Biosaintifika 16 (1) (2024): 98-106 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Population Genetic Study of *Gyrinops versteegii* from Two Agarwood Distribution Regions on Lombok Island Based on DNA Fingerprinting

I Gde Adi Suryawan Wangiyana[1*], Nova Kurnia[2], I Gusti Agung Ayu Hari Triandini[3]

[1]Forestry Department Universitas Pendidikan Mandalika. Jl. Pemuda No. 59A, Mataram, Indonesia. Postal code: 83125
[2]Chemistry Education Department Universitas Pendidikan Mandalika. Jl. Pemuda No. 59A, Mataram, Indonesia. Postal code: 83125
[3]Health Faculty Universitas Bhakti Kencana. Jl. Soekarno Hatta No.754, Cipadung Kidul, Bandung, Indonesia. Postal code: 40614

*Coresponding author: gdeadiswangiyana@undikma.ac.id

Submitted: 2024-01-05. Revised: 2024-03-10. Accepted: 2024-04-12

Abstract. *Gyrinops versteegii* tends to grow naturally in the western region of Lombok Island, while cultivated *G. versteegii* tends to spread over the eastern region. These two distribution patterns cause different characteristics of this species that can be excellent sources for genetic population study including genetic diversity and population inbreeding. This research aims to conduct a genetic population analysis of *G. versteegii* from western and eastern agarwood distribution on Lombok Island using the RAPD marker. *G versteegii* samples were taken from west region (8º 31′ 26″ S, 116º 07′ 03″ E) and east region (8º 42′ 28″ S, 116º 27′ 11″ E). RAPD PCR of genomic DNA was conducted using primers: OPA-01, OPA-02, OPA-04, OPA-08, OPA-09, and OPA-18. Genetic population analysis (allele frequencies, heterozygosity, Shannon index, band pattern, and AMOVA) was performed by GenAlEx 6.5. OPA-02 has an ideal discriminative power based on the number of alleles per locus and the Shannon information index. Observed heterozygosity was higher than expected heterozygosity in both the west and east populations of *G. versteegii*. Based on banding pattern analysis, the eastern population has higher genetic diversity than the western population. AMOVA has shown that molecular variation within the population was higher than molecular variation among the population. It could be concluded that the west and east populations of *G. versteegii* have a particular genetic variation that could be discriminated by RAPD primer. Despite the genetic diversity, inbreeding between those two populations has occurred constantly. This result could give new insight into the gene flow between two *G. versteegi* populations, which could support the development of this commodity.

Key words: Population Genetic, *Gyrinops versteegii*, Lombok Island

How to cite : Wangiyana, I. G. A. S., Kurnia, N., & Triandini, I. G. A. A. H. (2024). Population Genetic Study of Gyrinops versteegii from Two Agarwood Distribution Regions on Lombok Island Based on DNA Fingerprinting. *Biosaintifika: Journal of Biology & Biology Education, 16(1),* 98-106.

DOI: http://dx.doi.org/10.15294/biosaintifika.v15i1.3517

INTRODUCTION

Agarwood is a non-timber forest product commodity with a high economic value produced by the thymeleaceae family (Naziz et al., 2019). Indonesia is a country that has a reputation as the world's largest producer of agarwood (López-Sampson & Page, 2018). Two main genera of the thymeleaceae family are distributed in Indonesia: Aquilaria and Gyirnops. Aquilaria tends to spread in the western region of Indonesia, while Gyrinops spreads in the eastern region of Indonesia (Turjaman & Hidayat, 2017).

Lombok Island is one of the areas in Indonesia which is the endemic habitat of the genus Gyrinops, namely the species *Gyrinops versteegii* (Wangiyana & Malik, 2018). This species grows naturally and is cultivated by the people of Lombok, with distribution in almost all island areas (Sutomo & Oktaviani, 2019). With high economic value and being one of the leading export commodities, the use of *G. versteegii* is a solution for the welfare of forest farmers, especially on the island of Lombok. However, the utilization of agarwood commodity must also consider the sustainability issue (Sukenti et al., 2021).

The distribution of *G. versteegii* on the island of Lombok has two different patterns in two different regions. *G. versteegii* tends to grow

naturally in the western region of Lombok Island (Mulyaningsih et al., 2017). Meanwhile, *G. versteegii*, which forest farmers cultivate, tends to spread over the eastern region of Lombok Island. These two different distribution patterns give rise to different characteristics of this species. These differences can be studied through a genetic diversity study approach (Wangiyana et al., 2022).

