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Research Article

Cross-Species SSR Marker Transfer in Indonesian *Santalum album* L.: Applications for Genetic Monitoring and Conservation

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ABSTRACT

Santalum album L., more commonly known as sandalwood, is a highly valuable species currently threatened with extinction due to overexploitation. In Indonesia, its wild populations have declined by more than 80 %. Conservation efforts will be effective by utilising strong genetic markers, which are crucial for assessing diversity and population structure. This study evaluated the application of microsatellite markers from *Osyris lanceolata* (African sandalwood) in *S. album* populations across four regions in Indonesia: Timor, Flores, Sumba, and Java. All eight loci tested were successfully amplified, demonstrating their transferability and polymorphism. The number of alleles ranged from 2 to 7 per locus. A comparative analysis revealed notable differences in allele sizes between Indonesian and Indian *S. album* populations, indicating geographic divergence. The absence of allele overlaps with *O. lanceolata* confirmed the species' distinctiveness. Genetic diversity metrics indicated moderate to low marker informativeness. Observed heterozygosity ranged from 0.146 to 0.938, expected heterozygosity from 0.203 to 0.498, and polymorphic information content (*PIC*) from 0.182 to 0.374. *PIC* values were used to evaluate the informativeness of the microsatellite markers. One locus, KFOL8, deviated from the Hardy-Weinberg equilibrium. A non-exclusion probability of 0.098 indicated the effectiveness of these markers in paternity analysis. These findings provided methodological support for genetic conservation, facilitated population monitoring, identified illegal logging activities, and enhanced reforestation initiatives. Additional research with a larger sample size and more markers is necessary to improve these findings, providing an important basis for developing sustainable conservation strategies and managing genetic resources for this vulnerable species.

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INTRODUCTION

Santalum album L., commonly known as sandalwood, is a tree species of considerable importance, native to Indonesia, India, and northern Australia (Harbaugh & Baldwin 2007; Fatima et al. 2019). This species is esteemed for its fragrant heartwood and essential oils, which are used in perfumery, traditional medicine, and religious rituals; yet, it has undergone considerable overexploitation across its range (Thomson et al. 2020; Pullaiah 2021). In Indonesia, specifically in the East Nusa Tenggara (ENT) province, wild populations have declined by more than 80 % due to decades of illegal logging and habitat degradation (Yuskianti & Warrier 2022). The significant decline has resulted in its designation as Vulnerable on the IUCN Red List, with decreasing population sizes, genetic diversity, and the long-term survival of the species (Arunkumar et al. 2019; Leigh et al. 2019; Zhang et al. 2024).

This study evaluated the potential use of cross-species microsatellite markers (SSRs) derived from African sandalwood (*Osyris lanceolata*) in Indonesian populations of *S. album*. SSR markers are valuable tools for studying genetic diversity and population structure due to their high polymorphism and codominant inheritance (Hodel et al. 2016; Wang et al. 2021; Alves et al. 2024). Although developing species-specific SSR markers for endangered species like *S. album* is labour intensive and costly (Song et al. 2019; Moulin et al. 2022), cross-species amplification provides a more affordable and practical alternative (Rosetto 2001; Alexander et al. 2018; Pandey et al. 2021).

The study examined the transferability of SSR markers from *O. lanceolata* to *S. album* populations in four ecologically distinct regions Java, Flores, Sumba, and Timor. The objectives were to (1) evaluate the polymorphism and amplification success of these markers in Indonesian populations and (2) compare the results with those from Indian populations. Ultimately, the aim of the study is to develop a cost-effective tool for population monitoring, illegal logging detection, and reforestation support key components of conservation and sustainable management of genetic resources. These findings contribute to the development of conservation strategies for this vulnerable species and advance efforts to protect Indonesia's forest biodiversity.

