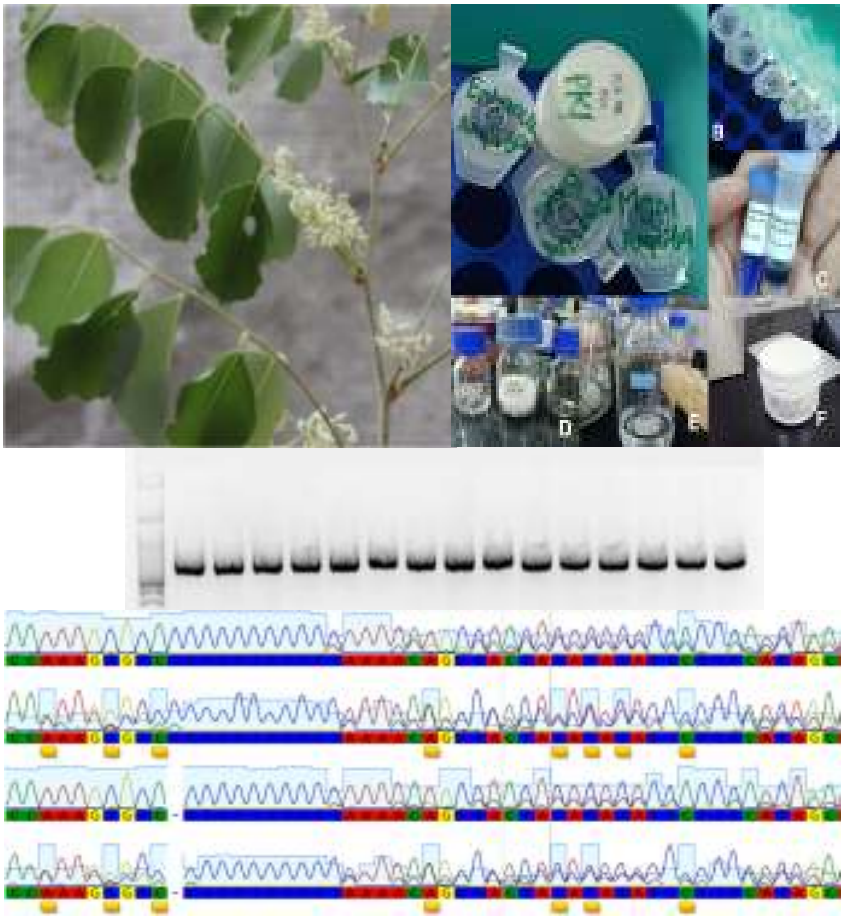
A Report on the Molecular characterization of *Dalbergia latifolia* from Java and West Nusa Tenggara: DNA extraction, PCR amplification, and DNA sequencing



KS Yulita, FG Dwiyanti, I Kamal, Susila, N Arrofaha, and HH Rachmat

Jakarta, Indonesia

September 2022

A report on the Molecular characterization of *Dalbergia latifolia* from Java and West Nusa Tenggara: DNA extraction, PCR amplification, and DNA sequencing

Programme	:	CITES Tree Species Programme Web Page: https://cites-tsp.org/
Financial Support	:	The European Union and the CITES Tree Species Programme
Implementing Agency	:	Directorate of Biodiversity Conservation of Species and Genetics, Ministry of Environment, and Forestry and National Research and Innovation Agency, Indonesia
Authors	:	KS Yulita, FG Dwiyanti, I Kamal, Susila, N Arrofaha, and HH Rachmat
Indonesia Project Team and Contributors to the Report	:	A Subiakto, TD Atikah, BA Pratama, T Setyawati, S Nurjanah, W Wardani, and F Nopiansyah
Cover	:	On the left, top left: leaf and flowers of *Dalbergia latifolia*, top right: tubes and reagents, middle: Gel photo of PCR amplification of *trn*G intron, below: chromatogram of part of the alignment of DNA sequences. Photos by KS Yulita, FG Dwiyanti, I Kamal, Susila, N Arrofaha, and HH Rachmat
Citation	:	KS Yulita, FG Dwiyanti, I Kamal, Susila, N Arrofaha, and HH Rachmat. 2022. A Report on the Molecular characterization of *Dalbergia latifolia* from Java and West Nusa Tenggara: DNA extraction, PCR amplification, and DNA sequencing. Directorate of Biodiversity Conservation of Species and Genetics, Ministry of Environment and Forestry, and the National Research and Innovation Agency, Indonesia
Copyright	:	Directorate of Biodiversity Conservation of Species and Genetics, Ministry of Environment and Forestry, and the National Research and Innovation Agency, Indonesia

Contents

List of Figures

List of Tables

ACKNOWLEDGEMENTS

The CTSP Indonesia Team wish to acknowledge the European Union for funding this project, and the CITES Secretariat for coordinating this project. During the course of this project, from preparation and post-project implementation, we owed our respect and gratitude to Mr. Thang Hooi Chiew as the Regional Coordinator for Asia, and to Dr. Milena Sosa Schmidt as the CITES Tree Species Programme Coordinator and Regional Coordinator for Central and South America and the Caribbean. We also acknowledge the support of Dr. Haruko Okusu and Ms. Sofie H. Flensborg from the CITES Secretariat.

We are grateful to Mrs. Indra Exploitasia, Director of Biodiversity Conservation of Species and Genetics, Ministry of Environment and Forestry and her staff, Dr. Kirsfianti L. Ginoga, former Director of Forest Research and Development Center which is currently the Center for Standardization of Sustainable Forest Management Instruments, Dr. Atit Kanti, former Head of Research Center for Biology, Dr. Anang Setiawan, Head of Research Centre for Ecology and Ethnobotany, and Dr. Bayu Adjie, Head of Research Center for Biosystematics and Evolution, the National Research and Innovation Agency for their support and assistance in this project.

We express our appreciation to everyone who has assisted us during the field survey and collections, and also to all who have made valuable contributions until this report is completed.

On behalf of the project team,

Dr. Kusumadewi Sri Yulita

Project Team Leader

EXECUTIVE SUMMARY

Dalbergia latifolia (Fabaceae: Papilionaceae), locally known as sonokeling, is a commercial rosewood timber species distributed widely in tropical and subtropical regions in South America, Africa, Asia, and Madagascar. In Indonesia, the species is mainly distributed in Java and West Nusa Tenggara, and may be present in Timor Island, South Sumatra, Kalimantan, and Sulawesi. As a commercial tree species, *D. latifolia* is extracted for its very dense, non-porous and very durable heartwood, which is suitable as a raw material for furniture, musical instruments, and other handicrafts. Local farmers and Forestry State-Owned Companies (Perhutani) are sources for the *D. latifolia* timber trade market in Indonesia. The international trade for *D. latifoli*a is regulated by CITES by including them in Appendix II of CITES since 2017. At the global level, the species is considered vulnerable to extinction due to habitat degradation and illegal logging but is locally abundant in Indonesia and was even considered a weed in certain areas based on our field surveys. However, the abundance of this species will tend to decrease in its distribution area if it is not accompanied by careful management. Therefore, in order to develop a good management strategy for this species, it is necessary to have basic information such as DNA sequence references for populations of *D. latifolia* from Java and West Nusa Tenggara. The DNA sequence references can be developed into DNA fingerprints of populations/locations for tracing and traceability purposes.

This report focussed on the nucleotide variations found in *D. latifolia* from Java and West Nusa Tenggara that will be used as reference data for developing the DNA database for the purpose of trace and traceability. DNA materials from leaves and woods were collected from mature trees with diameters at breast height (dbh) of above 10 cm. The total number of collection sites was 113 located in Java, Bali, Lombok, and Sumbawa islands during 2019–2022. The total number of collected DNA materials was 551 consisting of 486 leaves samples and 65 wood samples, and 192 of them were selected for molecular work. Twelve molecular markers from non-coding chloroplast regions, i.e., petD – rpoA, CLP, trnC – trnD, *trn*G, rpl16 – rps3, trnV, atpB – rbcL, psbk – trnS, psbA – trnH, rrn16 – trnI, ndhAF – ndhAR, and rps16F – rps16 R were used for screening (**Table 1**), and five (CLP, petD – rpoA, rpl16 – rps3, *trn*L, and *trn*G) were selected to perform PCR amplification and the DNA sequencing. Of the five markers, the longest amplicon size was yielded by the rpl16 – rps3 intergenic spacer (789 bp), followed by *trn*G intron (722 bp), *pet*D intron (691 bp), and *trn*L intron (590 bp), while the shortest was in CLP intron (401 bp) (**Table 7**). All the five markers used were A/T rich-region (**Table 7**). The highest number of polymorphic sites was found in rpl16 – rps3 intergenic spacer with 4 nucleotide substitution and 1 indel, and the lowest was found in CLP intron and *trn*L intron with 2 nucleotide substitutions and 0 indel. The rpl16 – rps3 intergenic spacer showed a good performance as indicated by the length of the sequence (789 bp) and the high number of polymorphic sites. These five markers are recommended to be used for mass amplification to support further studies on *D. latifolia* genetic diversity and population structure.

ACRONYMS AND ABBREVIATIONS

A	Adenin
BLAST	Basic Local Alignment Search Tool
bp	base pair
C	Cytosin
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
cm	centimeter
CTAB	Cetyl Trimethyl Ammonium Bromide
CTSP	CITES Tree Species Programme
D.	*Dalbergia*
dbh	diameter at breast height
DNA	Deoxyribo Nucleic acid
et al.	et alia: and others (used especially in referring to academic books or articles that have more than one author)
G	Guanine
IGS	inter-genic spacer
indel	insertion deletion
mg	milligram
ng	nanogram
PCR	Polymerase Chain Reaction
Perhutani	Forestry State-Owned Company (Perusahaan Hutan Negara Indonesia)
RNA	Ribo Nucleic acid
T	Thymine
TBE	Tris Borate EDTA
µL	microliter
µM	micromolar
°C	degree Celcius
~	approximately

1. INTRODUCTION

Dalbergia latifolia (Fabaceae: Papilionaceae), locally known as sonokeling, is a commercial rosewood timber species distributed widely in tropical and subtropical regions in South America, Africa, Asia, and Madagascar (Cardoso *et al*., 2013; Saha *et al*., 2013; Vatanparast *et al*., 2013). The species is considered native to the Andaman islands, Bangladesh, East and West Himalayas, India, Java, and Nepal (powo.science.kew.org, n.d.). In Indonesia, the species are mainly distributed in Java and West Nusa Tenggara, and may be present in Timor Island, South Sumatra, Kalimantan, and Sulawesi (Yulita *et al*., 2020). However, this species was thought to have been introduced to Indonesia by the colonial government before the 20th century (Sunarno, 1996; Adema *et al*., 2016; Maridi and Saputra, 2014; Arisoesilaningsih and Soejono, 2015) and has been naturalized in Indonesia since then (powo.science.kew.org, n.d.). This is supported by the existence of the oldest herbarium specimen recorded in 1890, referring to a specimen collected from Java (www.gbif.org). Besides that, one of the four synonyms of the species, namely, *Dalbergia javanica* Miq, indicates that the species also comes from Java (Miquel, 1855). However, the origin of *D. latifolia* is still unclear, hence, the origin of *D. latifolia* in Indonesia needs to be resolved. This species is mostly found in mixed-plantations, mixed agroforestry areas in both commercial plantations and local farmers' gardens, and only a few have been recorded from protected forests, or even found as monocultures based on current field observations and several reports (Atikah and Dede, 2018; Mulyana *et al*., 2017; Hani and Suryanto, 2014).