Genetic diversity is a crucial topic to study to standardize agarwood commodity raw materials. Agarwood commodity standardization in the form of genetic diversity studies is one of the ways to determine a "barcode" on a molecular basis. Many genetic diversity studies have been conducted on agarwood-producing species from the genus Aquilaria (Shiou et al., 2016; Pern et al., 2020). However, the same study on agarwood-producing species from the genus Gyrinops was limited (Lee et al., 2018).

Population genetic analysis is an appropriate method for screening the genetic diversity of agarwood populations in the western and eastern regions of the island of Lombok (Iswantari et al., 2017). The population structure data obtained from the analysis can be used to determine the population for developing agarwood commodities for conservation and investment purposes (Rindyastuti et al., 2019). This analysis also can be useful for plant breeding program development (Hefzi et al., 2023).

Population genetic studies require molecular markers in their analysis. Randomized Amplified Polymorphism DNA (RAPD) marker is commonly used in population genetic analysis (Wang et al., 2016). Studies of the genetic diversity of agarwood producers from the genus Aquilaria have been widely reported using RAPD markers (Fatihah et al., 2020; Qiptiyah et al., 2021;). Meanwhile, studies on the genetic diversity of the agarwood genus Gyrinops, especially the *Gyrinops versteegii* species using RAPD markers, still need to be improved. The study was reported to have been carried out on the *G. versteegii* population in the Papua region (Siburian et al., 2017). Therefore, this marker is also estimated to be suitable for genetic analysis of the population of the agarwood producer *G. versteegii* on the island of Lombok.

This study aimed to determine the genetic structure of *G. versteegii* populations in the western and eastern regions of Lombok Island using RAPD marker-based DNA fingerprint analysis. RAPD analysis of wildtype and cultivated *G. versteegii* populations is expected to give a new insight into population genetic study from this species in Lombok Island. This study will improve the raw material standardization of non-timber forest products from *G. versteegi*. Thus, it will eventually benefit the development of the agarwood commodity.

METHODS

Determination of the *G. versteegii* population sample

G. versteegii population samples were taken from the western and eastern regions of the island of Lombok (figure 1). The population of *G. versteegii* in the western region represented the natural population and was taken around the Kekait Puncang area at coordinates 8° 31′ 26″ S, 116° 07′ 03″ E. The population of *G. versteegii* in the eastern region represented the cultivated population and was taken around the East Pejaring region at coordinates 8° 42′ 28″ S, 116° 27′ 11″ E (Wangiyana et al., 2021a).

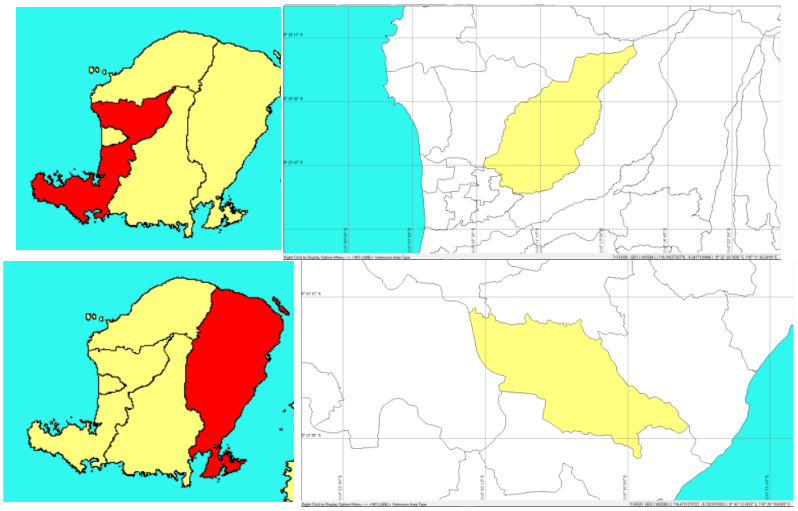

Figure 1. Map of the *G. versteegii* Sampling Locations

Sampling of *G. versteegii* Leaves

Leaves are the central part of the stand of *G. versteegii*, which is used as a sample for DNA isolation. Leaf samples were taken from healthy stands of *G. versteegii* aged 8-10 years. The sampled leaves must be free from attack by pests and diseases, not experiencing chlorosis, and not experiencing necrosis. The leaves underwent surface sterilization before being brought to the laboratory for DNA isolation (Wangiyana et al., 2019). There are 20 samples represent each region.