MATERIALS AND METHODS

Sample collection and DNA extraction

Samples of the *S. album* leaf were obtained from 48 randomly selected trees in their native region of ENT, as well as from Java Island, where the species has been introduced. The sampling site in the ENT region includes three large islands Flores, Sumba, and Timor that serve as the natural habitat for *S. album*. Leaf samples were obtained from healthy, clean, fresh, green leaves that were physiologically mature. Individual leaf samples were gathered, with up to six leaves from each tree, to obtain 50 g of dried leaves for DNA analysis. Samples were stored in silica gel envelopes to preserve quality during transport. Upon arrival at the laboratory, the samples were enclosed in plastic bags and preserved in a deep freezer at -20 °C before the DNA extraction process began.

DNA extraction and microsatellite analysis

The CTAB method was utilised for the isolation of DNA (Shiraishi & Watanabe 1995). Fifty milligrams of dried leaf samples were ground with a mini bead machine, M-301 series (Retsch), to aid in the DNA isolation process. After isolating the DNA, the samples were evaluated with a NanoVue spectrophotometer to assess their quantity and quality (GE Healthcare). For effective amplification in the PCR process, it is crucial to have high-quality DNA at an adequate concentration. DNA samples were stored at 4 °C prior to further analysis.

PCR Amplification and Genotyping

DNA amplification by PCR (Mullis & Faloona 1987; Blair & Zajdel 1992) was performed using MyTaq HS Red Mix 2x (Meridian Bioscience n.d.; Baramantya et al. 2025). This research utilised eight previously developed microsatellite primers from *O. lanceolata*, as listed in Table 1 (Otieno et al. 2016). PCR reactions were conducted with a total volume of 10 μL, consisting of 6 μL of MyTaq HS Red Mix 2x Taq Polymerase (Bioline), 0.25 μL of 20 μM forward primer, 0.25 μL of 20 μM reverse primer, 2 μL of DNA at a concentration of 10 ng μL^{-1}, and 1.5 μL of sterile double-distilled water. The PCR procedure was conducted with a thermal cycler, namely the GeneAmp PCR System 9700 from Applied Biosystems (Thermo Fisher Scientific, Waltham, USA). The PCR procedure began with an initial denaturation step at 95 °C for 1 minute, followed by 35 cycles. Each cycle included a denaturation step at 95 °C for 15 seconds, an annealing phase at 54 °C to 58 °C (see Table 1) for 15 seconds, and an extension phase at 72 °C for 10 seconds. After 35 cycles, the temperature decreased to 4 °C. Each PCR experiment incorporated negative controls to identify any potential contamination. The controls, which included all reagents except the DNA template, exhibited no amplification, thereby confirming that there was no contamination in the reagents and equipment utilized. DNA fragment lengths were measured using a capillary electrophoresis sequencer. Amplified DNA fragments were evaluated using an internal size standard (Liz 500 size standard) and analysed utilizing an ABI 3730xl DNA Analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA). Allele genotyping was conducted using GeneMapper version 3.5 software developed by Applied Biosystems (Thermo Fisher Scientific, Waltham, USA). The fluorescence peaks from fragments were manually analysed using the same software to ensure validity and consistency.

Data analysis

The analysis of PCR codominant data was performed using MicroChecker version 2.2.3 software (Van Oosterhout et al. 2004) to check the data's reliability by detecting contamination, sequencing errors, and PCR errors. This software is designed to manage quality by identifying technical inaccuracies in raw data that may result in erroneous interpretations. Utilising Micro-Checker version 2.2.3 enables the verification of data integrity prior to further

Table 1. List of selected microsatellite primers for Indonesian *S. album*.

