CROSS-AMPLIFICATION OF *AQUILARIA CRASSNA* MICROSATELLITE DNA MARKERS IN TWO OTHER CLOSELY RELATED AGARWOOD SPECIES (*A. MALACCENSIS* AND *A. MICROCARPA*)

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CROSS-AMPLIFICATION OF *AQUILARIA CRASSNA* MICROSATELLITE DNA MARKERS IN TWO OTHER CLOSELY RELATED AGARWOOD SPECIES (*A. MALACCENSIS* AND *A. MICROCARPA*). Agarwood is a distinctive wood resin product extracted from the important genus of *Aquilaria*, but the population of agarwood-producing trees from natural forests in Indonesia is threatened due to over-exploitation, leading to an urgent call for conservation and sustainable uses. Molecular techniques such as DNA profiling have been used to ensure the legality, conservation, and sustainability of species from this genus. In this study, cross-species amplification of microsatellite markers initially developed for *Aquilaria crassna* was developed on two other closely related agarwood species (*Aquilaria malaccensis* and *A. microcarpa*), and their genetic variation was evaluated. The four loci (6pa18, 10pa17, 16pa17, and 71pa17) were used to amplify leaf genomic DNA from 55 trees across three *Aquilaria* species. The results showed that the four loci could successfully be amplified in *A. malaccensis*, *A. microcarpa*, and *A. crassna*. In addition, *A. crassna* exhibited higher genetic variation (N_a=2.75, N_e=2.35, H_e= 0.5672, and F=-0.727) than *A. malaccensis* (N_a=2.75, N_e=2.19, H_e=0.5424, and F=-0.598) and *A. microcarpa* (N_a=2.50, N_e=2.11, H_e= 0.5234, and F=-0.734) indicated the transferability of microsatellite markers in closely related agarwood species, possibly due to the flanking region in these four microsatellite regions being well-conserved in several agarwood species. These findings indicated that the markers tested here can be considered an effective tool for future studies in population and conservation genetics to support the management of agarwood genetic resources and track its supply chain to prevent overexploitation.

Keywords: Agarwood, *Aquilaria*, cross-amplification, microsatellite

AMPLIFIKASI SILANG PENANDA DNA MIKROSATELIT AQUILARIA CRASSNA *PADA DUA SPESIES KERABAT GAHARU LAINNYA (*A. MALACCENSIS *DAN* A. MICROCARPA*). Gaharu merupakan produk resin kayu yang khas yang diekstraksi dari genus penting* Aquilaria*, tetapi populasi pohon penghasil gaharu dari hutan alam Indonesia terancam punah karena eksploitasi berlebihan yang memerlukan upaya konservasi dan pemanfaatan berkelanjutan. Teknik molekuler seperti pembuatan profil DNA telah digunakan untuk memastikan legalitas, konservasi, dan keberlanjutan spesies dari genus ini. Dalam penelitian ini, amplifikasi lintas spesies penanda DNA mikrosatelit yang awalnya dikembangkan untuk* Aquilaria crassna*, dikembangkan pada dua spesies gaharu lain yang berkerabat dekat (*Aquilaria malaccensis *dan* A. microcarpa*) dan keragaman genetika mereka dievaluasi. Empat lokus (6pa18, 10pa17, 16pa17, dan 71pa17) digunakan untuk mengamplifikasi genomik DNA daun dari 55 pohon pada tiga spesies* Aquilaria*. Hasil penelitian menunjukkan bahwa keempat lokus tersebut berhasil diamplifikasi pada* A. malaccensis*,* A. microcarpa*, dan* A. crassna*. Selain itu,* A. crassna *menunjukkan variasi genetik yang lebih tinggi*