Leaf Sample Preparation

G. versteegii leaf samples were stored at -70ºC for 12 hours. The leaves are then crushed using a mortar pestle with the help of liquid nitrogen until they become fine particles. As much as 80 mg of fine leaf particles were transferred to a microtube with a volume of 1.5 ml for the cell lysis process.

DNA Extraction

DNA extraction was performed using a Blood Animal Plant DNA Preparation Kit (Jena Bioscience). A 300 µl lysis buffer and 2 µl RNAse were added to the leaf particle sample in a 1.5 ml microtube. Homogenization was carried out for 30 seconds, and then 8 µl of proteinase K was added. Incubation was carried out at 60ºC for 20 minutes, followed by centrifugation at 10,000 g for 5 minutes. The supernatant portion was transferred to the activated column in the kit and then centrifuged at 10,000 g for 30 seconds. The column was washed two times using 500 µl washing buffer. The column was then transferred to the elution tube with the addition of 50 µl of elution buffer followed by centrifugation of 10,000 g for 2 minutes.

Genomic DNA Inspection

The spectrophotometric method measured the isolated genomic DNA's purity and concentration. The absorbance of genomic DNA samples was measured at three different wavelengths: 230 nm, 260 nm, and 280 nm. The purity of genomic DNA was seen from the absorbance ratio of 260/230 and 260/280. Meanwhile, the DNA concentration was determined based on the absorbance value of 260 (Lucena-Aguilar et al., 2016)

Genomic DNA visualization was also performed to complete the DNA inspection data. Genomic DNA samples were electrophoresed on 0.8% agarose (Roche) stained with ethidium bromide and 1x TAE. Ladder 1000 bp (Invitrogen) was used as a marker. The results of the electrophoresis were observed using a UV-transilluminator.

RAPD Primer Selection

The RAPD primer used was the OPA series decamer primer. This primer consists of 10 random nucleotide sequences and was only a single forward primer (5′→3′). The detailed design of the primer sequences is shown in Table 1. The primers in the OPA series, as shown in Table 1, have a reasonably good polymorphism value for the *G. versteegii* agarwood samples (Wangiyana et al., 2021b).

Table 1. Primer OPA for RAPD

RAPD Primer	Squence
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′

RAPD PCR procedure

RAPD PCR of extracted genomic DNA was performed using a ready-to-use PCR reagent (KAPA Biosystem). A total of 25 µl total volumes of the mixture in the PCR tube consisted of 12.5 µl 2 x KAPA 2G PCR mix, 8.5 µl ddH2O, 2 µl OPA series primer (10 pmol/ µl), and 2 µl DNA genome template (40 ng/ µl). The PCR program was carried out in 40 cycles consisting of initial denaturation at 95ºC for 3 minutes, denaturation at 95ºC for 15 seconds, annealing at 37ºC for 1 minute, extension at 72ºC for 2 minutes, and final extension at 72ºC for 5 minutes (Wangiyana et al. al., 2021b).

The PCR results (amplicons) were subjected to electrophoresis on 1.2% agarose (Roche) with ethidium bromide staining in 1x TAE buffer. Ladder 1000 bp (Invitrogen) was used as a marker. Electrophoresis results were visualized with a UV transilluminator. The visualization results were photographed and transferred to a doc gel to estimate the size of the resulting molecular bands. The results of interpolating molecular band sizes in base pair units (BP) are essential data in molecular data analysis.

Data analysis

The bands resulting from the PCR amplification process from the OPA primers are sorted according to their molecular size. The band

data is tabulated in binary using the PFE for Windows program. Code "1' indicates the presence of a band, while code '0" indicates no band. The number of bands produced determines the loci used in the analysis.

Population genetic analysis was performed on band data generated from each OPA primer. The analyses performed included: analysis of allele frequencies, heterozygosity, Shannon Index, band distribution patterns, and Analysis of Molecular Variance (AMOVA). Population genetic analysis was performed using the GenAlEx 6.5 program (Ogban et al., 2019).