Locus	Accession No.	Primer sequence (5' - 3')	Repeat motif	T_a (°C)
KFOL2	LC126834	F: AGAATGTCATTTGAAGGCTCGA R: CCTTTCCTCCGTTCTCCTCG	CGTC	54
KFOL7	LC154965	F: CTGTGCAATGGAGAAGGCCA R: CGCGGGATTGGGATGTCATA	ATT	58
KFOL8	LC154966	F: GCTGCTTCTACGGTCACTGT R: GTGGTGGATATGGAGGTGGC	CCG	58
KFOL16	LC154968	F: TGGAGCCCATTCTCTTTCCTT R: TGCACGTATTCCACATTTCCA	GT	56
KFOL19	LC154969	F: GGTAGCGAGCGGTGATATGT R: ACCTAACAACTTGAAGCTCTCCC	TC	56
KFOL24	LC126838	F: CAACTCGATCGTGCATTGGC R: TCCGCATATCCATTTGGCCG	CT	57
KFOL28	LC126839	F: ATAAAGGCCCACGAGCTCAG R: AACATCGCCATGCAGAACAG	CT	57
KFOL29	LC154961	F: GCTGAATCAGGGACAGGCAT R: GGCCTCGAACAAAGTGCATG	GA	57

F: Forward; R: Reverse primers; T_a: annealing temperature analysis, thereby enhancing the reproducibility and credibility of genetic findings. A locus was classified as possessing a null allele when the software reported 'Null Present' as 'Yes,' a designation corroborated by consistently positive values across all estimation methods.

Co-dominant data were analysed by GenAlEx version 6.5 software (Peakall & Smouse 2012) to estimate the quantities of different alleles (N_a), effective alleles (N_e), expected heterozygosity (H_e), observed heterozygosity (H_o), Shannon's index (I), and fixation index (F). These parameters were used to describe the level of heterozygosity and polymorphism of microsatellite markers. N_a was determined by identifying the different alleles amplified at each locus. N_e, representing the allele diversity available for selection in future generations, is determined by the frequency of alleles at polymorphic loci relative to the total number of loci analysed (Cole 2003). The heterozygosity at each locus was determined by comparing H_e to H_o. H_e per locus was determined using the frequency of detected alleles, whereas H_o was determined by dividing the count of observed heterozygotes by the aggregate number of individuals analysed (Nei 1978). I represent the total genetic diversity, which was determined based on the quantity of alleles present at a locus and the relative frequency of alleles (Konopiński 2020). F values reflecting Hardy-Weinberg equilibrium (HWE) deviations were evaluated for statistical significance through the chi-squared test (X^2).

In accordance with Botstein et al. (1980), the polymorphic information content (*PIC*) of each locus was utilized to assess the informativeness of microsatellite markers. The estimation of *PIC* values was performed using CERVUS v.3.0.7 software (Kalinowski et al. 2007). Additionally, the Combined Non-Exclusion Probabilities (*NEs*) for the locus specific to the first parent (*NE-1P*), second parent (*NE-2P*), and parent pair (*NE-PP*) were calculated utilising the CERVUS v.3.0.7 program.

RESULTS AND DISCUSSION

Microsatellite marker amplification

Interspecies amplification successfully amplified eight microsatellite markers from the source species, *O. lanceolata*, to the target species, Indonesian *S. album*. The size of the alleles found through PCR amplification in the Indonesian *S. album* are shown in Table 2, along with comparisons to those found in Indian *S. album* and *O. lanceolata*.

Microsatellite markers from *O. lanceolata* were successfully cross amplified in the Indonesian *S. album*, demonstrating complete transferability and polymorphism. These findings are in consistent with Fatima et al. (2019) research on the Indian *S. album*. We noted variations in allele sizes between the populations of Indonesia and India. For instance, the allele at KFOL7 in the Indonesian population ranges from 351 to 391 bp, which is longer than the range observed in India and spanned from 260 to 320 bp. In contrast, KFOL8 and KFOL16 exhibited narrower size ranges. KFOL19 exhibited a pattern where Indian alleles ranged from 275 to 292 bp, in contrast to the shorter alleles found in Indonesian, which measured between 210 and 212 bp. These variations may indicate selective pressures, geographic isolation, or environmental influences. Loci such as KFOL7 and KFOL19 may serve to distinguish between populations, whereas KFOL2 and KFOL28 exhibit genetic conservation. The finding underscored the intraspecific divergence observed in *S. album*, establishing a basis for future conservation and genetic research. It aligns with the agenda to regenerate sandalwood in ENT, as outlined in the Master Plan document for Sandalwood Management in ENT (Ministry of Forestry of Republic Indonesia & Provincial government of Nusa Tenggara Timur (NTT) 2010).

