member on morphology character dendrogram and phytochemical character dendrogram. Members of cluster 1 were *G. versteegii* Pejaring and *G. versteegii* Kekait with 67.7% similarity value. Members of cluster 2 were *G. versteegii* Rarung and *G. versteegii* Mataram, with a 54.8% similarity value. *G. versteegii* Lingsar joins the node after cluster 1 and forms cluster 3.~~ Clustering analysis that uses RAPD profile as basis data commonly produces low similarity value among OTU. However, the DNA fingerprinting profile of RAPD could reveal variation that could not be observed based on morphological analysis or chemical analysis. RAPD also could provide genetic variation data that could support morphology and chemical variation data for diversity study (Irsyad et al. 2020).

Numeric phenetic analysis based on combination character

The diversity study of *G. versteegii* mostly takes primary data from morphology character, phytochemical character, or molecular character without combining all of those characters. A combination of morphology, phytochemical, and molecular character could provide better comprehensive data for variation analysis of *G. versteegii*. Combining these three characters as basis data also could be a useful to examine how the characters support each other to generate a better clustering analysis method. Dendrogram constructed based on these combination characters is shown in figure 7.

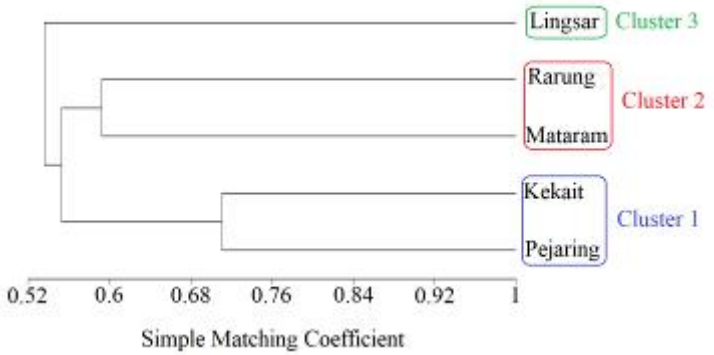

Figure 7. Dendrogram based on combination of morphology, phytochemical, and molecular character

Dendrogram constructed based on combination characters has resulted in the same number of clusters and cluster members with morphology, phytochemical, and molecular dendrogram. However, *G. versteegii* Lingsar was not directly clustered on the node with cluster 1 as it did on morphology character dendrogram and molecular dendrogram. This OTU was not directly clustered on the node with cluster 2 on the phytochemical character dendrogram. This OTU was clustered after cluster 1, and cluster 2 was clustered into a new node. This result confirmed that *G. versteegii* Lingsar has a minor similarity among others *G. versteegii* from different sampling locations. However, this result also implies that *G. versteegii* Lingsar is a unique variant of *G. versteegii* from Lombok Island that needs further exploration about its potency.

Similarity value of morphology, phytochemical, molecular, and combination characters from all *G. versteegii* samples has been subjected to cophenetic-correlation analysis. Cophenetic correlation in table 5 has shown various correlation

values (r), error of r value, and probability value (p) of all characters. Nevertheless, the non-significant correlation value was absent from all characters that have been observed.

Table 5. Cophenetic - correlation analysis of each clustering method

Character	Corr (r)	S.E. of r	P(r=0)	Significant notation
Morphology	0.893	0.159	0.0005	**
Pphytochemical	0.951	0.109	0.00001	**
Mmolecular	0.697	0.254	0.0251	*
Ceombination	0.736	0.239	0.0153	*

Note: * = significant correlation, ** = very significant correlation

Cophenetic - correlation analysis of clustering method using different characters has resulted in a significant correlation on all characters that have been used. The morphology character and phytochemical character even have a very significant correlation with their clustering method. It means that there was no distortion between the unsorted similarity matrix as an input for clustering analysis and the sorted similarity matrix as an output of clustering analysis (Carvalho et al. 2019). Thus, dendrograms that were constructed based on this clustering method have high reliability.

In conclusion, *G. versteegii* from 5 sampling locations of agarwood plantation on Lombok Island have genetic diversity on the DNA fingerprinting as molecular characters. This genetic diversity has been expressed as diversity on morphology character, especially on leaves organ and phytochemical profile. These variations were the main basis data to divided *G. versteegii* samples from Pejaring, Kekait, Lingsar, Mataram, and Rarung into 3 cluster group: cluster 1 (*G. versteegii* Kekait and *G. versteegii* Pejaring), cluster 2 (*G. versteegii* Rarung and *G. versteegii* Mataram), and cluster 3 (*G. versteegii* Lingsar).

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