Population genetic of the Indonesian rosewood (*Dalbergia latifolia*) from Java and West Nusa Tenggara revealed using sequence-related amplified polymorphism

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**ABSTRACT**

*Dalbergia latifolia* is commercial tropical tree species known for its beautiful heartwood, mainly used for furniture and musical instrument. High market demand has put concerns on its sustainability and conservation aspects in Indonesia. Ninety-five specimens of *D. latifolia* were collected from eight populations of Java, Lombok, and Sumbawa Island to study intra-specific variability and diversity using SRAP. One hundred and eighty SRAP loci with 54.03% ± 4.35% polymorphism obtained from PCR amplification of 10 primer combinations, with the average PIC for these primers of 0.28. Genetic diversity and variability measures were calculated using GenALEX software indicating a relatively low-mid level of percentage of polymorphic loci (PPL) (54.03%), effective number of alleles (Ne) (1.255), Shannon information index (I) (0.242), and heterozygosity (He) (0.156) on average. The highest value (I = 0.309, He = 0.196) was observed in population P2CJ (Central Java), followed by West Java of P1WJ (I = 0.300, He = 0.191) and West Sumbawa of P7Wsumb (I = 0.257, He = 0.169), while the lowest (I = 0.202, He = 0.129) was found in West Lombok (P4WL). The genetic relationships were measured from genetic distance and identity from the two-pairwise calculation, PCoA, and STRUCTURE analysis. The relative homologous population is found between populations of the gene pool for genetic enrichment programs.

1. Introduction

*Dalbergia latifolia* (Fabaceae: Papilionaceae) is a commercial tree 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 known as Sonokeling in the Indonesian language and is mainly distributed in Java and West Nusa Tenggara, and maybe present in Timor Island, South Sumatra, Kalimantan, and Sulawesi (Yulita et al. 2020). The species is considered native to Andaman Island, Bangladesh, East and West Himalayas, India, Java, and Nepal (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:490295-1). However, others considered the species was perhaps introduced to Indonesia by the colonial government before the 20th century (Sunarno 1996; Maridi et al. 2014; Arisoesilaningsih and Soejono 2015; Adema et al. 2016) and may have been...
naturalized in Indonesia since then (Yulita et al. 2020). The oldest herbarium specimen was recorded in 1890, referring to a specimen collected from Java (https://www.gbif.org). One of the four synonyms of the species, i.e. Dalbergia javanica Miq, is also referred to as a species from Java (Miquel 1855). Hence, the origin of D. latifolia in Indonesia is to be resolved. Based on our observation during our fieldwork and several reports, e.g. Atikah and Dede (2018); Mulyana et al. (2017); Hani and Suryanto (2014), the species commonly found in mixed-plantation, mixed agroforestry areas either in commercial plantations or local farmers' gardens, few were recorded from the protected forest, and even found as monoculture plantation.

Dalbergia latifolia was extracted for its heartwood and is used for furniture, musical instruments, and other crafts since the heartwood is very dense without pores and very durable (Barrett et al. 2010; Karlinasari et al. 2010; Hassold et al. 2016). The market trade of D. latifolia from Indonesia mainly come from local farmers and Forestry state-owned companies (Perhutani). The wood is sent to the primary and secondary timber industry to be processed as semi-finished products to be exported (Yulita and Susila 2019). Leftover and low-quality pieces are usually used for traditional crafts and firewood (Yulita and Susila 2019). The CITES regulated the international trade for D. latifolia by including them in Appendix 2 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 it is 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 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 the genetic variation of D. latifolia throughout its distribution in Indonesia. Genetic variation is an essential aspect of indicating plant adaptability to its environment. The results of genetic diversity studies can be used to help protect species against environmental change as the stability of a population is often attributed to the degree of genetic variation present. The greater the genetic diversity of a population, the greater its chances to resist environmental change (Reed and Frankham 2003; Kirk and Freeland 2011; Szczecińska et al. 2016).