Meanwhile, when comparing the allele size range of Indonesian *S. album* with that of *O. lanceolata*, two species exhibited completely different allele profiles, with no overlap. Microsatellite markers identified alleles of varying sizes, which differed between Indonesian S. album and *O. lanceolata*. At the KFOL7 locus, Indonesian *S. album* demonstrated a wide allele range of 351–391 bp, in contrast to *O. lanceolata*, which ranged from 115 to 120 bp, resulting in a difference exceeding 200 bp. KFOL8 and KFOL16 exhibited shorter alleles in *O. lanceolata*, whereas KFOL29 presented longer alleles in *O. lanceolata* (230–250 bp) in comparison to Indonesian *S. album* (179–187 bp).

The observed differences supported classifying the two groups as distinct species, reflecting a complex evolutionary history influenced by selective pressures and mutational events. The variation in allele size could be resulted from disparities in genetic evolution, mutation rates, primer compatibility, repeat patterns, and microsatellite stability (Ranathunge et al. 2022; Marion & Noor 2023). Despite both being from the Santalaceae family, *S. album* and *O. lanceolata* follow distinct evolutionary paths (Fatima et al. 2019). Mutation, selection, and genetic drift influence allele length, and differences in mutation rates among species further enhance variability in allele size (Charlesworth & Jensen 2021; Ranathunge et al. 2022; Marion & Noor 2023). In cross-amplification, primer compatibility is crucial, as sequence variations can lead to size differences in the amplification products (Pern et al. 2020). Differences in nucleotide repeat patterns can result in variations in allele size ranges, particularly when evaluated using the same primers (Cohen et al. 2022). These findings had significant implications for taxonomy, conservation, and sandalwood source identification, with markers such as KFOL7 and KFOL29 serving as reliable diagnostic tools.

Although the two species are from different genera, both species belong to the same family, the Santalaceae (Fatima et al. 2019; Mugula et al. 2021). Successful amplification of microsatellites across different genera is possible (Pern et al. 2020; Pandey et al. 2021). Primers derived from *Setaria italica* were effectively transferred to both millet and non-millet species, resulting in success rates of 92.5 % for guinea grass (*Magathyrsus maximus*) and 80.2 % for finger millet (*Eleusine coracana*) (Kumari et al. 2013). Established precedents supported the creation of microsatellite markers by screening primers across related species, thereby saving time and costs.

Table 2. The sequence sizes of alleles from eight microsatellite loci in *S. album.*

Locus	Size range (bp)		
	Indonesian *S. album*	Indian *S. album*	*O. lanceolata*
KFOL2	176 – 192	170 – 190	178 – 194
KFOL7	351 – 391	260 – 320	115 – 120
KFOL8	178 – 180	150 – 170	120 – 130
KFOL16	177 – 187	140 – 165	130 – 160
KFOL19	210 – 212	275 – 292	200 – 230
KFOL24	316 – 366	205 – 218	219 – 263
KFOL28	321 – 353	330 – 350	245 – 255
KFOL29	179 – 187	159 – 170	230 – 250

bp: base pairs.

Characterisation of Microsatellite Markers

Microsatellites, also known as simple sequence repeats (SSRs), are polymorphic genetic markers characterised by brief tandem repeats, making them valuable for population genetic studies. The data revealed multiple notable attributes of these loci. All primers exhibited polymorphism, with the number of alleles ranging from two (KFOL8, KFOL19, and KFOL29) to seven (KFOL28) at each locus, with varying allele frequencies (Figure 1). The limited allele diversity at loci such as KFOL8, KFOL19, and KFOL29, each with only two alleles, may hinder effective population-level analysis. Future research is crucial to enhance genetic resolution by increasing the number of loci and sample size, aiming to achieve more diverse allele distribution and to improve genetic insights. A total of 34 alleles were identified, resulting in an average of 4.250 (± 0.701). The mean allelic diversity observed was similar to that documented in *O. lanceolata* (mean = 4.875 ± 1.529, total N_a = 39 alleles), indicating comparable levels of genetic variation (Otieno et al. 2016). A higher number of alleles generally signifies increased genetic diversity, potentially indicating enhanced adaptive capacity or historical population growth.