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(N_a=2.75, Ne=2.35, H_e= 0.5672, dan F=-0.727) dibandingkan A. malaccensis *(N_a=2.75, N_e=2.19, H_e= 0.5424, dan F=-0.598) dan* A. microcarpa *(N_a=2.50, N_e=2.11, H_e= 0.5234, dan F=-0.734) yang mengindikasikan adanya perpindahan penanda mikrosatelit pada spesies gaharu yang berkerabat dekat, kemungkinan karena flanking region pada keempat daerah mikrosatelit tersebut terpelihara dengan baik pada beberapa spesies gaharu. Temuan ini menunjukkan bahwa rangkaian penanda yang diuji di sini dapat dianggap sebagai alat yang efektif untuk studi genetika populasi dan konservasi di masa mendatang guna mendukung pengelolaan sumber daya gaharu dan pelacakan rantai pasokannya untuk mencegah eksploitasi berlebihan.*

Kata kunci: Amplifikasi silang, Aquilaria*, gaharu, microsatellite*

I. INTRODUCTION

Agarwood resin (gaharu) from Indonesia, which is harvested from the trunk or branches of *Aquilaria* and *Gyrinops* species (Thymelaeaceae), is known to be used for the highly valued incense production (Pern et al., 2020; Putri et al., 2017; Siburian et al., 2019). Due to overexploitation driven by increasing market demand, the natural population of agarwood is threatened with extinction (Destri et al., 2020; Lian et al., 2016; Singh et al., 2015; Soehartono & Newton, 2000). Therefore, sustainable harvesting of agarwood from the natural populations is controlled through production quotas, as listed in Appendix II of the Convention on International Trade in Endangered Species (CITES) (CITES, 2024; Ministry of Environment and Forestry & National Research and Innovation Agency, 2024).

Despite the vital role of natural populations in supplying agarwood, efforts to increase agarwood production through plantations are implemented in various ways. Therefore, several agarwood plantations have been established since the 1980s, including in Indonesia (Abdulah et al., 2022; Eurlings et al., 2010). In particular, small-scale plantations have been established sporadically, where the species grown are predominantly *Aquilaria malaccensis* and *A. microcarpa*. However, the establishment of plantations and seedling translocations raises concerns about maintaining the genetic integrity of wild populations. Significant genetic differentiation among wild *Aquilaria malaccensis* populations was observed (Rachmat et al., 2024), highlighting their evolutionary and geographical isolation and emphasizing the need to regulate seedling translocations across regions. Without such regulation, mixing genetic material between plantation and wild sources may threaten natural populations and complicate agarwood product traceability. As a consequence, current agarwood products in the market may consist of mixed wood from plantations and natural sources, where discrimination of agarwood from these two sources is still difficult to resolve (Eurlings et al., 2010; Li et al., 2016; Wu et al., 2020). Therefore, investigating agarwood genetic variation patterns in both plantations and natural populations is necessary to provide helpful information, particularly on the origin of agarwood. This can be enhanced through the use of molecular marker techniques, which have significantly advanced the study of forest tree species over the past decade (Nurtjahjaningsih et al., 2020; Spooner et al., 2005; Wang & Szmidt, 2001). With the latest developments in molecular biology, researchers now have many options for conducting such studies.

Highly polymorphic microsatellites are excellent DNA markers suitable for studies investigating genetic variation, ranging from the population level to the individual level (Lee et al., 2004; Mottura et al., 2005; Wang et al., 2018). Microsatellites, also referred to as simple sequence repeats (SSRs), serve as valuable molecular markers utilized in population genetic studies of tree species, particularly those

found in tropical forests (Dwiyanti et al., 2014a; Dwiyanti et al., 2015; Lee et al., 2004; Mottura et al., 2005; Ouinsavi et al., 2006). However, developing these markers can be both costly and time-consuming. Utilizing microsatellites from related species can help reduce these costs, ultimately saving both time and money (Barbará et al., 2007; López-Vinyallonga, 2011). This approach would also encourage further studies to adopt similar methods. Microsatellites have been identified in various organisms and are generally dispersed throughout their genomes (Deng et al., 2016; Hancock, 1999; Koubínová & Grant, 2024; Xiong et al., 2012).