A previous study on the genetic variation of D. latifolia limited to a few populations in Central Java, and Lombok Island suggested a low level of genetic diversity (Yulita et al. 2020). However, this study did not cover most geographic distribution and may not reflect the total genetic diversity of D. latifolia from Indonesia. Hence, this present study was aimed to assess the genetic diversity of D. latifolia from the major geographic distributions in Indonesia, i.e. Java, Lombok, and Sumbawa Island using the sequence-related amplified polymorphisms (SRAP) marker. The SRAP is an effective dominant marker for producing genome-wide fragments with high reproducibility and versatility (Robarts and Wolfe 2014). To date, several studies have been reported to explain genetic variation in taxonomic levels using SRAP (Liu et al. 2008; Uzun et al. 2009; Cheng et al. 2016; Yulita and Rahmat 2019; Zhao et al. 2020), and even ploidy levels (Yu et al. 2019). Some studies have applied this technique also to Quantitative Trait Loci (QTL) identification of Cynodon dactylon (Zhang et al. 2011), and mutant detection of Setaria italica (Yulita and Ridwan 2018). The marker was developed by Li and Quiros (2001) for gene tagging in Brassica oleracea L. to amplify specific coding regions targeting GC-rich exons and AT-rich promoters, introns, and spacers. In this study, the same SRAP as Yulita et al. (2020) was selected to analyze the genetic variation among individual trees and populations. The results of the study will complement the previous study Yulita et al. (2020) by including more samples from West and East Java and Sumbawa Island. We expect that the D. latifolia in Indonesia may have considerable genetic variations considering that the breeding system of this species is open pollination with Xylocopa confusa and Apis cerana as the most dominant pollinators (Damaiyani and Prabowo 2019; Sasidharan et al. 2020). It may be able to maintain certain level of genetic diversity (Stone et al. 2019), even though vegetative propagation was mostly used for more than a century. Some of the present populations grow in protected areas have appeared as naturalized stands without significant human interference for their growth. The result from this study is expected to be used to formulate management policy and practices for the sustainable use of D. latifolia in Indonesia.

2. Materials and methods

2.1. Study area and sample collection

Leaf samples of 95 representative D. latifolia mature trees with diameters breast-high above 10 cm (Table 1) were collected from community mix plantations in 8 populations in Java, Lombok, and Sumbawa Islands during 2019–2021 (Figure 1). Sampling distances between individual trees ranged from 15 to 20 m to avoid genotype duplication. All samples were labeled correctly, preserved in dry silica gels, and transported to the laboratory for further analysis. Voucher specimens were also made from each site, these specimens were identified at the Herbarium Bogoriense, and the laboratory work was carried out in the Molecular Systematic Laboratory of the National Research and Innovation Agency-Indonesia in 2020–2021.

2.2. Molecular work

The molecular analysis was carried out in the Molecular Systematics Laboratory of the Research Center for Biology of the National Research and Innovation Agency, Indonesia. Total genomic DNA was isolated using the Genomic DNA Mini Kit (Plant),
100 preps/kit (GP100) from GeneAid. Ten combinations of SRAP primers (Li and Quiros 2001) were used in this study with details of polymorphism of each primer combination shown in (Table 2). Amplification was performed in 15 μL of 1x PCR master mix (Green Master Mix Promega Corporation n.d.), ~10 ng DNA template, and 2 μM of each primer. PCR cycling included 5 min at 94 °C, and the initial five cycles comprised denaturation at 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 2 min. The subsequent amplification was 30 cycles, and each cycle consisted of a denaturation phase at 94 °C for 1 min, annealing at 50 °C for 1 min, and an extension at 72 °C for 1 min.

The reaction was terminated by a final extension at 72 °C for 5 min. The amplified fragments were separated by electrophoresis on 1.5% agarose gel stained in GelRed Biotium that was run electrophoretically in 0.5× TBE buffer at 100 Volt for 90 min, then photographed using a gel documentation system (Bioinstrument, ATTO Biosystems Inc., Warington, UK). The PCR reaction was performed at least three times after the optimum PCR condition was achieved; some primers need 4–5 times to ensure the consistency of the resulting bands.
Table 2. Polymorphism revealed by SRAP primer combinations.