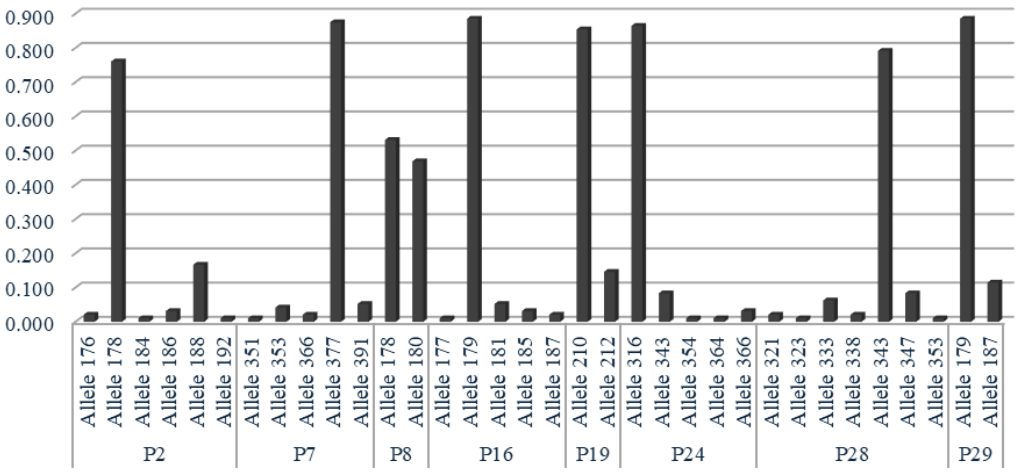

Figure 1. The allele frequencies at each locus were analyzed in 48 samples of Indonesian *S. album*.

The MicroChecker version 2.2.3 analysis indicated that the majority of primers exhibited high quality and are appropriate for subsequent genetic analysis (Table 3). Although KFOL29 showed signs of a null allele, it did not compromise primer reliability but required cautious interpretation. Consequently, locus KFOL29 was retained for parentage analysis, with statistical corrections applied using the mean null allele frequency (r = 0.078) derived from the three estimators as a correction parameter. The majority of loci (e.g., KFOL2, KFOL7, KFOL8, KFOL16, KFOL19, KFOL24, and KFOL28) did not show a null allele (Null Present = no) and exhibited consistent values across various estimation methods, indicating that the primers successfully amplified the target with adequate specificity. Moreover, there was a lack of evidence for contamination PCR, which supported the validity of the amplification results. These primers can be relied upon for genetic analysis. Thus, these primers met the quality standards required for genetic research.

Table 3. Analysis of MicroChecker version 2.2.3. on eight microsatellite primers for *S. album*.

Locus	Null present	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
KFOL2	no	0.0331	0.0491	0.0376	0.1470
KFOL7	no	-0.0391	-0.0031	-0.0019	0.3860
KFOL8	no	0.0439	0.0445	0.0555	0.0555
KFOL16	no	0.0425	0.0445	0.0798	0.2703
KFOL19	no	-0.0027	-0.0182	-0.009	0
KFOL24	no	0.0622	0.0772	0.0528	0.2954
KFOL28	no	-0.019	0.0093	0.0045	0.3330
KFOL29	yes	0.0512	0.0835	0.0983	0.3256

The effective alleles (N_e) varied from 1.255 to 1.992, with a mean of 1.460 (± 0.092), as indicated in Table 4, implying the presence of rare alleles that contributed insignificantly to the existing genetic variation. All analysed microsatellite loci displayed a reduced number of effective alleles relative to the total allele count. This indicates that specific alleles do not contribute to the genetic variation observed in the population. A decrease in the number of effective alleles in a population may arise from multiple natural factors or deliberate selection (Westbury et al. 2018; Allendorf et al. 2024). The observed discrepancy between N_a and N_e alleles suggests that some alleles may be present at low frequencies, possibly due to genetic drift, selective sweeps, or demographic bottlenecks.