RESULTS AND DISCUSSION

Molecular population genetic analysis of agarwood commodity producer species has mainly been carried out in the Aquilaria genus. The study was carried out using various markers, including microsatellites (Nanda et al., 2017; Singh et al., 2015), SSR (Bordoloi et al., 2022; Hishamuddin et al., 2022), and RAPD. Population genetic analysis on agarwood producer *Gyrinops versteegii* still needs to be done. Thus the population genetic analysis data of Aquilaria can be used as the primary reference in discussing the results of population genetic analysis on the species *G. versteegii*.

Table 2. Allele Frequencies and Estimated Diversity Each Primer

Population	Na	Ne	I	Ho	He
OPA 1					
West Region	1.429	1.429	0.297	0.214	0.429
East Region	1.429	1.429	0.297	0.214	0.429
OPA 2					
West Region	0.571	1.000	0.000	0.000	0.000
East Region	2.000	2.000	0.693	0.500	0.990
OPA 4					
West Region	1.500	1.500	0.347	0.250	0.500
East Region	0.000	1.000	0.000	0.000	0.000
OPA 8					
West Region	0.333	1.167	0.116	0.083	0.167
East Region	1.500	1.500	0.347	0.250	0.500
OPA 9					
West Region	1.500	1.500	0.347	0.250	0.500
East Region	1.000	1.500	0.347	0.250	0.500
OPA 18					
West Region	1.143	1.571	0.396	0.286	0.571
East Region	1.000	1.000	0.000	0.000	0.000

Description:
Na : Number of alleles per locus
Ne : Number of effective alleles per locus
I : Shannon's information index
Ho : Observed heterozygosity
He : Expected heterozygosity

The RAPD PCR results for each OPA primer used showed varied results (Table 2). These differences include the mean of alleles per locus (Na), the expected number of alleles per locus (Ne), the value of the Shannon Information index (I), the observed heterozygosity value (Ho), and the expected heterozygosity value (He). All of these data are essential parameters in population genetic analysis, especially in determining the discriminatory ability of the primers used (Saputro et al., 2016).

Allele frequency analysis indicated that the *G. versteegii* population predominantly had an average range of alleles per locus (Na) between 1 - 2 with an average expected value per locus (Ne) of 1.4. This value is still within the range of allele values per locus of *G. versteegii* from Papua, with a Na range of 1.7 – 1.9 and an average Ne value of 1.4 (Siburian et al., 2017). These results are also identical to the analysis of the allele frequency of *Aquilaria microcarpa* from Kalimantan, which

has a Na value range of 1 – 2 and an average Ne value of 1.4 (Qiptiyah et al., 2021). This data proves that genetically the genera Aquilaria and Gyrinops have high similarities and should not be separated into two different genera (Lee et al., 2022).

Each OPA primer can discriminate against *G. versteegii* populations in the western and eastern regions. This data can be seen from the discrimination of allele values per locus in each population, thus indicating that the OPA primer is an ideal RAPD primer for use in population genetic analysis for both Gyrinops (Siburian et al., 2017) and Aquilaria (Fatihah et al., 2020). OPA-2 was the primer with the best discriminating ability among the OPA primers used in this study. This statement is supported by the result that OPA-2 has the most significant Shannon Information Index (I) value compared to other OPA primers. The "I" and the unbiased estimator values are the parameters used in population genetic analysis to determine diversity at various levels, from the gene to the population level (Konopiński, 2020).