As a commercial tree species, *D. latifolia* is harvested for its very dense, non-porous and very durable heartwood (Barret *et al*., 2010; Karlinasari *et al*., 2010; Hassold *et al*., 2016), which is suitable as a raw material for furniture, musical instruments, and other handicrafts. Local farmers and Forestry State-Owned Companies (Perhutani) are sources for the *D. latifolia* timber trade market in Indonesia. The wood is sent to the primary and secondary timber industries to be processed as semi-finished products for export (Yulita and Susila, 2019). Leftover and low-quality pieces of wood are usually used for traditional crafts and firewood (Yulita and Susila, 2019). CITES regulated the international trade for *D. latifoli*a by including them in Appendix II of CITES since 2017. At the global level, the species is considered vulnerable to extinction due to habitat degradation and illegal logging (Lakhey *et al*., 2020) but is locally abundant in Indonesia and was even considered a weed in certain areas based on our field surveys. However, the abundance of this species will tend to decrease in its distribution area if it is not accompanied by careful management. Therefore, in order to develop a good management strategy for this species, it is necessary to have basic information such as DNA sequence references for populations of *D. latifolia* from Java and West Nusa Tenggara. The DNA sequence references can be developed into DNA fingerprints of populations/locations for tracing and traceability purposes. This report focussed on the nucleotide variations found in *D. latifolia* from Java and West Nusa Tenggara that will be used as reference data for developing the DNA database for the purpose of trace and traceability.

This report also described the development of a protocol to isolate wood and wood products using several methods.

2. METHODOLOGY

2.1. Leaf samples

2.1.1. Study area and sample collection

DNA material from leaves and wood was collected from mature trees with diameters at breast height (dbh) of above 10 cm. The total number of collection sites was 113 located in Java, Bali, Lombok, and Sumbawa islands during 2020–2022 (**Figure 1**). The field collections were carried out during 14 trips for the duration of 79 days. Within the course of project implementation, there was more than a year of restriction placed on travel and the closing of the laboratory. Hence, the number of trips and the duration of collections were mostly done sporadically, at the end of 2020, early 2021 and end of 2021. The total duration of the laboratory work itself was 10 months of effective work. Five samples of *D. latifolia* from Central Java that were collected in 2019 before the commencement of this project were also included in this study.

Sampling distances between individual trees ranged from 15–20 meters to avoid genotype duplication. All leaf samples were labeled, and preserved in dry silica gels (**Appendix 1**). Wood samples were labeled and wrapped in dry paper. All materials for further molecular analysis were transported to the Molecular Systematic Laboratory of the National Research and Innovation Agency, Indonesia. Voucher specimens were also made from each site, these specimens were identified at the Herbarium Bogoriense.

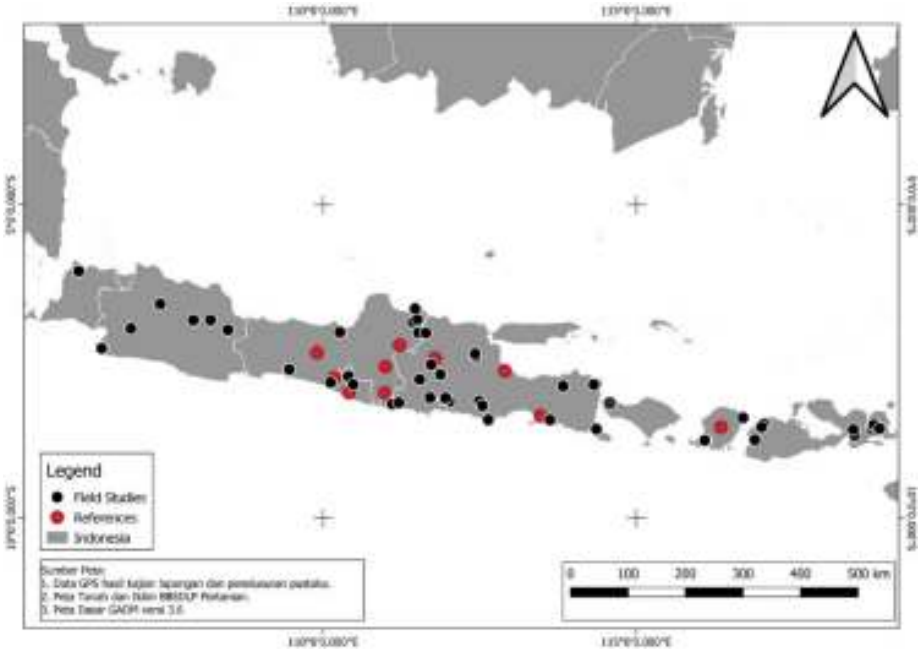


Figure 1. Collection sites for *Dalbergia latifolia* in Java, Bali, Lombok and Sumbawa Islands

2.1.2 Molecular work: DNA isolation, markers screening, PCR amplification and DNA sequencing

The molecular analysis was carried out at the Molecular Systematics Laboratory of the National Research and Innovation Agency, Indonesia. Molecular analysis for this work was done from collected leaf samples as DNA isolation for wood was carried out separately using different protocols. The wood samples were stored at room temperature at the Molecular Systematic Laboratory of the National Research and Innovation Agency, Indonesia. The total genomic DNA from leaves was isolated using the Genomic DNA Mini Kit (Plant) from GeneAid. Twelve molecular markers from non-coding chloroplast regions, i.e., petD – rpoA, CLP, *trn*L intron, *trn*G, rpl16 – rps3, trnV, atpB – rbcL, psbk – trnS, psbA – trnH, rrn16 – trnl, ndhAF – ndhAR, and rps16F – rps16 R were used for screening (**Table 1**), and five (CLP, petD – rpoA, rpl16 – rps3, *trn*L, and *trn*G) were selected to perform PCR amplification and the DNA sequencing in this study (**Table 1**) with details of the nucleotide sequence of each primer combination shown in **Table 2**.

Table 1. List of non-coding markers screened for this study

No	Markers	Reference
1	petD – rpoA IGS	(Nishizawa and Watano, 2000)
2	CLP intron	(Huang *et al*., 1994)
3	*trn*L intron	(Taberlet *et al*., 1991)
4	*trn*G intron	(Shaw *et al*., 2005)
5	rpl16 – rps3 IGS	(Provan *et al*., 2004)
6	trnV IGS	(Shaw *et al*., 2007)
7	atpB – rbcL IGS	(Janssens *et al*., 2006)
8	psbk – trnS IGS	(Lahaye *et al*., 2008)
9	psbA – trnH IGS	(Sang *et al*., 1997)
10	rrn16 – trnI IGS	(López-Ochoa *et al*., 2015)
11	ndhAF – ndhAR IGS	(Pfeil *et al*., 2002)
12	rps16F – rps16 R IGS	(Oxelman *et al*., 1997)

Table 2. Primer sequences for non-coding regions used in this study

No	Marker	5'-3' primer *sequence*
1	CLP intron	
	Forward	5'- AAA AGA ACT RGC AGG TTG GTG -3'
	Reverse	5'- AAA CGY CTA GCA TTC CCT CA -3'
2	IGS petD – rpoA	
	Forward	5'- AAA TTC CAA AAT CCM TTT CGT C -3'
	Reverse	5'- AAT GGA AGT TTA ACY CCT AA -3'
3	IGS rpl16 – rps3	
	Forward	5'- AGT CAC ACA CTR AGC ATA GCA -3'
	Reverse	5'- TCC ACT YGG TTT CAG ACT TGG -3'

4	*trn*L intron	
	Forward	5'- CGA AAT CGG TAG ACG CTA CG -3'
	Reverse	5'- GGG GAT AGA GGG ACT TGA AC-3'
5	*trn*G intron	
	Forward	5'- GCG GGT ATA GTT TAG TGG TAA -3'
	Reverse	5'- GCT TGG AAG GCT AGG GGT TA -3'

The PCR amplification was performed in a volume of 25 µL, containing 1 x PCR master mix (My taq HS Red Mix 2x), 2 µM of forward and reverse primers, and approximately 10 ng of genomic DNA (**Table 3**). Sedi G Thermo Cycler (Wealtec) was used under the following conditions: initial denaturation at 94° C for 3 minutes, then 35 cycles of denaturation at 94° C for 30 seconds, annealing for 30 seconds and extension at 72° C for 1 minute and 30 seconds, followed by a final extension at 72° C for 4 minutes. The annealing temperature was 48–55° C (**Table 4**). The amplified fragments were separated by electrophoresis on 1% agarose gel stained in GelRed Biotium that was run electrophoretically in 0.5x TBE buffer at 100 volts for 30 minutes, then photographed using a gel documentation system (*Bioinstrument*, *ATTO* Biosystems Inc.). The PCR products were multiplicated until they reached the required concentration for DNA sequencing analysis. The PCR products were sent to the 1st Base company to perform Sanger sequencing.

Table 3. PCR mixture composition for leaf sample amplification

Composition	Volume/reaction
My taq HS Red Mix 2x	12.5 µL
Primer Forward	0.5 µL
Primer Reverse	0.5 µL
Nuclease free water	9.5 µL
DNA *template*	2 µL
Total	25 µL

Table 4. PCR condition for 5 non-coding cp markers used in this study

No.	Marker	Pre-denaturation	35 cycles			Final extension
			Denaturation	Annealing	Extension	
1.	CLP intron	94°C = 3'	94°C = 30"	51°C = 30"	72°C = 1'30"	72°C = 4'
2.	petD – rpoA IGS	94°C = 3'	94°C = 30"	48°C = 30"	72°C = 1'30"	72°C = 4'
3.	rpl16 – rps3 IGS	94°C = 3'	94°C = 30"	50°C = 30"	72°C = 1'30"	72°C = 4'
4.	*trn*L intron	94°C = 3'	94°C = 30"	55°C = 30"	72°C = 1'30"	72°C = 4'

5.	*trn*G intron	94°C = 3’	94°C = 30”	49°C = 30”	72°C = 1’30”	72°C = 4’

2.1.3. Data analysis

The contig editor on ATGC software package version 4.3.5 (Genetyx Co., Japan) and Geneious (trial version) were used to assemble the DNA sequence. The forward and reverse sequences were observed to ensure there was no mismatch in the consensus produced. Furthermore, the MEGA 7.0 software was used to evaluate the nucleotide composition of the target markers (Kumar *et al*., 2016). The homology and identity of samples were examined by using the BLAST nucleotide on GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) available in Geneious (Geneious version 2021). Meanwhile, the data from the GenBank were downloaded in FASTA format and aligned using Geneious (trial version).