The pioneering development of microsatellite markers in the genus *Aquilaria* represents a significant advancement in the creation of a DNA fingerprint database for *Aquilaria crassna*. This database is essential for accurately tracing the geographic origins of traded wood and incense samples, with critical forensic applications in Thailand and Vietnam (Eurlings et al., 2010). This study tested for cross-species amplification in *Aquilaria sinensis* and *A. rugosa* found in Vietnam and China (Eurlings et al., 2010). Following this, a second set of markers was developed for *A. malaccensis* from Malaysia (Tnah et al., 2012) and *A. malaccensis* from India (Singh et al., 2015). The last set was developed for *A. sinensis*, which occurred in China, and these markers were tested for cross-species amplification in *A. yunnanensis* and *A. crassna*, which also occurred in China (Wang et al., 2018). These three sets of markers were further tested for cross-species amplification in ten other *Aquilaria* species in Malaysia, including *A. beccariana*, *A. hirta*, *A. microcarpa*, *A. rostrata*, *A. rugosa*, *A. subintegra*, and *A. yunnanensis* (Pern et al., 2020). The study revealed 13 cross-amplifiable markers, of which only one was polymorphic across all species. Given the findings of previous studies, it is crucial to test the cross-species amplification of microsatellite markers developed for *Aquilaria* species on other *Aquilaria* species in Indonesia. This is particularly important due to the potential genetic uniqueness of the Indonesian population compared to those in Malaysia, Vietnam, Thailand, India, and China. Additionally, there is a notable lack of reports on studies regarding the cross-species amplification of microsatellite markers in *Aquilaria* species within Indonesia. As a preliminary study step, the present study evaluated the cross-species amplification of microsatellite markers originally developed for *A. crassna* on two native and endangered *Aquilaria* species in Indonesia: *A. malaccensis* and *A. microcarpa* (Nurtjahjaningsih et al., 2020; Qiptiyah et al., 2021). Furthermore, this study aimed to investigate the effectiveness of these *Aquilaria crassna* microsatellite DNA markers in two closely related agarwood species, *A. malaccensis* and *A. microcarpa*, from the plantation populations in West Java, Indonesia, and to examine the genetic variation among the three studied *Aquilaria* spp. Identifying suitable microsatellite markers can enhance future genetic studies on these species, particularly in analyzing diversity and population structure. In wild populations, it can aid in developing genetic barcodes to determine the accurate identification of species names and to develop genetic fingerprints to identify the population origins. In contrast, in planted populations or products, it can be utilized to trace their genetic lineage. The findings of this study can support the Indonesian government and law enforcement agencies to take decisive action in managing agarwood resources, monitoring the supply chain to prevent overexploitation and illegal logging, and formulating strategies to conserve agarwood-producing species.

II. MATERIALS AND METHODS

A. Study Site

Leaves from a total number of 55 individual trees representing three species, i.e., *Aquilaria crassna* (n=15), *A. malaccensis* (n=32), and *A. microcarpa* (n=8), which grow in the plantation areas in West Java Province, Indonesia, and also in natural forest in Riau Province, Indonesia (population of *A. malaccensis* only) were collected for DNA analysis (Table 1). The

leaf samples were then preserved in a plastic zip containing silica gel (1:5 v/v) in the field for further DNA extraction at the Laboratory of Forest Genetics and Molecular Forestry, Department of Silviculture, Faculty of Forestry and Environment of IPB University in Bogor Regency, West Java Province, Indonesia.

B. Microsatellites Analysis

DNA extraction of dried leaves from 55 trees consisting of three *Aquilaria* spp. (i.e. *Aquilaria crassna*, *A. malaccensis*, and *A. microcarpa*) (Table 1) was carried out using the CTAB (Cetyltrimethylammonium bromide) protocol with modifications (Doyle & Doyle, 1990). All four pairs of microsatellite DNA markers or simple sequence repeats (SSRs) developed for *A. crassna* (Table 2) (Eurlings et al., 2010; Siburian et al., 2019) were amplified in *A. crassna*, *A. malaccensis*, and *A. microcarpa* samples in this study. Polymerase Chain Reactions (PCR) were carried out in a final volume of 15 µl for one reaction, containing approximately 20 ng of genomic DNA (2 µl), 7.5 µl Go Taq Green Master Mix Kit (Promega), 1.5 µl forward primer, 1.5 µl reverse primer, and 2.5 µl H2O. Amplification was initially conducted by modifying the PCR protocol of Eurlings et al. (2010) to determine the optimal PCR profile for the three *Aquilaria* species studied. The thermal cycling profile used in the present study consisted of 95°C for 2 min for initial