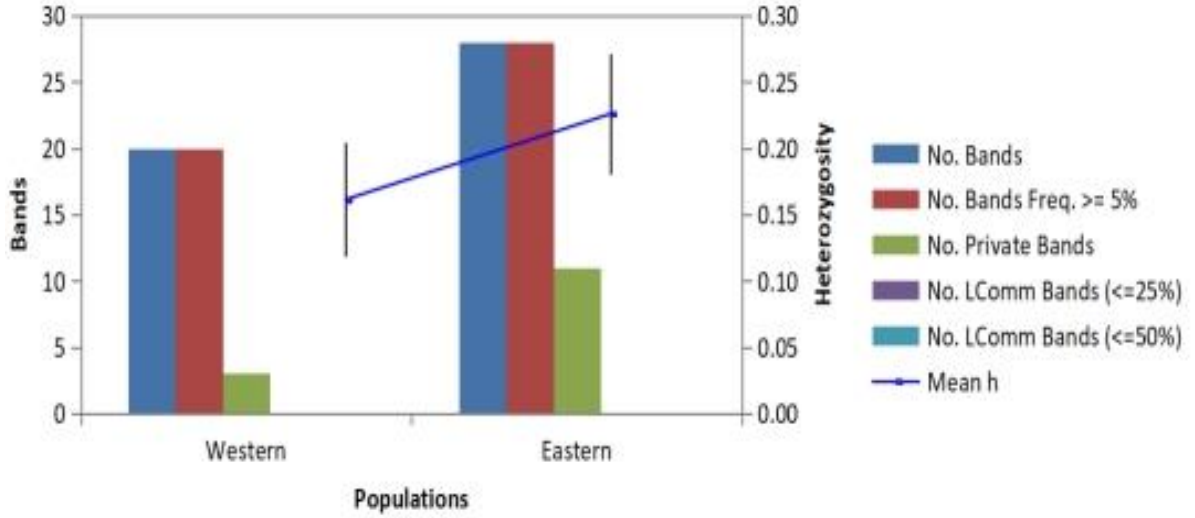

Figure 1. Band Pattern Analysis Using All Primers

The band distribution analysis results showed variations in the number of bands from the western and eastern *G. versteegii* populations (Figure 2). The population of the eastern region has a more significant number of bands than the population of the eastern region both in terms of the total number of bands, the number of frequency bands > 5%, and the number of unique bands (bands that only belong to one population and are not found in other populations). The average value of diversity (h) also shows a tendency towards the population in the eastern region.

The observed heterozygosity value (Ho) is below 0.5, which is lower than the expected heterozygosity (He). The heterozygosity value is essential in assessing a population's genetic diversity (Samuels et al., 2016). As a comparison, the average Ho value of the *Aquilaria sinensis* population is 0.5 and does not deviate much from the He value, which is also 0.5 (Wang et al., 2020). This low heterozygosity value indicates low genetic diversity in the population of *G. versteegii* in the western and eastern regions.

The number of bands (exceptionally polymorphic ones) is the main parameter in fingerprinting analysis using the RAPD marker on *G. versteegii* (Siburian et al., 2017). The greater the number of bands, the higher the number of polymorphic alleles, which correlates with high diversity values (Irsyad et al., 2020). In this case, it can be said that the population of *G. versteegii* in the eastern region has higher diversity than that in the western region. Analysis of the average value of diversity (h) also shows a tendency towards the population in the eastern region. A more excellent h value indicates greater diversity.

Table 3. Analysis of Molecular Variance (AMOVA) of *G. versteegii* Population

Source	Degree of Freedom	Sum Square	Mean Square	Estimated Variance	Variance Percentage
Among Population	1	11.50	11.50	2.75	31
Within Population	2	12.00	6.00	6.00	69
Total	3	23.50		8.75	100

AMOVA compares the two types of variation in the populations being compared. The first variation is the variation within the population which shows the variation between individuals within a population. The second variation is the variation between populations which shows the variation between individuals in different populations. The AMOVA results showed that in the *G. versteegii* case study population, the variation within the population was higher than the variation between populations (table 3).

The AMOVA results showed that the genetic variation within the population was more significant than the variation between populations, indicating continuous in-breeding between the populations of *G. versteegii* in the western and eastern regions. This in-breeding causes gene flow between populations (Oumer, 2020). Gene flow is one of the factors that play an important role in evolutionary support for the survival of plant species. The more significant the gap between the variation within the population and the variation between populations indirectly indicates the rate of gene flow (Ellstrand, 2014).

Based on the comparison of AMOVA in various populations of agarwood producers, the population of *G. versteegii* in the western and eastern regions of Lombok Island has an intermediate gene flow rate (table 4). This data can be seen based on the difference in the variation between populations and the variation within the population. The *A. malaccensis* population from North India had the highest gene flow rate (Singh et al., 2015). Meanwhile, the *A. malaccensis* population from Assam had the lowest gene flow rate (Banu et al., 2015). The *G. versteegii* population from Lombok has a lower gene flow rate than the *G. versteegii* population in Papua (Siburian et al., 2017).