2.2. Wood samples

2.2.1 DNA isolation

The molecular analysis was carried out at the Molecular Systematics Laboratory of the National Research and Innovation Agency, Indonesia. A total of 65 wood samples were collected and 37 were isolated for their total genomic DNA using the standard CTAB method (Doyle and Doyle 1990) and commercial kits. The standard CTAB methods comprised two techniques, with the addition of RNase and without the addition of RNase. These two techniques were used to check the amount of contamination of RNA in the wood. The commercial kits used were the DNeasy® Plant Mini Kit from QIAGEN and the Genomic DNA Mini Kit (Plant) from GeneAid. Fifteen samples for the isolation method using the CTAB and Genomic DNA Mini Kit (Plant) GeneAid were selected randomly. The following is a detailed procedure for the four methods.

2.2.1.1. CTAB methods without RNase

A mixture of 60 mg of fresh wood chips with quartz sand was ground to a fine powder using a pestle and mortar. The fine powder was then inserted into a 1.5 mL microtube with an additional 700 µL of extraction buffer and 14 µL mercaptoethanol. The samples were then homogenized in a vortex until the whole sample was mixed with the buffer, followed by incubation in a water bath for at least 3 hours at 65° C. The microtubes were inverted every 30 minutes to ensure an evenly homogenized content. When finished, 600 µL of chloroform-isoamyl alcohol (24:1) was added into the microtube. Next, the mixture was centrifuged for 5 minutes at 10,000 rpm. When the contents began to separate and formed layers of supernatant, organic materials, and chloroform, the uppermost layer of the supernatant was transferred to a new microtube using a micropipette. This process was repeated twice. Afterwards, 500 mL of cold isopropanol was added to the supernatant, then mixed and stored in a freezer overnight. Next, the microtube was centrifuged for 5 minutes at 10,000 rpm to form precipitates. The separated fluid was discharged from the microtube and replaced with 500 mL of 70% ethanol, followed by further centrifugation for 2 minutes and another fluid

was discarded. The process was carried out twice. The pellets or DNA precipitates were then dried at room temperature for 30 minutes (with the tube cap opened) before 20 µL of nuclease-free water was added. Finally, the microtubes were flicked and the isolated DNA was subsequently used for PCR amplification.

2.2.1.2. CTAB Methods with RNase

A mixture of 60 mg of fresh wood chips with quartz sand was ground to a fine powder using a pestle and mortar. The fine powder was then inserted into a 1.5 mL microtube with an additional 700 µL of extraction buffer, 14 µL mercaptoethanol, and 0.0008 g polyvinylpyrrolidone (PVP). The samples were then homogenized in a vortex until the whole sample was mixed with the buffer, followed by incubation in a water bath for at least 3 hours at 65° C. The microtubes were inverted every 30 minutes to ensure an evenly homogenized content. When completed, 600 µL of chloroform-isoamyl alcohol (24:1) was added into the microtube. Next, the mixture was centrifuged for 5 minutes at 10,000 rpm. When the contents began to separate and formed layers of supernatant, organic materials, and chloroform, the uppermost layer of the supernatant was transferred to a new microtube using a micropipette. This process was repeated twice. Afterwards, 500 mL of cold isopropanol was added to the supernatant, then mixed and stored in a freezer overnight. Next, the microtube was centrifuged for 5 minutes at 10,000 rpm to form precipitates. The separated fluid was discharged from the microtube and replaced with 500 mL of 70% ethanol, followed by further centrifugation for 2 minutes and another fluid was discarded. The process was carried out twice. The pellets or DNA precipitates were then dried at room temperature for 30 minutes (with the tube cap opened), before 20 µL of nuclease-free water and 4 µL RNAse was added. At this stage of the process, the microtubes were flicked and the isolated DNA was subsequently used for PCR amplification.

2.2.1.3 DNeasy® Plant Mini Kit

Wood samples (20 mg lyophilized tissue) were disrupted using a mortar and pestle. The procedure for isolation had followed the manufacturer's instructions (HB-0542-003+1101205_PCard_DNY_Plant_Spi).

2.1.1.4. Genomic DNA Mini Kit (Plant) GeneAid

The process of DNA isolation using the GeneAid kit had followed the manufacturer's instructions.

2.2.2 DNA quantification

The quality of the genomic DNA was tested by agarose electrophoresis 1% (w/v). As much as 2 µL DNA was loaded to the gel and the electrophoresis was run at 100 volts for 30 minutes. The electrophoretic gel was stained with GelRed Biotium and then photographed using a gel documentation system (*Bioinstrument*, *ATTO* Biosystems Inc.) to check the presence of DNA bands. In addition, the concentration of DNA extracts was measured using a nanodrop

(Implen Nanophotometer version-7122 V2.30). The amount of extract used was 1 µL per sample and measured at a wavelength of 260 nm. The DNA purity measurements were carried out to estimate the presence of contaminants in the DNA, where the purity was measured at wavelengths of 260/280 nm.

2.2.3. PCR amplification

Two molecular markers from non-coding chloroplast regions, i.e., *trn*L intron and *trn*G intron were used for the DNA amplification test. The details of nucleotide polymorphism of primers *trn*L intron and *trn*G intron are shown in **Table 2**. The PCR amplification was performed in a volume of 14.5 µL and the PCR mixture composition are shown in **Table 5**. The PCR condition for wood samples was the same with PCR condition for leaf samples (**Table 4**), except for the annealing temperature. The annealing temperature was 55°C for *trn*L intron and 49°C for *trn*G intron. The electrophoresis process for wood is the same as the leaf electrophoresis process. The PCR products were sent to the 1st Base company to perform Sanger sequencing.

Table 5. PCR mixture composition for wood sample amplification

Composition	Volume/reaction
KOD Buffer FX Neo	6.25 µL
dNTPs	2.5 µL
Primer Forward	0.375 µL
Primer Reverse	0.375 µL
Taq Polymerase	0.25 µL
Nuclease free water	2.75 µL
DNA *template*	2 µL
Total	14.5 µL

3. RESULTS AND DISCUSSION

3.1. Leaf samples

3.1.1. Collected samples, isolated DNA, and PCR amplification of five non-coding chloroplast regions

The total number of collected DNA materials was 551, and 192 were used for subsequent analysis. These materials were collected from 113 locations in Java, Bali, Lombok, and Sumbawa, with the majority collected from Java, and the least number collected from Bali (**Table 6** and **Appendix 1**). Different numbers of collections may reflect the different degrees of the abundance of *D. latifolia* stands.

Table 6. List of numbers of samples collected from Java, Bali, Lombok and Sumbawa Island

	Java	Bali	Lombok	Sumbawa
Number of collected samples	409 (344 Leaves + 65 Woods)	12 leaves	51 leaves	79 leaves

Number of samples for molecular work	109 (96 Leaves + 13 Woods)	6 leaves	26 leaves	51 leaves
Number of locations	45 (37 Leaves + 8 woods)	3 leaves	9 leaves	35 leaves

The DNA isolation was conducted for a small amount from ~20 mg of dried silica leaf. There was no obvious obstacle in isolating DNA from leaf samples collected from the field since they were relatively in good condition with almost no appearance of contamination except for a few samples showing smear bands due to RNA contamination (**Figure 2**).

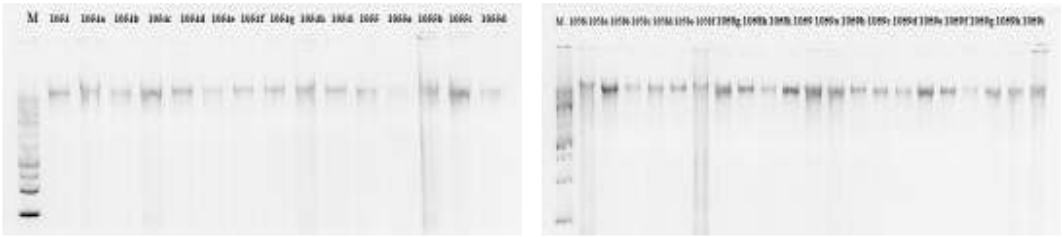
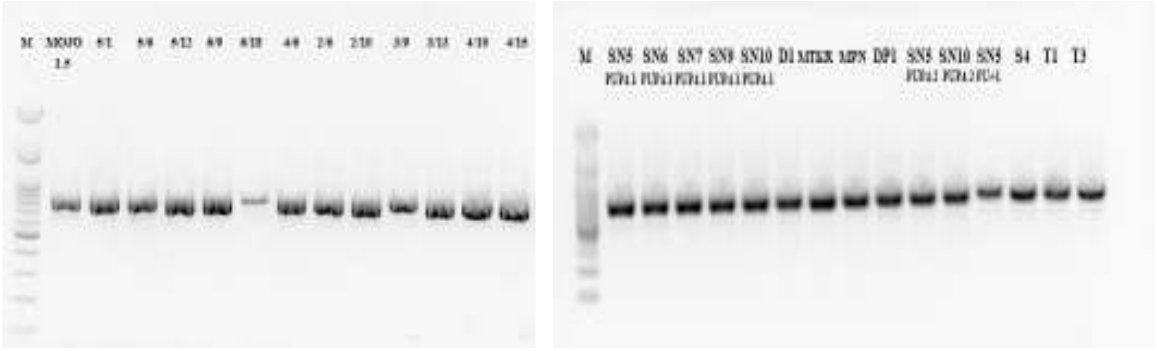


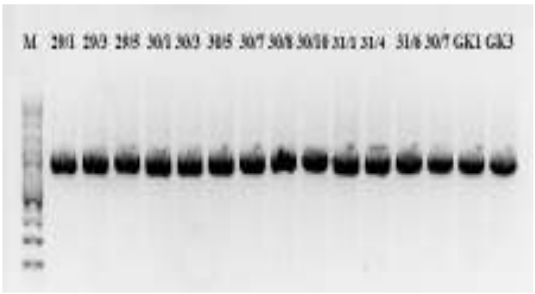
Figure 2. Representative photos from agarose gel electrophoresis of isolated total genomic DNA of *Dalbergia latifolia*

The isolated DNA was then used as a template for PCR amplification using the five selected non-coding chloroplast regions. Prior to this, markers screening was performed on 12 non-coding chloroplast regions (**Table 1**) amplified on 28 samples representing the putative populations. The populations were divided to represent geographic and climatic aspects. The screening process had been conducted a maximum of three times to ensure the consistency of the amplified products. Five markers that mostly worked for the representative samples were selected.