Table 1. Sampling location of *Aquilaria crassna, A. malaccensis*, and *A. microcarpa* in Indonesia

No.	Species name	Study site	Number of sample (n)
1	*Aquilaria crassna*	Biotrop, Bogor, West Java	4
		Greg Hambali Garden, Bogor, West Java	2
		Gunung Walat University Forest, Sukabumi, West Java	9
		Total	**15**
2	*Aquilaria malaccensis*	Biotrop, Bogor, West Java	4
		Greg Hambali Garden, Bogor, West Java	2
		Bogor Botanical Garden, Bogor, West Java	4
		Gunung Walat University Forest, Sukabumi, West Java	2
		Muara Fajar, Rumbai Barat, Riau	20
		Total	**32**
3	*Aquilaria microcarpa*	Greg Hambali Garden, Bogor, West Java	2
		Gunung Walat University Forest, Sukabumi, West Java	5
		Bogor Botanical Garden, Bogor, West Java	1
		Total	**8**
		Grand Total	**55**

Table 2. Characteristics of four polymorphic nuclear microsatellites developed for *Aquilaria crassna* by Eurlings et al. (2010)

Locus	Primer sequences (5'–3')	Repeat	Size range (bp)	Number of alleles	Ta (°C)
6pa18	F: TGAGGCGTGAGTGAGATATTGATT R: CCTTCCTCTCTTCTTACCTCACCA	(CA)8	180–210	7	50
10pa17	F: ACACACTGTTATGGTCTACAGCTT R: CGCCATCTCATAATATTCTAATGTA	(CA)12	152–156	3	50
16pa17	F: AGTGAACAACTTGACTAGGCTTG R: GCTGAACACAACAAGATATCACC	(CA)19	143–155	6	59
71pa17	F: AGCAAACAGTGGGATAAGGTC R: AGAAAGGAGGCGAAACGAAT	(CA)15	152–224	15	54

Note: F = forward, R = reverse, Ta = annealing temperature.

denaturation, followed by 35 cycles of 95°C for 1 min for denaturation, 49-54 oC for 2 min for annealing, 72°C for 2 min for extension, and 72°C for 10 min for final extension. To ensure the reliability of the results, one individual from each species was tested on four markers, and the amplification process was repeated twice, following the methodology outlined by Pern et al. (2020). Afterward, amplification testing of each marker on 55 samples was performed once. All PCR reactions were performed in the Peltier Thermal Cycler (PTC-100, MJ Research). PCR products were electrophoresed in 2% agarose gel in 1x TAE buffer and stained with ethidium bromide for visualization on a UV transilluminator. Genotyping was carried out on a 30% polyacrylamide gel. Product sizes were scored against a 100 bp DNA ladder (Vivantis).

C. Data Analysis

Successful PCR amplification was validated by the presence of a single DNA band within the expected size range of the amplification products. A DNA fragment is considered to be outside the predicted size range if it deviates by 100 bp (base pair) larger or smaller than the original sequence (Arnold et al., 2002). The sizes of DNA fragments were assessed visually by comparing them to a standard DNA molecular weight marker and scoring them accordingly. If multiple bands appeared near the expected size range, they were categorized as stuttering bands and excluded from the study. The DNA electropherogram was then analyzed to determine the putative genotype. General estimation of genetic variation of each species, including the number of alleles per locus (Na), observed heterozygosity (HO), expected heterozygosity (He), and the fixation index (F), was calculated using GenAlEx software version 6.503 (Peakall & Smouse, 2012). Furthermore, deviations from the Hardy-Weinberg equilibrium were assessed for each locus in every population using GENEPOP 4.7.5 (Raymond & Rousset, 1995; Rousset, 2008).