| Primer combination and their sequence | Total number of bands | Number of polymorphic bands | Percentage of polymorphic bands | Size range (bp) |
|--------------------------------------|-----------------------|----------------------------|---------------------------------|-----------------|
| me1F(TGAGTCCAAACCGAATACTGAGCCCTAATGAC) + me3R(GACTGCTGACGAACTGAC) | 20 | 20 | 11.11 | 100–1000 |
| me2F(TGAGTCCAAACCGAGACTGAC) + me3R(GACTGCTGACGAACTGAC) | 16 | 16 | 8.89 | 100–1000 |
| me3F(TGAGTCCAAACCGAATACTGAGCCCTAATGAC) + em3R(GACTGCTGACGAACTGAC) | 19 | 19 | 10.56 | 100–1000 |
| me3F(TGAGTCCAAACCGAATACTGAGCCCTAATGAC) + em4R(GACTGCTGACGAACTGAC) | 18 | 18 | 10.00 | 100–1000 |
| me5R(GACTGCTGACGAACTGAC) | 15 | 15 | 8.33 | 100–1000 |
| me4F(TGAGTCCAAACCGAATACTGAGCCCTAATGAC) + em5R(GACTGCTGACGAACTGAC) | 20 | 20 | 11.11 | 100–1000 |
| me6F(TGAGTCCAAACCGAATACTGAGCCCTAATGAC) + em6R(GACTGCTGACGAACTGAC) | 17 | 17 | 9.44 | 100–1000 |
| me7F(TGAGTCCAAACCGAATACTGAGCCCTAATGAC) + em7R(GACTGCTGACGAACTGAC) | 16 | 16 | 8.89 | 100–1000 |
| me8F(TGAGTCCAAACCGAATACTGAGCCCTAATGAC) + em8R(GACTGCTGACGAACTGAC) | 15 | 15 | 8.33 | 100–1000 |
| Total/average 180 (18/locus) | 180 (18/locus) | 10.00 |

Table 3. Summary of genetic variation statistics of Dalbergia latifolia from Java and West Nusa Tenggara Indonesia.

| Population code | N | Na | Ne | I | He | uHe | PPL |
|----------------|---|----|----|---|----|-----|-----|
| P1WJ           | 15 ± 0.000 | 1.461 ± 0.066 | 1.306 ± 0.025 | 0.300 ± 0.018 | 0.191 ± 0.013 | 0.197 ± 0.014 | 72.22% |
| P2CJ           | 26 ± 0.000 | 1.511 ± 0.063 | 1.307 ± 0.023 | 0.309 ± 0.018 | 0.196 ± 0.013 | 0.199 ± 0.013 | 74.44% |
| P3EJ           | 11 ± 0.000 | 1.100 ± 0.071 | 1.240 ± 0.025 | 0.224 ± 0.020 | 0.144 ± 0.014 | 0.151 ± 0.014 | 51.11% |
| P4WL           | 9 ± 0.000  | 1.000 ± 0.071 | 1.207 ± 0.023 | 0.202 ± 0.019 | 0.129 ± 0.013 | 0.137 ± 0.013 | 45.56% |
| P5EL           | 10 ± 0.000 | 1.011 ± 0.071 | 1.237 ± 0.026 | 0.215 ± 0.020 | 0.141 ± 0.014 | 0.148 ± 0.015 | 46.11% |
| P6Sumb         | 10 ± 0.000 | 1.011 ± 0.071 | 1.233 ± 0.025 | 0.215 ± 0.020 | 0.140 ± 0.014 | 0.148 ± 0.014 | 46.11% |
| P7WSumb        | 8 ± 0.000  | 1.117 ± 0.072 | 1.281 ± 0.026 | 0.257 ± 0.020 | 0.169 ± 0.014 | 0.180 ± 0.015 | 52.78% |
| P8BSumb        | 6 ± 0.000  | 0.983 ± 0.071 | 1.227 ± 0.025 | 0.211 ± 0.020 | 0.137 ± 0.013 | 0.149 ± 0.015 | 43.89% |
| Mean           | 11.875 ± 0.155 | 1.149 ± 0.025 | 1.227 ± 0.009 | 0.242 ± 0.007 | 0.156 ± 0.005 | 0.164 ± 0.005 | 54.03% ± 4.33% |

Sample size (N), Number of alleles (Na), effective number of alleles (Ne), Shannon Information Index (I), heterozygosity (He), unbiased heterozygosity level (uHe), percentage of polymorphic loci (PPL), and population code refers to Table 1.