Table 4. Comparison of AMOVA Result Among Several Agarwood Species Population on Various Provenance

Agarwood Species	Provenance	variation		Reference
		Among Population (%)	within Population (%)	
Gyrinops versteegii	Lombok Island	31	69	This research
Gyrinops versteegii	Papua	11	89	(Siburian et al., 2017)
Aquilaria malaccensis	Bangka Belitung	14	86	(Nurtjahjaningsih et al., 2020)
Aquilaria microcarpa	Borneo	36	64	(Qiptiyah et al., 2021)
Aquilaria malaccensis	North India	7	93	(Singh et al., 2015)
Aquilaria malaccensis	Assam	38	62	(Banu et al., 2015)
Aquilaria sinensis	Cina	20	80	(Zou et al., 2012)

This research gives novel information about agarwood species' medium gene flow rate from natural populations and cultivated populations. That information could lead to the cross-inbreeding strategy development of *G. versteegii* on Lombok Island. That development could give better quality of non-timber forest products from this species. The gene flow rate information can also give valuable consideration to agarwood farmers on Lombok Island for breeding strategies of agarwood species from *G. versteegii*.

CONCLUSION

The genetic structure of *the G. versteegii* population in the western and eastern regions of Lombok Island has high genetic diversity, which can be discriminated against using RAPD primers through allele frequency, band distribution, heterozygosity, and AMOVA analysis. Despite genetic variations, the in-breeding process still occurs between populations in the western and eastern regions, characterized by the flow of genes between these populations. It is suggested that a DNA barcoding study based on sequencing is carried out to complete a population genetic study of *G. versteegii*. The barcoding data could be compared with fingerprinting data to generate polyphasic analysis. DNA barcoding can also provide more precise quantitative data to support the standardization of *G. versteegi* products.

ACKNOWLEDGEMENT

Thank you to the Ministry of Education, Culture, Research, and Technology of Indonesia for the research grant fund scheme "Penelitian Terapan Jalur Hilirisasi" with contract number: "184/E5/PG.02.00.PL/2023" for 2023 funding.

REFERENCES

Banu, S., Baruah, D., Bhagwat, R.M., Sarkar, P., Bhowmick, A. & Kadoo, N. Y. (2015). Analysis of genetic variability in *Aquilaria malaccensis* from Bramhaputra valley, Assam, India using ISSR markers. *Flora - Morphology, Distribution, Functional Ecology of Plants*, *217*, 24–32.

Bordoloi, S., Ravi, N., Modi, M.K. & Jayaraj, R. S. C. (2022). Genome-wide mining of simple sequence repeats and development of polymorphic SSR markers *in Aquilaria malaccensis*. *Indian Journal of Genetic Plant Breeding*, *82*(2), 236–239.

Ellstrand, N.C. (2014). Is gene flow the most important evolutionary force in plants. *American Journal of Botany*, *101*(5), 737–753.

Fatihah, H. N. N., Jamali, N. A., Alias, N., Mahmod, N. H., Mahmud, K. & Ali, A. M. (2020). Genetic diversity and differentiation of *Aquilaria malaccensis* Lam. using RAPD markers. *Bioscience Research*, *17*, 117–125.

Hefzi, F., Mansyurdin, M., & Maideliza, T. (2023). Analysis of Genetic Variations of *Ceiba pentandra* (L.) Gaertn. on Several Critical Lands in West Sumatra Using RAPD Molecular Markers. *Biosaintifika: Journal of Biology & Biology Education*, *15*(1), 26 – 35.

Hishamuddin, M. S., Samsuddin, A. S. & Mohamed, R. (2022). Mining and analysis of chloroplast simple sequence repeats (SSRs) from eight species of Aquilaria. *Turkish Journal of Botany*, *46*(1), 26–36.

Irsyad, A. F., Rindyastuti, R., Yulistyarini, T., Darmayanti, A. S. & Daryono, B. S. (2020). Genetic variation of agarwood producing tree (*Gyrinops versteegii*) from Pongkor, Manggarai District, flores Island, Indonesia using ISSR molecular markers. *Biodiversitas*, *21*(2), 485–491.

Iswantari, W., Mulyaningsih, T. & Muspiah, A. (2017). Karyomorphology and chromosome number of four groups of *Gyrinops versteegii* (Gilg.) Domke in Lombok. *Jurnal Ilmu Kehutanan*, *11*, 205–211.

Konopiński, M. K. (2020). Shannon diversity index: A call to replace the original Shannon's formula with unbiased estimator in the population genetics studies. *PeerJ*, *6*, 1–16.

Lee, S. Y., Ng, W. L., Mahat, M. N., Nazre, M. & R. Mohamed. (2016). DNA barcoding of the endangered aquilaria (Thymelaeaceae) and its application in species authentication of Agarwood Products traded in the market. *PLoS ONE*, *11*(4), 1–21.