III. RESULTS AND DISCUSSION

A. Microsatellite Marker Amplification of *Aquilaria crassna* in *A. malaccensis* and *A. microcarpa*

In this study, four nuclear microsatellite markers developed for *A. crassna* species in Thailand, namely 6pa18, 10pa17, 16pa17, and 71pa17 (Eurlings et al., 2010), were able to successfully amplify the three Indonesian agarwood species, namely *A. crassna*, *A. malaccensis*, and *A. microcarpa*. The success of this cross-amplification was indicated by the appearance of the four microsatellite bands between 100 bp and 200 bp in each *Aquilaria* species, specifically, i.e., 143-155 bp for locus 16pa17, 152-156 bp for locus 10pa17, 152-224 bp for locus 71pa17, and 180-210 bp for locus 6pa18 (Figure 1).

Four microsatellite loci (100%) produced amplicons of the expected size. The total number of alleles for *A. malaccensis* and *A. microcarpa* species ranged from two to three, i.e., two alleles at locus 6pa18 and three alleles at loci 10pa17, 16pa17, and 71pa17 (Table 3). Four alleles (143 bp, 145 bp, 153 bp, and 186 bp) were identified in the *A. crassna* study by Eurlings et al. (2010) and were also observed in all species tested in this study. Additionally, two previously unidentified alleles were identified in the *A. crassna* study (Eurlings et al., 2010). However, they were identified in this study, i.e., allele 158 bp found in *A. microcarpa* samples and allele 164 bp in *A. malaccensis* and *A. crassna* samples (Table 4). These findings indicated that the markers are polymorphic across various agarwood species, which may demonstrate the presence of genetic variation within these species. This variation is essential for understanding evolutionary processes and population dynamics.

In contrast to this study, which demonstrated the success of all four markers on three Indonesian *Aquilaria* species, a previous study by Pern et al. (2020) found that locus 10pa17 was ineffective in amplifying samples from *A. malaccensis* and *A. microcarpa* collected in

Malaysia. The locus 10pa17 also failed to amplify other Malaysian *Aquilaria* species, including *A. beccariana, A. hirta*, *A. rugosa*, and *A. yunnanensis* (Pern et al., 2020), as indicated by multiple bands resulting from non-specific amplification.

The success of amplification in the present study may be attributed to the suitability, efficiency, and optimization of the PCR process. Unspecific primers can amplify other regions in the genome that are not targeted or even absent in the amplified regions. PCR

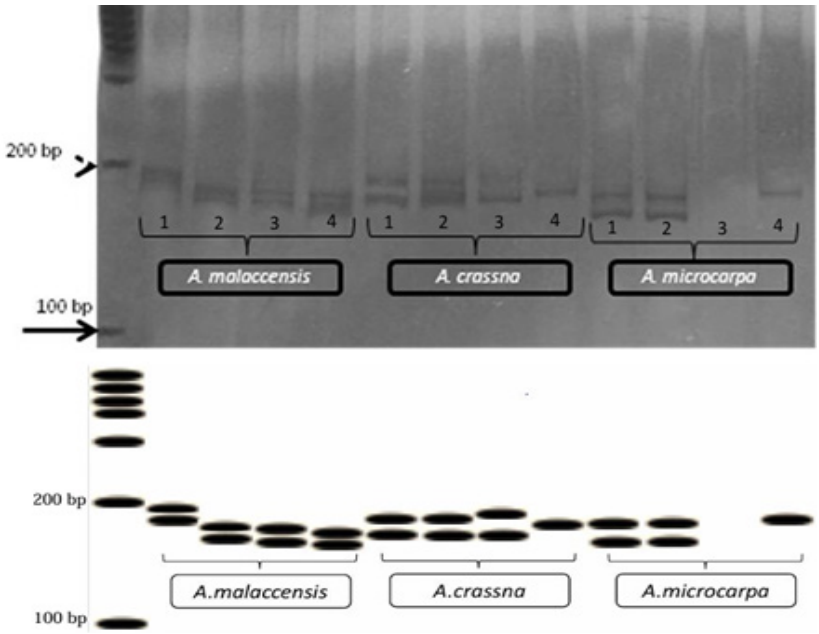

Figure 1. Polyacrylamide gel electrophoresis bands representing four microsatellite markers used in this study on *A. malaccensis*, *A. crassna*, and *A. microcarpa* species. Locus 6pa18 (1), locus 10pa17 (2), locus 16pa17 (3), and locus 71pa17 (4).