Table 4. The characteristics of microsatellite markers for Indonesian *S. album* were analysed using GenAlEx version 6.5.

Locus	N	N_a	N_e	H_o	H_e	I	F
KFOL2	48	6	1.646	0.479	0.392	0.791	-0.221^{ns}
KFOL7	48	5	1.298	0.250	0.229	0.531	-0.090^{ns}
KFOL8	48	2	1.992	0.938	0.498	0.691	-0.882***
KFOL16	48	5	1.269	0.229	0.212	0.498	-0.082^{ns}
KFOL19	48	2	1.332	0.208	0.249	0.415	0.164^{ns}
KFOL24	48	5	1.323	0.250	0.244	0.536	-0.023^{ns}
KFOL28	48	7	1.566	0.417	0.361	0.822	-0.153^{ns}
KFOL29	48	2	1.255	0.146	0.203	0.356	0.281^{ns}
Mean		4.250	1.460	0.365	0.299	0.302	-0.126

ns: not significant; ** $P<0.01$; *** $P<0.001$; N: number of samples; N_a: number of different alleles; N_e: number of effective alleles; I: Shannon's information index; H_o: observed heterozygosity; H_e: expected heterozygosity; F: fixation index.

The observed heterozygosity values ranged from 0.146 (KFOL29) to 0.938 (KFOL8). The expected heterozygosity values ranged from 0.203 at gene KFOL29 to 0.498 at locus KFOL8. The locus KFOL8 had the highest anticipated and observed heterozygosity, indicating significant genetic diversity, while KFOL29 demonstrated the lowest heterozygosity, suggesting less variation, potentially due to selective pressures or inbreeding. In the Hardy-Weinberg equilibrium (HWE) deviation test, seven loci demonstrated fixation index values that were not substantially different from HWE ($P > 0.05$), however, one locus (KFOL8) violated HWE principles.

The maximum recorded Shannon Information Index (I) value was 0.822 in KFOL28, indicating significant genetic variation at this locus, whereas KFOL29 exhibited the minimum index value of 0.356. The fixation index (F) varied from negative to positive values, with KFOL8 displaying the lowest F value of -0.822, which was statistically significant ($P < 0.001$) and indicated a deviation from Hardy-Weinberg equilibrium (HWE), implying an excess of heterozygotes at this locus. This deviation may be resulted from a variety of mechanisms, such as overdominant selection (in which heterozygotes exhibit a higher fitness than homozygotes), negative assortative mating (preferential mating between dissimilar genotypes), or population substructure (the Wahlund effect, in which sampling from genetically distinct subpopulations artificially inflates heterozygosity). The presence of rare alleles and deviations in heterozygosity, with certain loci possibly underwent a balanced selection and others indicated genetic drift, underscored the necessity of employing a multi-locus approach in population genetics research to mitigate bias from single-locus deviations.

The focus of this research is to evaluate the cross-amplification ability of SSR markers in *S. album* rather than comparing genetic variation among populations. If the loci are successfully amplified, future research should be expanded to include genetic variation studies, population structure analysis, and potential correlations with environmental factors at the sampling locations. These efforts allow more comprehensive understanding of genetic differentiation and its relationship with environmental variation.