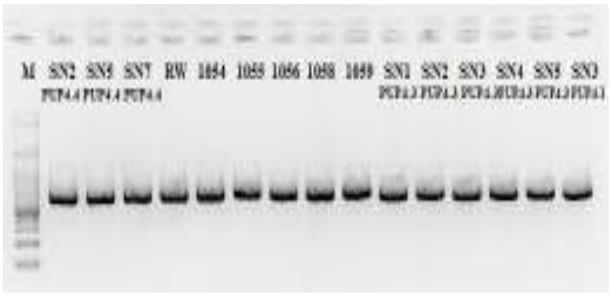
The PCR amplification was further performed on 179 samples and successfully resulted in good PCR products as shown by clear single bands (**Figure 3**). The PCR products were directly sequenced using the Sanger method (Sanger *et al.,* 1977). Four samples were omitted from further analysis due to their unclear results of DNA sequencing.



(a) CLP intron

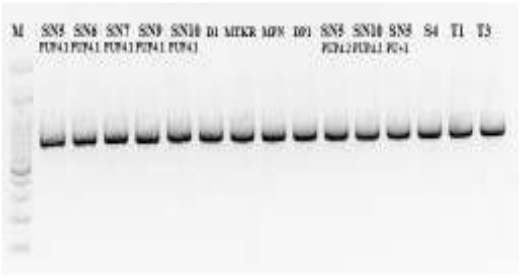


(b) petD intron



(c) rpl16 – rps3 intergenic spacer

(d) *trn*L intron



(e) *trn*G intron

Figure 3. Representative photos from agarose gel electrophoresis of PCR products of (a) CLP intron, (b) petD intron, (c) rpl16 – rps3 intergenic spacer, (d) *trn*L intron, and (e) *trn*G in some samples of *Dalbergia latifolia*

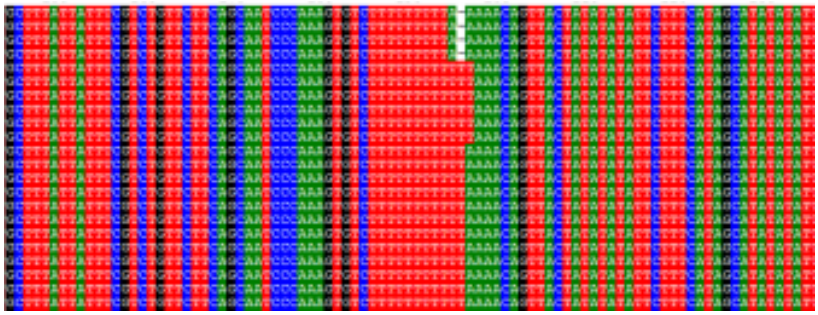
3.1.2. The sequence homology and identity of *Dalbergia latifolia* from Indonesia

Of the five markers, the longest amplicon size was yielded by the rpl16 – rps3 intergenic spacer (789 bp), followed by *trn*G intron (722 bp), petD intron (691 bp), and *trn*L intron (590 bp), while the shortest was in CLP intron (401 bp) (**Table 7**). All the five markers used were A/T rich-region (**Table 7**). The highest number of polymorphic sites was found in rpl16 – rps3 intergenic spacer with 4 nucleotide substitution and 1 indel, and the lowest was found in CLP intron and *trn*L intron with 2 nucleotide substitution and 0 indel (**Table 7**). The results of multiple sequence alignment and single nucleotide polymorphism by some regions in this study are showed in **Figure 4**. In addition, these five markers are recommended to be used for mass amplification to support *D. latifolia* genetic diversity and population structure studies in the future.

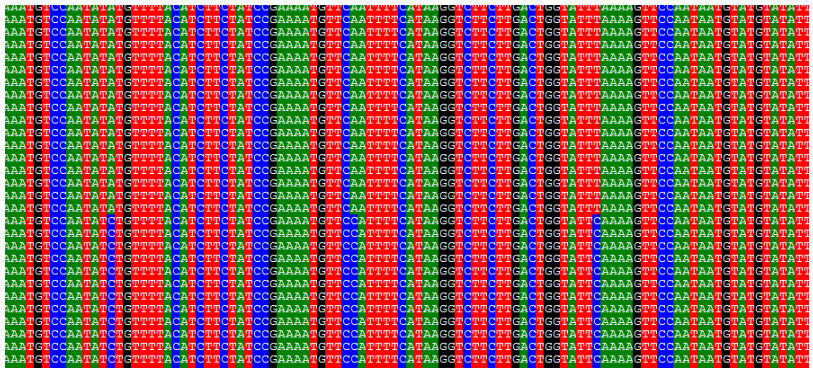
Table 7. The marker sizes and nucleotide polymorphisms

No	Marker	Size (bp)	AT/GC rich	Number of polymorphic sites	
				Nucleotide substitution	Indels event
1	CLP intron	401	71.6/28.4	2	–

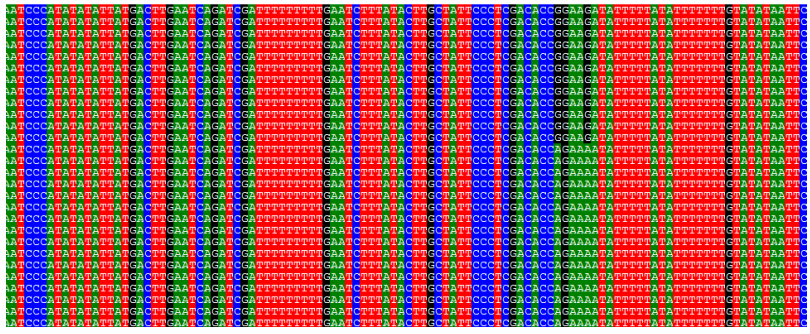
2	petD – rpoA IGS	691	70.4 / 29.6	3	1
3	rpl16 – rps3 IGS	789	70 /30	4	1
4	*trn*L intron	590	64.3 / 35.6	2	–
5	*trn*G intron	722	69 / 31	1	3



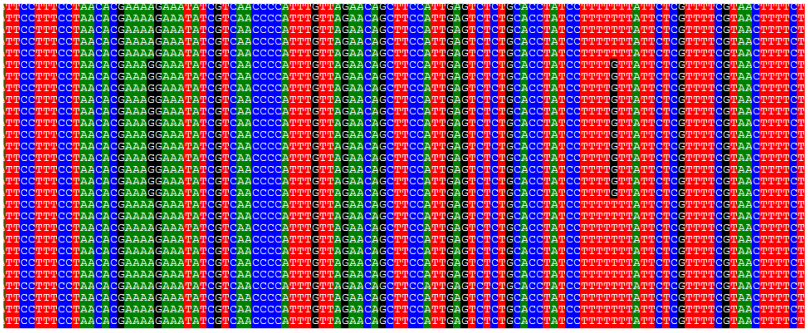
(a) CLP intron



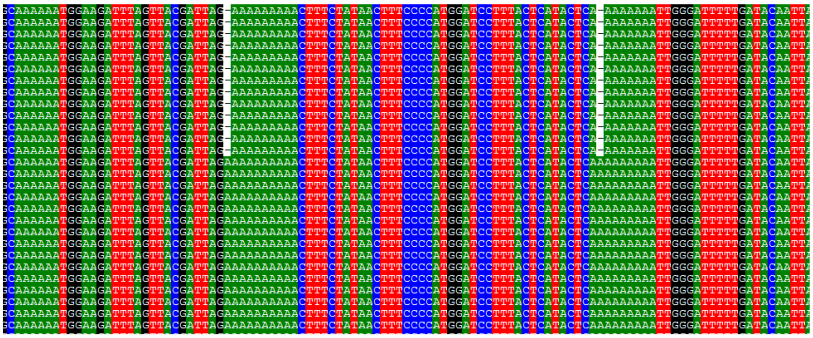
(b) IGS petD – rpoA



(c) IGS rpl16 – rps3



(d) *trn*L intron



(e) *trn*G intron

Figure 4. Representative regions of DNA sequence alignment of (a) CLP intron, (b) IGS petD – rpoA, (c) IGS rpl16 – rps3, (d) *trnL* intron, and (e) *trn*G intron showing nucleotide polymorphisms in some samples of *Dalbergia latifolia*

The DNA sequences were checked for their identity by performing BLAST nucleotide available from the GenBank database. Among the 5 markers used in this study, only *trn*L intron was available for *D. latifolia*. The remaining 4 markers had not been sequenced from *D. latifolia* yet. Thus, these 4 markers would be the new reference markers of *D. latifolia* from Indonesia. The DNA sequence of the *trn*L intron was similar to the *D. latifolia* accession no. MH547571 from Bogor, Indonesia (Lee *et al*., 2019), with 100 % similarity. Thus, confirming the identity of *D. latifolia* in the sample used in the study. The highest similarity of the DNA sequence of CLP intron, *trn*G intron and IGS rpl16 – rps3 was similar to *D. cochinchinensis* from Hong Kong (Wu *et al.,* 2022), while the IGS petD – rpoA was 98.6% similar to *D. sisso* from Hainan Province, South China (Song *et al*., 2019) (**Table 8**).