Table 3. Results of microsatellite marker amplification of *Aquilaria crassna* in *A. malaccensis* and *A. microcarpa*

Locus	Polymorphism and detected number of alleles	
	Aquilaria microcarpa	***Aquilaria malaccensis***
6pa18	++ (2)	++ (2)
10pa17	+++(3)	+++(3)
16pa17	+++(3)	+++(3)
71pa17	+++(3)	+++(3)

Note: (++) = polymorphic locus with two alleles; (+++) = polymorphic locus with three alleles

Table 4. Allelic variation in four microsatellite markers in three *Aquilaria* species

No.	Species	Allele (bp)																
		143	145	147	151	152	153	154	158	164	180	186	194	196	206	210	216	224
1	*Aquilaria malaccensis*	✓	✓	✓	-	✓	✓	✓	-	Φ	-	✓	-	✓	-	-	-	-
2	*Aqularia microcarpa*	✓	✓	✓	-	✓	✓	✓	Φ	-	-	✓	-	-	-	✓	-	-
3	*Aquilaria crassna*	✓	✓	-	-	-	✓	-	-	Φ	✓	✓	-	✓	-	-	✓	✓

Note: ✓= The allele was identified in the *A. crassna* study (Eurlings et al., 2009) and also in this current study; - = This allele was detected in a study of *A.crassna* (Eurlings et al., 2009) but was not detected in the current study; Φ= This allele was not detected in the *A.crassna* study (Eurlings et al., 2009) but was detected in the current study. Alleles 155-156, 168-178, and 198-202 were detected in a study of *A.crassna* (Eurlings et al., 2009) but were not detected in the current study.

optimization is also needed to produce the desired yield. This optimization pertains to the temperature of DNA denaturation and annealing during the PCR process. If the denaturation temperature is set too low or the denaturation time is insufficient, it can lead to imperfect denaturation processes, such as the failure to separate double-stranded DNA into single-stranded DNA, and prevent new DNA polymerization from occurring. While too high a denaturation temperature or too long a denaturation time may cause the DNA template to be degraded, or may generate no identifiable product (Gustafson et al., 1993; Takara Bio, 2024). In addition, selecting an annealing temperature that is too high can prevent any amplification products from forming, while a temperature that is too low may unintentionally amplify non-specific DNA fragments. This unwanted amplification can create the appearance of multiple bands on the agarose gel, which distracts from the intended results and complicates data interpretation (Borah, 2011; Rychlik et al., 1990; Yunita et al., 2023).

B. Genetic Variation of Three *Aquilaria* spp.

Four microsatellite markers that were successfully amplified were then evaluated for their ability to determine the genetic variation of the three agarwood species. The results showed that genetic variation of *A. crassna* had a slightly higher genetic variation (N_a=2.75, N_e=2.35, and H_e= 0.5672) than *A. malaccensis* (N_a=2.75, N_e=2.19, and H_e= 0.5424) and *A. microcarpa* (N_a=2.50, N_e=2.11, and H_e= 0.5234) (Table 5). The fixation index (F) value was notably negative, indicating an excess of heterozygotes in all species (Dwiyanti et al., 2014b). However, this value was not significantly different from zero in *A. microcarpa* (Table 5). This result pattern indicated the transferability of microsatellite markers in closely related agarwood species, possibly due to the flanking region in these four microsatellite regions being well-conserved in several agarwood species (Dwiyanti et al., 2014a; Ng et al., 2004).