2.3. Data analysis

The scoring for SRAP profiles was made from electrophoretic gel photos; the cleared and observable bands were scored 1 for the present and 0 for an absent band. The scored data was compiled in a matrix using Excel software for further analyses. The polymorphic information content (PIC) for each population and SRAP marker was following Zheng et al. (2017). The maximum value of PIC for dominant markers is 0.5 for \( f = 0.5 \) (Riek et al. 2001). Population genetic statistics were analyzed using GenAIEx (Peakall and Smouse 2006; Peakall and Smouse 2012) based on dominant markers and diploid individuals. The genetic diversity within the population was analyzed using parameters of percentage polymorphic loci (PPL) (Nei 1973) with a 99% criterion, observed number of alleles per locus (\( N_o \)), effective number of alleles per locus/gene pool (\( N_e \)) (Hartl and Clark 1989), the average expected heterozygosity (\( He \)) assuming a Hardy–Weinberg equilibrium (Nei 1973), and the Shannon information index of genetic diversity (\( I \)) (Lewontin 1972). Multivariate analysis of genetic relationships was analyzed with principal coordinate analysis (PCoA) using GenAIEx (Peakall and Smouse 2006; Peakall and Smouse 2012). Population structure was analyzed using The Bayesian model-based clustering method implemented in STRUCTURE software version 2.3.4 (Pritchard et al. 2000) to estimate the number of distinct genetic clusters (K) and determine the genetic structure of the sampled populations. A burn-in of 20,000 steps followed by 100,000 steps of Markov Chain Monte Carlo simulation with 10 replications each, for a range of K-values from 1 to 10 was performed on the entire data set. STRUCTURE HARVESTER Web v0.6.94 (Earl and VonHoldt 2012) was further used to determine the appropriate number of clusters (K), which the statistic \( \Delta K \) (Evanno et al. 2005) was calculated based on the rate of change in the log probability of data between successive K-values.

3. Results

3.1. SRAP profiles for D. latifolia from Indonesia

SRAP amplification using ten primers (Zhang et al. 2011) combinations yielded 180 loci with a consistent scorable size ranging between 100 and 1000 bp (Table 2). The overall of polymorphic band of the populations is 54.03% (Table 3). The highest number of amplicons (22) and the PPL for each primer pair (12.565) was obtained from primers pair me2F and me4R, while the lowest (15) was recorded from me5F and em5R (Table 2). The average PIC for the 180 loci is 0.283.

The 180 loci are evenly distributed in each population; the highest number of loci (138) is recorded from the P2CJ West Sumbawa population, followed by P1WJ and P3EJ (133), and P7WSumb (106). The lowest number of loci (98) is found in the P4WL and P8BSumb (Table 4) and the band patterns of association across the population are shown in Figure 2.

3.2. Genetic diversity and variability

The measures of genetic diversity and variability were deduced from the number of alleles, the diversity index (Shannon Information Index, \( I \)), percentage of polymorphic loci (PPL), and unbiased heterozygosity level (\( He \)) (Table 3). Of the 180 SRAP bands found in this study, more than 1 (1.149) allele was found on average...
for each population, with the effective number of alleles (Ne) in populations was only around 1 (Table 3). The average number of alleles (Na) found in P2CJ (1.511) and P1WJ (1.461) are the highest among populations. The average heterozygosity (He) and diversity (I) of each population were 0.156 and 0.242, respectively. The lowest values were found in West Lombok (I = 0.202, He = 0.129) followed by the Bima regency of Sumbawa Island (I = 0.211, He = 0.137), and the highest genetic variation value was found in Central Java (I = 0.309, He = 0.196) followed by West Java (I = 0.300 and He = 0.191) (Table 3).