Table 8. Homology of DNA sequencing of *Dalbergia latifolia* with other *Dalbergia* species available in the GenBank database

No	Region	Similarity	GenBank Accession	% Similarity	Location	Reference
1	*trn*L intron	*Dalbergia latifolia*	MH547571	100	Bogor Indonesia	Lee *et al*., 2019

		Dalbergia cochinchinensis	AB850632	99.1	Cambodia	Moritsuka *et al*., 2017
		Dalbergia obtusifolia	NC_063303	98.8	Hong Kong	Wu *et al.,* 2022
		Dalbergia cochinchinensis	NC_058539	98.6	Hong Kong	Wu *et al.,* 2022
		Dalbergia cochinchinensis	MN251247	98.6	Hainan, South China	Song *et al*., 2019
2	CLP intron	*Dalbergia cochinchinensis*	NC_058539	97.8	Hong Kong	Wu *et al.,* 2022
		Dalbergia cochinchinensis	MN251247	97.5	Hainan, South China	Song *et al*., 2019
		Dalbergia hupeana	MN251245	97.5	Hainan, South China	Song *et al*., 2019
		Dalbergia obtusifolia	NC_063303	97.5	Hong Kong	Wu *et al.,* 2022
		Dalbergia sissoo	MN251242	97.5	Hainan, South China	Song *et al*., 2019
3	IGS petD–rpoA	*Dalbergia sissoo*	MN251242	98.6	Hainan, South China	Song *et al*., 2019
		Dalbergia cochinchinensis	NC_058539	98.4	Hong Kong	Wu *et al.,* 2022
		Dalbergia hupeana	MN251245	98.4	Hainan, South China	Song *et al*., 2019
		Dalbergia obtusifolia	NC_063303	98.4	Hong Kong	Wu *et al.,* 2022
		Dalbergia cochinchinensis	MN251247	98.3	Hainan, South China	Song *et al*., 2019
4	*trn*G intron	*Dalbergia cochinchinensis*	NC_058539	99.4	Hong Kong	Wu *et al.,* 2022
		Dalbergia cochinchinensis	MN251247	99.4	Hainan, South China	Song *et al*., 2019
		Dalbergia hupeana	MN251245	99.4	Hainan, South China	Song *et al*., 2019
		Dalbergia sissoo	MN251242	99.4	Hainan, South China	Song *et al*., 2019
		Dalbergia chlorocarpa	NC_049047	98.9	Hong Kong	Wu *et al.,* 2022

5	IGS rpl16 – rps3	*Dalbergia cochinchinensis*	NC_058539	99.4	Hong Kong	Wu *et al.,* 2022
		Dalbergia cochinchinensis	MN251247	99.4	Hainan, South China	Song *et al*., 2019
		Dalbergia hupeana	MN251245	99.4	Hainan, South China	Song *et al*., 2019
		Dalbergia sissoo	MN251242	99.4	Hainan, South China	Song *et al*., 2019
		Dalbergia obtusifolia	NC_063303	99.4	Hong Kong	Wu *et al.,* 2022

The results of the DNA sequencing were stored in the database system, and available upon request for non-commercial purposes.

3.2. Wood samples

The DNA extraction from woods of *D. latifolia* was complicated due to the abundance of secondary metabolites. A previous study by Ashraf *et al*., (2010) compared three modifications of the *Dalbergia* DNA extraction method, namely, Yang & Kang (2004), Dellaporta (1983), and Doyle & Doyle (1990). In comparison to the three protocols used to analyze the preserved leaves as described by Doyle & Doyle (1990), the best DNA quality produced was further tested by successful amplification of PCR for these accessions. This method is applicable to both, fresh leaves and herbarium specimens. Generally, fresh leaves produced better quality DNA although some level of degradation had been observed in the preserved samples during DNA extraction. The results described by Yang & Kang (2004) and Dellaporta (1983) showed the extracted DNA was yellow to dark brown in colour. The DNA yield was not of the required quality and quantity due to the abundance of secondary metabolites. Consequently, due to the unsatisfactory results after quantification, there was no PCR amplification undertaken, probably due to the high degradation of the samples.

According to Jobes *et al*., (1995), in the presence of PVP, phenolics adhere to DNA in solution forming a coloured extract around the DNA that can be removed after several washings only. The addition of a high molar concentration of sodium chloride (NaCl) increases the solubility of polysaccharides in ethanol which effectively decreases the co-precipitation of the polysaccharides and DNA (Fang *et al*., 1992). However, the protocol by Doyle & Doyle (1990) demonstrated desirable results in *D. latifolia*.

The extraction of genetic material from wood is very rare, not only because it is difficult to do but also because the wood contains fewer living cells, especially in dry wood and processed wood products. It is easier to extract genetic material from the leaves. However, the extraction of genetic material from wood needs to be carried out for forensic purposes of illegal logging or illegal trade (Rachmayanti *et al*., 2009). The cross-section of a tree generally consists of the cambium which is mainly a composite of living cells, while the sapwood is a

composite of living and dead tissue, and the heartwood is composite of dead tissue with small fragments still adhered to the cells wall (Wiedenhoeft, 2010). DNA extraction from the cambium and sapwood showed higher efficacy than the heartwood (Tnah *et al.,* 2011).

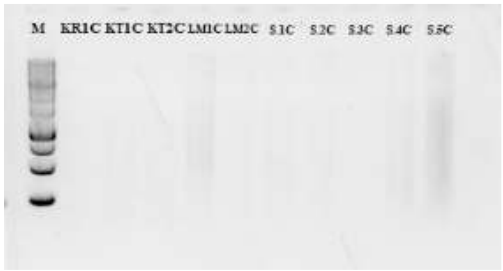
Generally, the wood in trade is not fresh wood and the wood products are from the heartwood, therefore the genetic material from wood is very small in quantity and of low quality. The selection of the proper extraction method can provide better results, improve the extracted DNA quality, and provide a higher chance of success in molecular analysis (Handayani *et al.,* 2016*).* The *Dalbergia latifolia* wood samples, especially the heartwood, were extracted using four methods, namely, CTAB without RNAse, CTAB with RNAse, DNeasy Plant Mini Kit (QIAGEN), and GeneAid kit.

3.2.1. DNA quality and quantity

The isolated DNA from the total genome was checked for its quality using the agarose gel and its quantity using a nanodrop. Extraction of the DNA using the CTAB method (**Figure 5a and b**) showed that not all samples revealed target bands in gel electrophoresis. The addition of RNase (**Figure 5b**) suggested that no contamination of RNA appeared in the samples. These were shown in **Figure 5b** where no smear was apparent from the gel photo. The smear in the gel electrophoresis indicated that the result from the extraction was not fully pure as they contained other materials. In addition, the electrophoretic gel from the extraction using the KIT extraction generally did not show the bands (**Figure 5c and d**), while the extraction using the Qiagen DNeasy Plant Mini Kit showed obscure bands from some samples, and the extraction using the GeneAid kit did not show any band in the electrophoretic gel.

(a) CTAB without RNAse

(b) CTAB with RNAse



(c) DNeasy® Plant Mini Kit

(d) Genomic DNA Mini Kit (Plant) GeneAid

Figure 5. Representative photos of agarose gel electrophoresis contained isolated DNA using methods of (a) CTAB without RNAse, (b) with RNAse, (c) DNeasy Plant Mini Kit (QIAGEN), and (d) Genomic DNA Mini Kit (Plant) GeneAid

After the extraction to check for the quality and purity of the wood DNA, the DNA extracted from the wood was tested using the nanodrop test. The ratio of absorbance at 260 and 280 nm was used to assess the DNA purity (Glasel 1995). The purity of the extracted DNA from wood samples varied as determined by the A260/A280 and generally had consistently low values (1.65 or less indicates protein contamination). A ratio of ~1.8 was generally accepted as ''pure'' for DNA (Hassan *et al.*, 2015). If the ratio was appreciably lower (<1.6), it might indicate the presence of proteins, phenol, or other contaminants that absorb strongly at or near 280 nm. The 260/230 ratio was widely used as a secondary measure of DNA purity (Usman *et al*., 2014; Aleksic *et al*., 2012). The expected 260/230 values for ''pure'' DNA were commonly within the range of between 2.0 and 2.2. If the ratio was appreciably lower than expected, it might indicate the presence of contaminants that absorb at 230 nm such as proteins (Liu *et al*., 2009), guanidine HCL (used for DNA isolations), EDTA, carbohydrates, lipids, salts, or phenol (Stulnig & Amberger 1994). The nanodrop test for the extraction using CTAB in both without RNAse (**Table 9**) and with RNAse (**Table 10**) showed that the five samples had low concentration, while the other samples were not pure and had <1.8 in 260/280 ratio, showing that the DNA contained other materials. But when using the kit extraction of both the Qiagen DNeasy Plant Mini Kit (**Table 11**) and GeneAid kit (**Table 12**), many samples had a low concentration of DNA and only some samples showed results from the nanodrop test.

Table 9**.** The nanodrop test of extraction product of CTAB without RNAse

No	Sample code	Concentration C (ng/µl)	Lid Factor	Wavelength (nm)				DNA Purity Rations	
				A230	A260	A280	A320	A260/A280	A260/A230
1	1			DNA concentration too low					
2	2	1512	50	0.947	0.605	0.452	0.265	1.337	0.639
3	3	597	50	0.683	0.239	0.164	0.068	1.456	0.350
4	4			DNA concentration too low					
5	5	63.7	10	0.160	0.127	0.078	0.003	1.641	0.795
6	KR1C	2044	50	1.235	0.817	0.630	0.383	1.297	0.662
7	KT1C	3444	50	1.777	1.378	1.079	0.614	1.277	0.775
8	PWL.1C	1765	50	1.217	0.706	0.600	0.330	1.176	0.580
9	LM.1C	148	50	0.027	0.059	0.037	0.010	1.605	2.179
10	LM.2C	1083	50	0.668	0.433	0.318	0.129	1.360	0.648
11	S.1C			DNA concentration too low					
12	S.3C	350	10	1.135	0.700	0.568	0.356	1.233	0.617
13	S.4C	97.6	10	0.231	0.195	0.144	0.071	1.352	0.845

14	S.4B	DNA concentration too low
15	S.1B	DNA concentration too low

Table 10. The nanodrop test of extraction product of CTAB with RNAse

No	Sample code	Concentration C (ng/µl)	Lid Factor	Wavelength (nm)				DNA Purity Rations	
				A230	A260	A280	A320	A260/A280	A260/A230
1	1			DNA concentration too low					
2	2	530	10	1.549	1.061	0.839	0.466	1.265	0.685
3	3	64.2	10	0.312	0.128	0.094	0.049	1.372	0.412
4	4			DNA concentration too low					
5	5	40.3	10	0.123	0.081	0.040	0.000	2.025	0.659
6	KR1C	925	50	0.547	0.370	0.296	0.177	1.249	0.677
7	KT1C	1175	50	0.786	0.470	0.357	0.200	1.315	0.598
8	PWL1C	1529	50	1.035	0.612	0.515	0.280	1.189	0.591
9	LM.1C	33.4	10	0.065	0.067	0.049	0.020	1.367	1.031
10	LM.2C	133	10	0.421	0.266	0.198	0.097	1.342	0.631
11	S.1C			DNA concentration too low					
12	S.3C	400	10	1.312	0.800	0.650	0.404	1.230	0.610
13	S.4C	36.4	10	0.108	0.073	0.053	0.024	1.377	0.676
14	S.4B			DNA concentration too low					
15	S.1B			DNA concentration too low					

Table 11. The nanodrop test of extraction product of DNeasy Plant Mini Kit (QIAGEN)