Lee, S. Y., Turjaman, M. & Mohamed, R. (2018). Phylogenetic relatedness of several agarwood-producing taxa (Thymelaeaceae) from Indonesia. *Tropical life sciences research*, *29*(2), 13 – 28.

Lee, S. Y., Turjaman, M., Chaveerach, A., Subasinghe, S., Fan, Q. & Liao, W. (2022). Phylogenetic relationships of Aquilaria and Gyrinops (Thymelaeaceae) revisited: evidence from complete plastid genomes. *Botanical Journal of the Linnean Society*, *200*(3), 344-359.

López-Sampson, A. & Page, T. (2018). History of Use and Trade of Agarwood. *Economic Botany*, *72*(1), 107–129.

Lucena-Aguilar, G., Sacnhez-Lopez, A. M., Barberan-Aceituno, C., Carrillo-Avila, J. A., Lopez-Guerrero, J. A. & Aguilar-Quesada., R. (2016). DNA source selection for downstream applications based on DNA quality indicators analysis. *Biopreservation and Biobanking*, *14*(4), 264–270.

Mulyaningsih, T., Marsono, D., Sumardi & Yamada, I. (2017). Infraspecific diversity of gaharu (*Gyrinops versteegii* (Gilg.) Domke) in Western Lombok Island). *Jurnal Penelitian Hutan Dan Konservasi Alam*, *14*(1), 57–67.

Nanda, P., Singh, A. K., Negi, M. S. & Tripathi, B. S. (2017). Rapid development and characterization of fifteen novel microsatellite loci in vulnerable tree *Aquilaria malaccensis* using next generation ion Torrent PGMTM sequencing. *Indian Journal of Genetics and Plant Breeding*, *77*(1), 145–152.

Naziz, P. S., Das, R. & Sen, S. (2019). The scent of stress: Evidence from the unique fragrance of agarwood. *Frontiers in Plant Science*, *10*(840), 1–13.

Nurtjahjaningsih, I. L. G., Widyatmoko, A. Y. P. B. C., Haryjanto, L., Yuliah & Hadiyan, Y. (2020). Genetic diversity of *Aquilaria malaccensis* from Western Bangka, Bangka Belitung Province and its implication for manage seed stands. *Jurnal Pemuliaan Tanaman Hutan*, *14*(2), 123–132.

Ogban, F., Udensi, O., Inyang, G., & Osang, F.

(2019). Diversity in Content and Analytical Algorithms of Biologist-Centric Software (s) for DNA and Sequence Data: Mega, DNASp, GenAlex and ARLEQUIN. *International Journal of Natural and Applied Sciences*, 12, 47-60.

Oumer, O. A., Dagne, K., Feyissa, T., Tesfaye, K., Durai, J., & Hyder, M. Z. (2020). Genetic diversity, population structure, and gene flow analysis of lowland bamboo [*Oxytenanthera abyssinica* (A. Rich.) Munro] in Ethiopia. *Ecology and Evolution*, 10(20), 11217-11236.

Pern, Y.C., Lee, S.Y., Kamarudin, N. & Mohamed, R. (2020). Genetic variation and DNA barcoding of the endangered agarwood-producing *Aquilaria beccariana* (Thymelaeaceae) populations from the Malesia Region. *Forestist*, *70*(2), 85–94.

Qiptiyah, M., Widyatmoko, C., A. Y. P. B. C, Nurtjahjaningsih, I. L. G. & Prihatini, I. (2021). Genetic diversity of *Aquilaria microcarpa* Baill. In Kalimantan using RAPD Markers. *IOP Conference Series: Earth and Environmental Science*, *914*(012039),1–6.

Rindyastuti, R., Yulistyarini, T. & Darmayanti, A. S. R. I. (2019). Population and ecological study of agarwood producing tree (*Gyrinops versteegii*) in Manggarai District, Flores Island, Indonesia. *Biodiversitas*, *20*(4), 1180–1191.

Turjaman, M. & Hidayat, A. (2017). Agarwood-planted tree inventory in Indonesia. In: *IOP Conference Series: Earth and Environmental Science*. Bogor, 25–26 October 2016. Bristol: IOP Publishing. Page 1-6.

Samuels, D. C., Wang, J., Ye, F., He, J., Levinson, R.T., Sheng, Q., Zhao, S., Capra, J. A., Shyr, Y., Zheng, W. & Guo, Y. (2016). Heterozygosity ratio, a robust global genomic measure of autozygosity and its association with height and disease risk. *Genetics*, *204*(3), 893–904.