3.3. Genetic relationship and structure

The genetic relationship was quantified by estimating the genetic distance between two samples, which provides the basis for estimating the proportion of genes that are identical/differ in two populations. According to the Nei genetic distance matrix (Table 5), relatively homologous values were found between Central (P2CJ)-West (P1WJ) Java (0.031), followed by East (P3EJ)-West (P1WJ) Java (0.053), Bima (P8BSumb)-West Sumbawa (P7WSumb) regency (0.054), and Sumbawa (P6Sumb)-West Sumbawa (P7WSumb) Regency (0.060). Meanwhile, the most distinct population was recorded between Sumbawa Regency (P6Sumb)-East Java (P3EJ) (0.157), followed by East Lombok (P5EL)-East Java (P3EJ) (0.149).

The genetic relationship among the individual samples was deduced from the PCoA diagram (Figure 3). The results of the PCoA of all individual samples from the population showed that the samples were placed in three groups almost in accordance with their island populations, except for Central Java. The first group consisted of samples from Java, the second
group consisted mainly of tree samples from Lombok Islands and some samples from Central Java, and the third group comprised trees from Sumbawa Islands.

The STRUCTURE analysis showed that the optimal cluster number was $K = 2$ followed by $K = 4$ based on delta $K$ (Figure 4(a)). For $K = 2$, West and East Java populations were grouped into cluster 1, Lombok and Sumbawa populations were grouped into cluster 2, and Central Java was a mixture of the two clusters (Figure 4(b)). For $K = 4$, the Java populations were separated into two, Central Java, and West-East Java, the Lombok populations were grouped into one cluster, and Sumbawa populations were grouped into one cluster (Figure 4(c)).

4. Discussion

4.1. SRAP profile

Amplification of SRAP using a combination of 10 primers has yielded 180 loci, with an average of 54.03% polymorphic loci existing in each population. The polymorphism obtained by SRAP was generally lower than those obtained from other markers that are more random, such as RAPD, ISSR, and SSR (Rout et al. 2003; Bakshi and Sharma 2011; Cheng et al. 2016; Zheng et al. 2017). This may be because SRAP has been claimed to target more specific sites (Li and Quiros 2001; Robarts and Wolfe 2014), i.e. the coding sequences.

These polymorphisms obtained by SRAP amplification can be represented by the PIC and PPL values. The PIC values for SRAP as dominant markers ranged from 0 to 0.5, where 0 indicates fixation of one allele, and 0.5 indicates equal frequencies of alleles (Zheng et al. 2017). The PIC value for SRAP in this study (0.283) was considered moderate. This value was similar to the previous study (PIC = 0.284), even though more samples are included in the present study. No studies used SRAP for genetic diversity assessment in related species of *Dalbergia latifolia* so far. A study on *D. odorifera* from China using 19 SSR loci showed a higher PIC value of 0.60 (Liu et al. 2019). A higher PIC value was also recorded from *D. olivieri* from Vietnam using ISSR (0.423) and RAPD (0.544) (Phong et al. 2011).

Concerning the PPL value, which indicated the proportion of polymorphic gene loci, the relatively moderate level of polymorphic loci (54.03%) that was obtained from this study, suggesting the same proportion of genetic biodiversity of *D. latifolia* in this study. However, the level of polymorphism was generally related to the number of accessions and their geographic origin. In this case, a higher level of polymorphism will be obtained from more accessions covering a more extensive geographic range than the narrower range (Zheng et al. 2017). In the case of *D. latifolia*, they have been extensively domesticated vegetatively, but in some few populations particularly in West Java, they are still able to regenerate naturally.

4.2. Genetic diversity and variability

Estimates on genetic diversity in this study were inferred from the Shannon diversity index that explains how diverse *D. latifolia* is in the studied populations, while inference of the genetic variability in the populations’ study was from the average He overall loci. These two values are highest in Central Java, while the lowest in the West Lombok population. This study used an unbiased estimator of expected heterozygosity that only accounts for sample size as it underestimates true population diversity in samples containing relatives less more samples and populations are included. Each population has different levels of genetic diversity/variability, and determination of which populations with low and high genetic diversity is important for long-term management strategies. *Dalbergia latifolia* is now widely planted over the archipelago. However, each region has had different environmental condition, the West Java have relatively higher humidity than the other parts of Java. East Java, Lombok, and Sumbawa Island have drier climates than...
West and Central Java. This species is open-pollinated (Damaiyani and Prabowo 2019; Sasidharan et al. 2020) as common in Fabaceae, but our observation showed that not all populations are known to reproduce viable seeds. Our data only recorded flowering and fruiting in most locations in West Java and Sumbawa, the other populations may produce flowers with unknown seeds or unviable seeds. This study showed that the D. latifolia population of Central Java was highly diverse and the West Lombok population was found to be least diverse. Low genetic diversity could be a reason for less adaptation of D. latifolia trees in the West Lombok region, as low polymorphic populations are at higher risk of extinction due to low fitness of individuals (Reed and Frankham 2003). It is unknown whether D. latifolia trees in West Lombok produced viable seeds.