No	Sample code	Concentration C (ng/µl)	Lid Factor	Wavelength (nm)				DNA Purity Rations	
				A230	A260	A280	A320	A260/A280	A260/A230
1	1			DNA concentration too low					
2	2	68.0	50	0.048	0.027	0.020	0.010	1.333	0.571
3	3			DNA concentration too low					
4	4			DNA concentration too low					
5	5			DNA concentration too low					
6	KR1C			DNA concentration too low					
7	KT.1C	11.0	10	0.043	0.022	0.016	0.008	1.375	0.512
8	PWL.1C			DNA concentration too low					
9	LM.1C			DNA concentration too low					
10	LM.2C			DNA concentration too low					
11	S.1C	6972	10	0.030	0.014	0.011	0.004	1.273	0.467
12	S.3C			DNA concentration too low					
13	S.4C	9462	10	0.029	0.019	0.012	0.004	1.583	0.655
14	S.4B			DNA concentration too low					
15	S.1B	9462	10	0.046	0.019	0.011	0.005	1.727	0.413
16	M.1C	6972	10	0.072	0.014	0.010	0.005	1.400	0.194

No	Sample code	Concentration C (ng/µl)	Lid Factor	Wavelength (nm)				DNA Purity Rations	
				A230	A260	A280	A320	A260/A280	A260/A230
17	M.2C			DNA concentration too low					
18	M.3C			DNA concentration too low					
19	M.4C	7968	10	0.027	0.016	0.012	0.004	1.333	0.593
20	PWL.2C	13.4	10	0.064	0.027	0.024	0.013	1.125	0.422
21	PWL.3C	12.9	10	0.048	0.026	0.019	0.008	1.368	0.542
22	PWL.4C	9462	10	0.040	0.019	0.016	0.007	1.188	0.475
23	PWL.5C			DNA concentration too low					
24	PWL.6C			DNA concentration too low					
25	PWL.7C	12.9	10	0.057	0.026	0.021	0.011	1.238	0.456
26	PWL.8C			DNA concentration too low					
27	PWL.9C			DNA concentration too low					
28	PWL.10C			DNA concentration too low					
29	KT.2C			DNA concentration too low					
30	S.2C			DNA concentration too low					
31	S.5C	18.9	10	0.063	0.038	0.023	0.009	1.652	0.603
32	LM.1B			DNA concentration too low					
33	KR.1B	60.8	10	0.202	0.122	0.102	0.063	1.196	0.601
34	M.2B	14.9	10	0.045	0.030	0.021	0.008	1.429	0.667
35	KT.1B	31.4	10	0.080	0.063	0.043	0.015	1.465	0.787
36	PWL.9B	16.9	10	0.062	0.034	0.020	0.009	1.700	0.548
37	6CV	7968	10	0.022	0.016	0.013	0.007	1.231	0.727

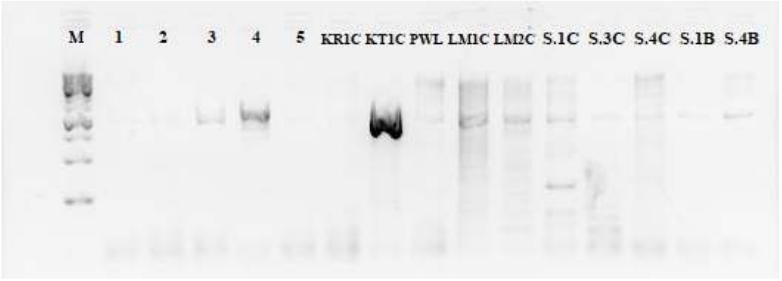
Table 12. The nanodrop test of extraction product of GeneAid Kit

No	Sample code	Concentration C (ng/µl)	Lid Factor	Wavelength (nm)				DNA Purity Rations	
				A230	A260	A280	A320	A260/A280	A260/A230
1	1			DNA concentration too low					
2	2	35.9	10	0.124	0.072	0.075	0.027	0.960	0.581
3	3			DNA concentration too low					
4	4			DNA concentration too low					
5	5			DNA concentration too low					
6	KR1C	42.8	10	0.117	0.086	0.069	0.044	1.246	0.735
7	KT1C	15.4	10	0.053	0.031	0.021	0.010	1.476	0.585
8	PWL.1C	45.3	10	0.147	0.091	0.080	0.047	1.137	0.615
9	LM.1C	7470	10	0.019	0.015	0.013	0.007	1.154	0.789
10	LM.2C			DNA concentration too low					
11	S.1C	10.5	10	0.085	0.021	0.020	0.006	1.050	0.247
12	S.3C			DNA concentration too low					
13	S.4C			DNA concentration too low					
14	S.4B			DNA concentration too low					
15	S.1B			DNA concentration too low					

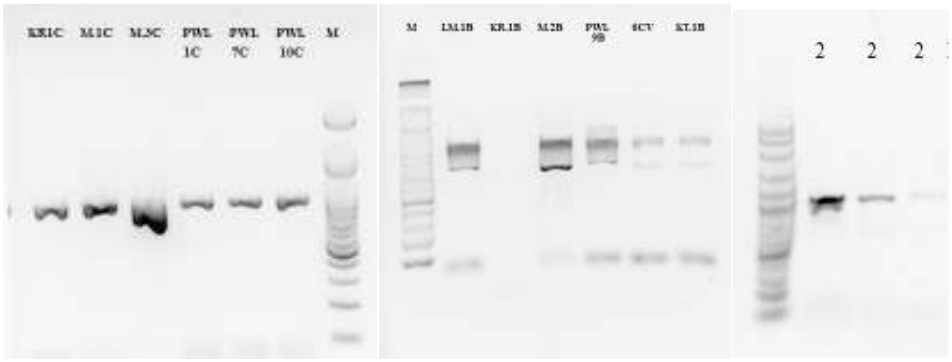
3.2.2. PCR amplification

The isolated DNA from the 4 methods was checked and verified by performing PCR amplification of several markers of non-coding chloroplast (cp) genome, i.e., *trn*L intron, *trn*G intron, petD intron, CLP intron, psbA – trnH IGS, and rpl16 – rps3 IGS. Amplification using the template DNA from the CTAB extraction method showed a bad result because it did not show a DNA band, except for the sample code LM.1C (**Figure 6**). Amplification using the template DNA from the commercial kit showed a better result. The PCR amplification using the Qiagen DNeasy Plant Mini Kit (QIAGEN) and from the Genomic DNA Mini Kit (Plant) GeneAid showed a higher success in *trn*G intron (**Figure 7b and Figure 8a**). Generally, all the PCR products from these commercial kits showed clear bands and mostly single copies. However, the PCR product of *trn*L intron (**Figure 7a and Figure 8b**) showed multiple bands, thus PCR condition and PCR mix need to be optimized. The Genomic DNA Mini Kit (Plant) GeneAid failed to amplify the psbA – trnH IGS (**Figure 8c**). **Figure 9** showed the PCR amplification of DNA extracted through the protocols DNeasy Plant Mini Kit (QIAGEN) and Genomic DNA Mini Kit (Plant) GeneAid where the DNA was amplified with rpl16 – rps3 IGS primers and the result was positive with clear bands. This indicated that the amplified DNA from *D. latifolia* wood using the Kit extraction yielded better quality than the amplified DNA using CTAB extraction.

Figure 6**.** Representative photos from agarose gel electrophoresis of PCR products of *trnL* intron in some samples of *Dalbergia latifolia* using DNA template extracted using the CTAB method



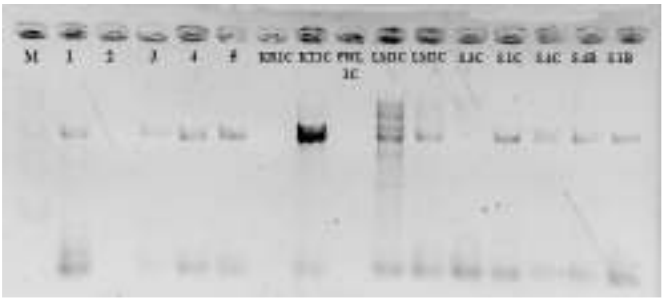
(a) *trn*L intron



(b) *trn*G intron

Figure 7. Representative photos from agarose gel electrophoresis of PCR products of (a) *trnL* intron, and (b) *trn*G in some samples of *Dalbergia latifolia* using DNA template extracted using the DNeasy Plant Mini Kit (QIAGEN)

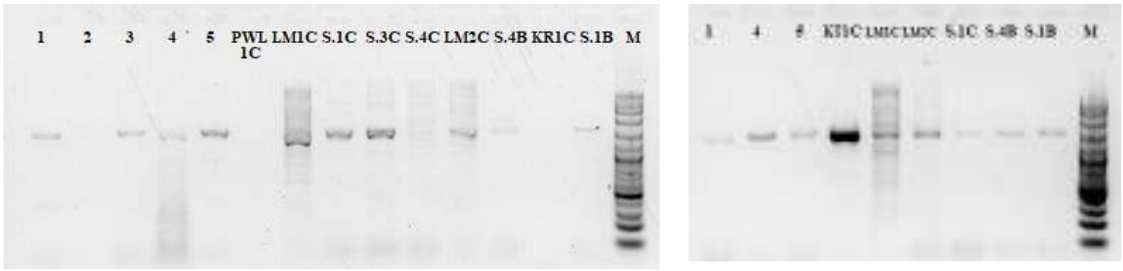
(a) *trn*G intron



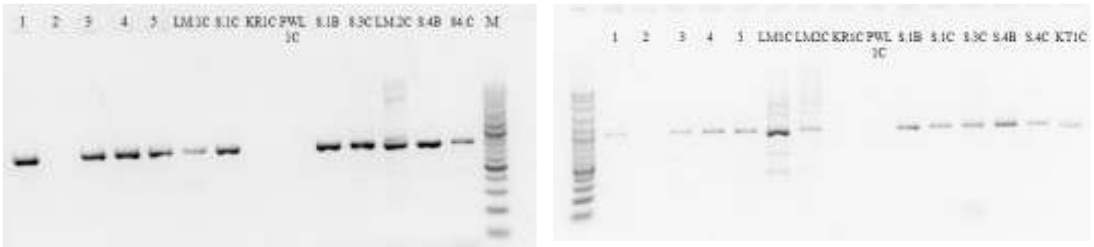
(b) *trn*L intron

(C) psbA – trnH IGS

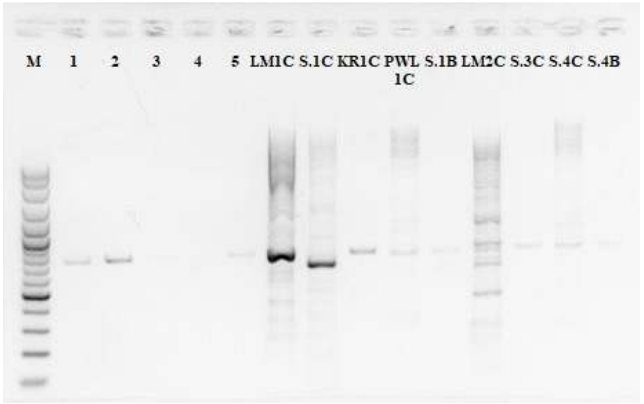
Figure 8. Representative photos from agarose gel electrophoresis of PCR products of (a) *trn*G, (b) *trnL* intron, and (c) psbA – trnH in some samples of *Dalbergia latifolia* using DNA template extracted using the Genomic DNA Mini Kit (Plant) GeneAid