Saputro, T. B., Finariyah, F., Dianawati, S., Sholihah, N. F., & Ermavitalini, D. (2016). In vitro selection of local maize (*Zea mays*) on NaCl stress and its genetic characterization using RAPD. *Biosaintifika: Journal of Biology & Biology Education*, 8(3), 344-351.

Siburian, R. H. S., Siregar, U. J. & Siregar, I. Z. (2017). Genetic variation of *Gyrinops verstegii* originated from papua based on RAPD. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, *19*(3), 1–5.

Singh, P., Nag, A., Parmar, R., Gosh, S., Bhau, B. S. & Sharma, R. K. (2015). Genetic diversity and population structure of endangered *Aquilaria malaccensis* revealed potential for future conservation. *Journal of Genetics*, *94*, 697–704.

Singh, P., Sharma, H., Nag, A., Bhau, B.S. & Sharma, R. K. (2015). Development and characterization of polymorphic microsatellites markers in endangered *Aquilaria malaccensis*. *Conservation Genetics Resources*, ***7***, 61–63.

Sukenti, K., Rohyani, I. S., Mulyaningsih, T., Hadi, S., Ito, M., & Yamada, I. (2021). Ethnobotanical study of *Gyrinops versteegii* (Gilg.) Domke from Lombok Island, West Nusa Tenggara, Indonesia as an effort in supporting the conservation of agarwood-producing species. *Indian Journal of Natural Products and Resources*, 12(2), 307-315.

Sutomo, S. & Oktaviani, G. A. (2019). Exploration of agarwood producer (*Gyrinops versteegii*) in Lombok Island West Nusa Tenggara. *ULIN: Jurnal Hutan Tropis*, **3**(2), 64–69.

Wang, Z. F., Cao, H. L., Cai, C. X. & Wang, Z. M. (2020). Using genetic markers to identify the origin of illegally traded agarwood-producing *Aquilaria sinensis* trees. *Global Ecology and Conservation*, *22*, 1–12.

Wang, S. J., Chen, X. L., Han, F. B., Li, R. S., Li, G., Zhao, Y., & Zhang, L. X. (2016). Genetic diversity and population structure of ginseng in China based on RAPD analysis. *Open Life Sciences*, 11(1), 387-390.

Wangiyana, I G. A. S., Supriadi, Nikmatullah, A., Sunarpi, Putri, D. S. & Rosidah, S. (2021a). Phytochemical screening and antioxidant activity of Gyrinops tea from agarwood plantation on Lombok island, Indonesia. IOP Conference Series: Earth and Environmental Science, *712*, 012029, Lombok, Indonesia. DOI 10.1088/1755-1315/712/1/012029

Wangiyana, I. G. A. S., Nugraheni, Y. M. M. A., Anggadhania, L. & Soetarto, E. S. (2021b). Morphological and DNA Polymorphism Analyses of *Fusarium solani* Isolated from *Gyrinops versteegii* in the West Nusa Tenggara Forest. *ASM Science Journal*, *14*(2), 65–74.

Wangiyana, I. G. A. S., Supriadi, Nikmatullah, A., Sunarpi & Triandini, I. G. A. A. H. (2022). Diversity of *Gyrinops versteegii* from several agarwood plantation on Lombok Island (Indonesia) as raw material of Gyrinops tea. *Biodiversitas*, *23*(1), 178–186.

Wangiyana, I. G. A. S., Sawaludin, Nizar, W. Y. & Wangiyana, W. (2019). Tannin concentrations of Gyrinops tea with different leaf processing methods and addition of herbal medicine ingredients. AIP Conference Proceedings, *2199*, 070012, Lombok, Indonesia. Doi: https://doi.org/10.1063/1.5141326

Wangiyana, I. G. A. S., & Malik, S. (2018). Application of arbuscular mycorrhiza from Senaru Forest rhizosphere for *Gyrinops versteegii* germination and growth. *Biosaintifika: Journal of Biology & Biology Education*, *10*(2), 432-438.

Zou, M., Xia, Z., Lu, C., Wang, H., Ji, J. & Wang, W. (2012). Genetic Diversity and Differentiation of *Aquilaria sinensis* (Lour.) Gilg Revealed by ISSR and SRAP Markers. *Crop Science*, *52*(5), 2304–2313.