The genetic variation indicated by the expected heterozygosity of *A. crassna* in the present study (H_e = 0.5672) is slightly higher than that of *A. crassna* (H_e = 0.542) studied by Wang et al. (2018), which used *A. sinensis* to amplify *A. crassna* samples. This pattern suggested that a species' genetic variation is likely high when using markers specifically developed for that species. However, this variation may slightly decrease when using markers that are not specifically tailored to that species.

Genetic variation is essential for maintaining the long-term stability of the forest ecosystem, as it influences the ability of tree species to adapt to changing environmental conditions. The amount and distribution of genetic variation are shaped by genetic systems and evolutionary factors. Understanding genetic variability is crucial for developing effective conservation and breeding strategies (Rohlf, 1998). Specifically, knowledge about the genetics of agarwood species can aid in designing effective tree breeding programs, conservation strategies for genetic resources, and establishing a DNA fingerprint database. This database would help identify the geographic and plantation origins of traded agarwood (Eurlings et al., 2010).

Table 5. Genetic variation of three Indonesian *Aquilaria* species using four microsatellite markers

No	Agarwood species	N	N_a	N_e	H_o	H_e	F
1	*Aquilaria crassna*	15	2.75	2.35	0.967	0.5672	-0.727***
2	*Aquilaria malaccensis*	32	2.75	2.19	0.867	0.5424	-0.598***
3	*Aquilaria microcarpa*	8	2.50	2.11	0.906	0.5234	-0.734^{ns}

Note: N= sample size; N_a= number of alleles; N_e= effective number of alleles; H_o = observed heterozygosity; H_e= expected heterozygosity; F = Fixation Index with the significance level for deviation from the Hardy-Weinberg Equilibrium (HWE): P < 0.001 (***), not significant (ns).

The present study revealed that four microsatellite markers evaluated in this study could be used as molecular tools for further population genetics studies, provenance identification, and genetic resource management and conservation of *Aquilaria* species in Indonesia. Practical application for discriminating between artificial and natural populations might also be another helpful option. In addition, the loci characterized here can also be used for genotyping of clones of agarwood produced by commercial tissue culture techniques within the context of seedling quality control.

Additional genetic aspects of reproductive systems in agarwood can also be explored to assess genetic variability loss, inbreeding depression, gene flow, the influence of selection, mating systems, and the identification of suitable germplasm for conservation efforts. The genetic variation data gathered in this present study can serve as valuable references for future population and conservation genetics studies. Considering the genetic variation in the three studied planted agarwood species in West Java, the breeding program may be started using the available plantation materials as base populations. However, a future backup of genetic resources from other populations, preferably from natural ones, is recommended and should be well-planned in the long-term breeding program.

IV. CONCLUSION

Four microsatellite markers originally developed for *Aquilaria crassna* in Thailand (6pa18, 10pa17, 16pa17, and 71pa17) were successfully amplified in three Indonesian *Aquilaria* species (*A. crassna*, *A. malaccensis*, and *A. microcarpa*). The success of amplification indicated the transferability of microsatellite markers, possibly due to the suitability of the markers and the efficiency and optimization of PCR processes. Moreover, the genetic diversity of the three *Aquilaria* species using the four markers also showed comparable values with slightly higher values in *A. crassna*, suggesting that the four markers are reliable enough to be used in future studies related to the genetic resources of these three *Aquilaria* species, such as population genetics, breeding, and conservation programs. This approach will support the management of *Aquilaria* resources and help track its supply chain to prevent overexploitation and illegal logging. While this study employs DNA band assessment methods on acrylamide gels, it is important to note that this approach has limitations, particularly when distinguishing smaller polymorphic bands that differ by just 2–3 base pairs due to the short length of the gels. Nevertheless, the study successfully demonstrates the presence of polymorphic sites in the three species of agarwood. To enhance the accuracy of future genetic population studies, it is recommended that a Sanger Sequencing instrument be utilized to determine SSR fragment sizes precisely. This advancement will contribute to the ongoing efforts to protect and manage valuable *Aquilaria* resources effectively.

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