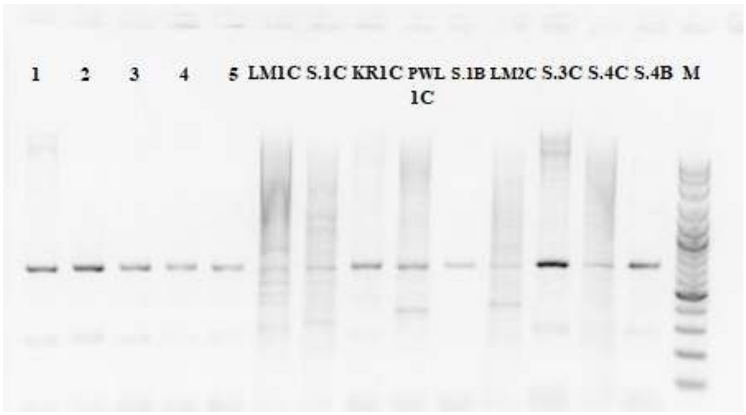
(a) *trn*L intron



(b) rpl16 – rps3 IGS



(c) petD intron



(d) CLP intron

Figure 9. Representative photos from agarose gel electrophoresis of PCR products of (a) *trnL* intron, (b) rpl16 – rps3, (c) petD, and (d) CLP in some samples of *Dalbergia latifolia* using DNA template extracted using the *D*Neasy Plant Mini Kit (QIAGEN) and Genomic DNA Mini Kit (Plant) GeneAid

4. CONCLUSIONS

The total number of 192 genomic DNA from *Dalbergia latifolia* consisting of 179 leaves and 65 wood samples were collected from 113 locations in Java, Bali, Lombok, and Sumbawa were successfully amplified by five selected markers (CLP intron, petD – rpoA, rpl16 – rps3, *trn*L, and *trn*G) as indicated by the good quality of their PCR products. Among the five markers, the rpl16 – rps3 intergenic spacer showed a good performance as indicated by the length of the sequence (789 bp) and the high number of polymorphic sites.

REFERENCES

Adema, F., Ohashi H, & Sunarno B. (2016). Notes on Malesian Fabaceae 17. The Genus *Dalbergia*. Blumea 61, 186–206.

Aleksic, J.M., Stojanović, D., Banović, B., Jančić, R. (2012). A simple and efficient DNA isolation method for *Salvia officinalis*. *Biochem Genet*. 50, 881– 892.

Arisoesilaningsih, E., & Soejono, S. (2015). Diversity of drought-resistant plants and the benefits of their biomass for improving fertility of degraded soil of Brantas River Basin. *J Degraded Mining Land Manag* 2(2), 313-318.

Ashraf, M., Mumtaz, A.S., Riasat, R., & Tabassum, S. (2010). A molecular study of genetic diversity in Shisham (*Dalbergia sissoo*) plantation of NWFP, Pakistan. *Pakistan Journal of Botany* 42, 79–88.

Atikah, T.D. & Dede S. A. (2018). Population study and trade of *Dalbergia latifolia* in Lombok, West Nusa Tenggara Province. Field report Research Centre for Biology, Indonesian Institute of Sciences.

Barrett, M.A., Brown, J.L., Morikawa, M.K., Labat, J. & Yoder, A.D. (2010). CITES Designation for endangered rosewood in Madagascar. *Science* 328, 1109–1110.

Bioinstrument, *ATTO* Biosystems Inc., Warington, UK.

Cardoso, D., Pennington, R.T., de Queiroz, L.P., Boatwright, J.S., Van Wyk, B.E., Wojciechowski, M.F., & Lavin, M. (2013). Reconstructing the deep-branching relationships of the papilionoid legumes. *South Afr J Bot* 89, 58-75.

Dellaporta, S.L., Wood, J., Hicks, J.B. (1983). A plant DNA minipreparation : Version II. *Plant Molecular Biology Reporter* 1, 19–21. https://doi.org/10.1007/BF02712670

Doyle, J.J. & Doyle, J.L. (1990). Isolation of Plant DNA from fresh tissue. *Focus* 12, 13-15.

Fang, G., Hammer, S., & Grumet, R. (1992). A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *Biotechniques* 13, 52–54.

Glasel, J.A. (1995). Validity of nucleic acid purities monitored by 260 nm/280 nm absorbance ratios. *Biotechniques*.;18: 62–63.

Handayani, F., Wulandari, R., & Murti, R. (2016). Genomic DNA extraction method from mature leaf of lai (*Durio kutejensis* Becc.). *AGRIVITA Journal of Agricultural Science*, 38. 10.17503/agrivita.v38i1.659.

Hani, A., & Suryanto, P. (2014). The dynamics of agroforestry in the Hills of Manoreh, Kulon Progo, Yogyakarta Province. *J Penelitian Kehutanan Wallacea* 3(2), 119–128. [Indonesian]

Hassan, R., Husin, A., Sulong, S., Yusoff, S., Farid, J., Yahaya, B., Ang, C., Ghazali, S., & Cheong, S. (2015). Guidelines for nucleic acid detection and analysis in haematological disorders. *Malays J Pathol* 37, 165–173.

Hassold, S., Lowry, P.P., Bauert, M.R., & Razafintsalama, A. (2016). DNA barcoding of Malagasy rosewoods: towards a molecular identification of CITES-listed *Dalbergia* species. *PLoS One* 11, e0157881. DOI: 10.1371/journal.pone.0157881

https://blast.ncbi.nlm.nih.gov/Blast.cgi

https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:490295-1

https://www.gbif.org

Huang, C., Wang, S., Chen, L., Lemieux, C., Otis, C., Turmel, M., & Liu, X. Q. (1994). The *Chlamydomonas* chloroplast clpP gene contains translated large insertion sequences and is essential for cell growth. *MGG Molecular & General Genetics* 244(2), 151–159. https://doi.org/10.1007/BF00283516

Janssens, S., Geuten, K., Yuan, Y.M., Song, Y., Kupfer, P., & Smets, E. (2006). Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast atpB-rbcL spacer sequences. Syst Bot 31(1), 171–180.

Jobes, D.V, Hurley, D.L, & Thien, L.B. (1995). Plant DNA isolation : A method to efficiently remove polyphenolics, polysaccharides, and RNA. *Taxon* 44, 379–386.

Kang, T.J., & Yang, M.S. (2004). Rapid and reliable extraction of genomic DNA from various wild-type and transgenic plants. *BMC Biotechnology* 4, 20. https://doi.org/10.1186/1472-6750-4-20

Karlinasari, L., Nawawi, D.S. & Widyani, M. (2010). Study of the anatomy and wood chemistry in relation to the acoustic properties of wood. *Bionatura* 12(3), 110–116. [Indonesian]

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* 33(7),1870–1874.

Lahaye, R.R.Y., Van Der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T.G., Savolainen, V. (2008). DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences*, USA, 105, 2923–2928.

Lakhey, P., Pathak, J., & Adhikari, B. (2020). *Dalbergia latifolia*. The IUCN Red List of Threatened Species e.T32098A67777757. https://dx.doi.org/10.2305/IUCN.UK.2020- 3.RLTS.T32098A67777757.en

Lee, S., Lamasudin, D. & Mohamed, R. (2019). Rapid detection of several endangered agarwood-producing *Aquilaria* species and their potential adulterants using plant DNA barcodes coupled with high-resolution melting (Bar-HRM) analysis. *Holzforschung*, 73(5), 435-444. https://doi.org/10.1515/hf-2018-0149

Liu PF, Avramova LV, Park C. (2009). Revisiting absorbance at 230 nm as a protein unfolding probe. Anal Biochem.; 389:165–170.

López-Ochoa, L. A., Apolinar-Hernández, M. M., & Peña-Ramírez, Y. J. (2015). Characterization of chloroplast region rrn16-rrn23S from the tropical timber tree *Cedrela odorata* L. and de novo construction of a transplastomic expression vector suitable for Meliaceae trees and other economically important crops. *Genetics and Molecular Research*, 14(1), 1469–1478. https://doi.org/10.4238/2015.February.20.2

Maridi, A.P. & Saputra, A. (2014). Vegetation analysis of Samin Watershed, Central Java as water and soil conservation efforts. *Biodiversitas* 15(2), 215–223.

Miquel, F.A.W. (1855). Flora van Nederlandsch Indie 1(1), 132.

Moritsuka, E., Chhang, P., Tagane, S., Toyama, H., Sokh, H., Yahara, T., & Tachida, H. (2017). Genetic variation and population structure of a threatened timber tree *Dalbergia cochinchinensis* in Cambodia. *Tree Genetics & Genomes*, 13, 1–11.

Mulyana, L., Febryano, I.G., Safe'i, R. & Banuwa, I.S. (2017). Performance of agroforestry management in the Rajabasa protected forest management unit. *J Hutan Tropis* 5(2), 127–133. [Indonesian].

Nishizawa ,T., & Watano, Y. (2000). Primer pairs suitable for PCR-SSCP analysis of chloroplast DNA in angiosperms. *Journal of Phytogeography and Taxonomy* 48, 67–70.

Oxelman, B., M. Liden, & D. Berglund. (1997). Chloroplast rps16 intron phylogeny of the tribe *Sileneae* (Caryophyllaceae). Plant Systematics and Evolution 206, 393–410.

Pfeil, B. E., Brubaker, C. L., Craven, L. A., & Crisp, M. D. (2002). Phylogeny of *Hibiscus* and the tribe *Hibisceae* (Malvaceae) using chloroplast DNA sequences of *ndh*F and the *rpl*16 intron. *Systematic Botany* 27, 333–350.

Provan, J., Murphy, S., & Maggs, C. (2004). Universal plastid primers for Chlorophyta and Rhodophyta. *European Journal of Phycology - EUR J PHYCOL* 39, 43–50. 10.1080/09670260310001636668.