The *PIC* values presented in Table 5, which assessed the informativeness of loci in genetic analysis, indicate that KFOL8, KFOL2, and KFOL28 exhibited the highest values of 0.374, 0.358, and 0.346, respectively. The KFOL29 locus exhibited the lowest *PIC* value, recorded at 0.182. Genetic studies utilised *PIC* values as a foundation for marker selection, underscoring their significance. Based on the *PIC* value, 3 loci, namely KFOL8, KFOL2, and KFOL28, were categorised as moderately informative (0.50 > *PIC* > 0.25), while 5 KFOL24, KFOL7, KFOL19, KFOL16, and KFOL29 were categorised as slightly informative (*PIC* < 0.25) (Botstein et al. 1980). Fine-scaled genetic differentiation could not be robustly assessed due to low polymorphism in five loci. Future work could be helpful in terms of incorporate larger samples and more variable markers to better characterise genetic variation.

Table 5. Polymorphic information content for Indonesian *S. album* was analysed using CERVUS v.3.0.7.

Locus	*PIC*	*NE-1P*	*NE-2P*	*NE-PP*
KFOL2	0.358	0.920	0.792	0.656
KFOL7	0.222	0.973	0.876	0.774
KFOL8	0.374	0.876	0.813	0.719
KFOL16	0.205	0.977	0.886	0.792
KFOL19	0.218	0.969	0.891	0.817
KFOL24	0.232	0.969	0.872	0.771
KFOL28	0.346	0.929	0.791	0.641
KFOL29	0.182	0.979	0.909	0.842
Combined NE		0.65483	0.27906	0.09826

NE: non-exclusion probability; *PIC*: polymorphic information content; *NE-1P*: *NE*-first parent; *NE-2P*: *NE*-second parent; *NE-PP*: *NE*-parent pair.

Table 5 shows the aggregated non-exclusion probability, illustrating the variability in effectiveness among loci. The combined non-exclusion probability refers to the likelihood that a potential parent is not excluded as a biological parent, determined by genetic data across multiple loci (Gwon et al. 2025). This parameter reflects the primer's capacity to differentiate the alleles of individual trees. The mean non-exclusion probability for the first parent candidate (*NE-1P*) ranged from 0.876 to 0.979. The mean for the second parent candidate, with one known parent (*NE-2P*), ranged from 0.791 to 0.909. The mean for parent pair candidates (*NE-PP*) ranged from 0.641 to 0.842 (Table 5). The overall non-exclusion probability for the eight loci was 0.09826 for parental pairs. Thus, there is a 9 % probability that the eight loci collectively will not eliminate an unrelated prospective parent. The combination of eight loci resulted in exclusion power exceeding 0.90, with a non-exclusion probability less than 0.10 (Gwon et al. 2025). This value is acceptable given the limited sample size and population diversity. Thus, based on the combined non-exclusion probability analysis, the eight-loci combination had the potential to be used in genetic analysis, especially in parental analysis. The eight cross-amplified microsatellite primers showed high reliability and are recommended for future genetic studies on Indonesian *S. album*.

CONCLUSION

This study demonstrated the successful cross-amplification of microsatellite markers from *O. lanceolata* in Indonesia's *S. album*, revealing both genetic similarities and differences among populations. The findings validated the utility of these markers for genetic research and emphasised the distinct evolutionary paths of the species. Furthermore, the results pointed out the importance of such genetic tools in assessing biodiversity and guiding sustainable sandalwood management. The successful cross-amplification of these primers enhanced this study's ability to inform and to guide a genetics-based conservation strategy, facilitating more targeted and effective efforts in sandalwood conservation. For future research, priorities should include expanding the range of loci and population sampling, particularly in regions of high conservation value, and developing species-specific primers for *S. album* to address the challenges posed by cross-amplification.

AUTHORS CONTRIBUTION

All authors contributed to the conception and design of this study. S. conducted the conceptualisation. The methodology was formulated by S., S.I., and I.L.G.N. Formal analysis and investigation conducted by S. and I.L.G.N. The original draft was written by S., S.I., Y.W.N.R., and I.L.G.N. The review, editing, and supervision were carried out by S.I. All authors have examined and endorsed the final manuscript.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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