Rachmayanti, Y., Leinemann, L., Gailing, O., & Finkeldey, R. (2009). DNA from processed and unprocessed wood: Factors influencing the isolation success. *Forensic science international. Genetics*. 3. 185–192. 10.1016/j.fsigen.2009.01.002.

Saha, S., Shilpi, J.A., Mondal, H., Hossain, F., Anisuzzman, M., Hasan, M.M., & Cordell, G.A. (2013). Ethnomedicinal, phytochemical, and pharmacological profile of the genus *Dalbergia* L. (Fabaceae). *Phytopharmacology*, 4, 291–346.

Sang, T., Crawford, D. J., & Stuessy, T. F. (1997). Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae*). American Journal of Botany* 84, 1120–1136.

Sanger, F., Nicklen, S., & Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.*

Shaw J., Lickey E.B., Schilling, E.E., & Small, R.L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in Angiosperms: The Tortoise and The Hare III. *American Journal of Botany* 94(3), 275–288.

Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, K. T. Winder, E. E. Schilling., & R. L. Small. (2005). The tortoise and the hare 11: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Am. J. Bot*. 92, 142–166.

Song, Y., Zhang, Y., Xu, J., Li, W., & Li, M. (2019). Characterization of the complete chloroplast genome sequence of *Dalbergia* species and its phylogenetic implications. *Sci Rep* 9, 20401. https://doi.org/10.1038/s41598-019-56727-x

Stulnig, T.M., & Amberger, A. (1994). Exposing contaminating phenol in nucleic acid preparations. *Biotechniques*, 16, 402– 404.

Sunarno, B. (1996). Systematic Studies of Malesian *Dalbergia* (Leguminosae). [*PhD Dissertation*]. Tohoku University, Japan.

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17, 1105–1110.

Tnah, L.H., Lee,S.L., Ng,K.K.S., Bhassu, S., & Othman, R.Y. (2012). DNA Extraction from dry wood of *Neobalanocarpus heimii* (Dipterocarpaceae) for forensic DNA profiling and timber tracking. *Wood Sci Technol*. 46, 813–825.

Usman, T., Yu, Y., Liu, C., Fan, Z., Wang, Y. (2014). Comparison of methods for high quantity and quality genomic DNA extraction from raw cow milk. *Genet Mol Res*. 13, 3319–3328.

Vatanparast, M., Klitgard, B.B., Adema, F.A.C.B., Pennington, R.T., Yahara, T., & Kajita, T. (2013). First molecular phylogeny of the pantropical genus *Dalbergia*: Implications for infrageneric circumscription and biogeography. *South Afr J Bot*, 89, 143–149.

Wiedenhoeft, A. (2010). *Chapter 3. Structure and Function of Wood*. Handbook of Wood Chemistry and Wood Composites. Department of Agriculture Forest Service, Madison.

Wu, H.Y., Wong, K.H., Kong, B.L.H., Siu, T.Y., But, G.W.C., Tsang, S.S.K., Lau, D.T.W., Shaw, P.C. (2022). Comparative analysis of chloroplast genomes of *Dalbergia* species for identification and phylogenetic analysis. *Plants*. 11(9), 1109. https://doi.org/10.3390/plants11091109

Yulita, K.S., & Susila. (2019). Field collection and monitoring of the flowering of Sonokeling (*Dalbergia latifolia*) in Yogyakarta and East Java Provinces. Field report Research Centre for Biology, Indonesian Institute of Sciences, Bogor. [Indonesian].

Yulita, K.S., Atikah, T.D., Wardani W., & Susila. (2020). Unraveling genetic variations of *Dalbergia latifolia* (Fabaceae) from Yogyakarta and Lombok Island, Indonesia. *Biodiversitas J of Biosci*, 21(2), 833–841.

Appendix 1. List of locations for sample collection.

No	No. of samples	Location	Island
1	5	Gn. Batu, Ciracap, Sukabumi, W. Java	Java
2	3	Cidahu, Cibitung, Sukabumi, West Java	Java
3	3	Sumber Jaya, Tegalbeleud, Sukabumi, West Java	Java
4	2	Padasenang, Cidadap, Sukabumi, W. Java	Java
5	3	Babakan Jawa, Majalengka, Majalengka, West Java	Java
6	3	Darmawangi, Tomo, Sumedang, W. Java	Java
7	3	Gendereh, Buahdua, Sumedang, W. Java	Java
8	1	Blok Paseh, Paseh, Sumedang, West Java	Java
9	1	Wanahayu, Maja, Majalengka, West Java	Java
10	1	Unnamed, Unnamed, Subang, West Java	Java
11	3	Jatiseeng, Ciledug, Kuningan, West Java	Java
12	3	Luragung, Kuningan, West Jawa	Java
13	2	Jogomulyo, Buayan, Kebumen, West Java	Java
14	5	Selapamioro, Imogiri, Bantul, Yogyakarta	Java
15	1	Srimulyo, Piyungan, Bantul, Yogyakarta	Java
16	1	Wonolelo, Pleret, Bantul, Yogyakarta	Java
17	3	Darupono, South Kaliwungu, Kendal, Central Java	Java
18	3	Suntri, Gunem, Rembang, Central Java	Java
19	3	Dowan, Gunem, Rembang, Central Java	Java
20	2	Bonang, Lasem, Rembang, Central Java	Java
21	1	Binangun, Lasem, Rembang, Central Java	Java
22	5	Semoyo, Patuk, Gunung Kidul, Yogyakarta	Java
23	5	Sermo SM, Kokap, Kulon Progo, Yogyakarta	Java
24	2	Bubakan, Tulakan, Pacitan, East Java	Java
25	4	Ngadirejan, Pringkuk, Pacitan, East Java	Java
26	1	Kenduruan, Sukorejo, Pasuruan, East Java	Java
27	6	unnamed, unnamed, Mojokerto, East Java	Java
28	3	Kembangarum, Sutojayan, Blitar, East Java	Java
29	1	Gampingan, Pagak, Malang, East Java	Java
30	1	Gajahrejo, Gedangan, Malang, East Java	Java
31	3	Oro-Oro Ombo, Ngetos, Nganjuk, East Java	Java
32	2	Glonggong, Dolopo, Madiun, East Java	Java
33	1	Sugih Waras, Saradan, Madiun, East Java	Java
34	1	Pajaran, Saradan, Madiun, East Java	Java

35	3	Sekarputih, Tegalampel, Bondowoso, East Java	Java
36	3	Sabrang, Ambulu, Jember, East Java	Java
37	5	Lampe, East Rasana'e, Bima, NTB	Sumbawa
38	1	Raba, Wawo, Bima, NTB	Sumbawa
39	1	Barabali, Batukliang, central Lombok, NTB	Lombok
40	1	Pemepek, Pringgarata, central Lombok, NTB	Lombok
41	1	Kebun Ayu, Gerung, West Lombok, NTB	Lombok
42	1	East Sekotong, Lembar, West Lombok, NTB	Lombok
43	1	Mareje, Lembar, West Lombok, NTB	Lombok
44	5	Senggigi, Batu Layar, West Lombok, NTB	Lombok
45	7	Boak, Unter Iwes, Sumbawa, NTB	Sumbawa
46	1	Tarusa, Buer, Sumbawa, NTB	Sumbawa
47	1	Matakiri, Alas , Sumbawa, NTB	Sumbawa
48	1	Mapin Rea, Alas Barat, Sumbawa, NTB	Sumbawa
49	1	Panua, , Sumbawa, NTB	Sumbawa
50	3	Belo, Jereweh, West Sumbawa, NTB	Sumbawa
51	2	Rimpi, Seteluk, West Sumbawa, NTB	Sumbawa
52	2	Sapugara Bree, Brang Rea, West Sumbawa, NTB	Sumbawa
53	2	Telaga Bertong, Taliwang, West Sumbawa, NTB	Sumbawa
54	1	Brang Ene, Brang Ene, West Sumbawa, NTB	Sumbawa
55	1	Tamekan, Taliwang, West Sumbawa, NTB	Sumbawa
56	2	Seteluk, Seteluk, West Sumbawa, NTB	Sumbawa
57	1	Taman Baru, Sekotong, West Lombok, NTB	Lombok
58	2	Central Sekotong, Sekotong, West Lombok, NTB	Lombok
59	7	Senang Galih, Sambelia, East Lombok, NTB	Lombok
60	6	Senggigi, Batu Layar, West Lombok, NTB	Lombok
61	1	Kuta, Lambitu, Bima, NTB	Sumbawa
62	1	Woko, Pajo, Dompu, NTB	Sumbawa
63	1	Dorebara, Dompu, Dompu, NTB	Sumbawa
64	1	Sari, Sape, Bima, NTB	Sumbawa
65	1	Mangge, Lambu, Bima, NTB	Sumbawa
66	1	Hidirasa, Lambu, Bima, NTB	Sumbawa
67	1	Pesa, Wawo, Bima, NTB	Sumbawa
68	1	East Rasanat, East Rasana'e, Bima, NTB	Sumbawa
69	1	Kodo, East Rasana'e, Bima, NTB	Sumbawa
70	1	Nowa, Woja, Dompu, NTB	Sumbawa
71	1	Madaprama, Woja, Dompu, NTB	Sumbawa
72	1	Anamina, Manggelewa, Dompu, NTB	Sumbawa
73	1	Tekasire, Manggelewa, Dompu, NTB	Sumbawa
74	1	Suka Damai, Manggelewa, Dompu, NTB	Sumbawa
75	1	Kwangko, Manggelewa, Dompu, NTB	Sumbawa

76	1	Ntoke, Wera, Bima, NTB	Sumbawa
77	1	Rite, Ambalawi, Bima, NTB	Sumbawa
78	1	Mawu, Ambalawi, Bima, NTB	Sumbawa
79	1	Ncera, Belo, Bima, NTB	Sumbawa
80	1	Noba, Belo, Bima, NTB	Sumbawa
81	1	Diha, Belo, Bima, NTB	Sumbawa
82	2	Cempaga, Banjar, Buleleng, Bali	Bali
83	2	Liligundi, Buleleng, Buleleng, Bali	Bali
84	2	Melaya, Melaya, Jembrana, Bali	Bali
85	3	Pejaten, Kramatwatu, Serang, Banten	Java
86	1	unnamed, unnamed, Blitar, East Java	Java
87	1	unnamed, unnamed, Batu, East Java	Java
88	1	unnamed, unnamed, Magelang, Central Java	Java
89	1	unnamed, unnamed, Sragen, Central Java	Java
90	1	unnamed, unnamed, Kebumen, Central Java	Java
91	1	unnamed, unnamed, Kepanjen, Central Java	Java
92	2	unnamed, Leuwimunding, Majalengka, West Java	Java
93	5	unnamed, unnamed, Sumedang, West Java	Java