Another measure of genetic diversity is number of alleles, indicating a population’s long-term potential for adaptability and persistence (Greenbaum et al. 2014). This study found that the effective number of alleles for each population was only 1.255. Besides that, this study also recorded a low number of effective alleles on average that gives rise to He (0.156) suggesting a relatively low variation of alleles in the gene pool. Considering that the maximum heterozygosity value is 1, the D. latifolia populations in Indonesia appeared to maintain a low level of genetic variability. As this plant was thought to be introduced from India to Java Island (Sunarno 1996; Maridi et al. 2014; Arisoesilaningsih and Soejono 2015; Adema et al. 2016), one of the reasons for the low genetic diversity is the founder effect (Kumar and Agrawal 2017). Other studies (Dida et al. 2008; Hagenblad et al. 2015; Kumar and Agrawal 2017) also showed a low genetic diversity in the introduced population than in the native source (DeWalt et al. 2011). When certain plants with few numbers of progeny and outbreed were introduced to a new environment, such plants may not have well adapted and eventually result in decreasing genetic diversity and may have experienced bottleneck events (Kumar and Agrawal 2017), and this was one of the most universal features of invasions for founding populations (Dlugosch et al. 2015). However,

Figure 4. Clustering of eight populations of Dalbergia latifolia in Java, Lombok, and Sumbawa Islands obtained from STRUCTURE analysis. (a) STRUCTURE estimation of populations number from K values ranging from 1 to 10, by delta K (\(\Delta K\)) values, (b) population structure from K = 2, and (c) population structure from K = 4.
in the case of _D. latifolia_ in Indonesia, the trees have been successfully introduced and well-adapted in different geographical regions of Indonesia although the different level of genetic diversity is observed. Similar findings of introduced populations revealed very less genetic diversity but have been adapted successfully in a new environment were reported in two introduced plants, _Fallopia japonica_ and _Eichhornia crassipes_ (Hollingsworth and Bailey 2000; Ren et al. 2005).

Another reason for the low genetic diversity of _D. latifolia_ in Indonesia is the propagation method. Based on our observations, _D. latifolia_ were mainly reproduced vegetatively from roots, and only a few populations are known to produce flowers and viable seeds – among them are populations from Sumbawa and West Java. This phenomenon may be caused by intensive domestication in the past so that plants may decrease their ability to reproduce generatively; thus, one can expect a low level of heterozygosity within the trees and populations studied. Until now, _D. latifolia_ regenerated easily using roots sprout. When the parent trees are cut with stumps left behind, new sprouts from the stump (coppice) and roots below the ground will produce new offspring. Such conditions are not beneficial for the trees’ long-term survival because the homogenous populations are easily affected by internal and external pressure, such as climate change, pests, and diseases. In addition, populations with a high level of genetic diversity have a strong positive correlation between heterozygosity and population fitness, which is vital for the long-term adaptation of populations to novel environmental conditions (Stojnić et al. 2019). Thus, a population is more susceptible to unfavorable environmental conditions if it lacks heterozygosity (Basyuni et al. 2012).

Concerning our results, the genetic characteristic that makes up the population in Central Java is considered the most diverse; it contains the highest number of loci (138), even though a low number of private loci (4), an almost similar value observed in the West Java. Hence, the genetic characteristics of individual trees was observed from Central Java vary most. High genetic diversity may serve as a way for the population to adapt to changing environment. Overall, the _He_ and _I_ value of the Central Java was higher than the other populations, indicating that the Central Java may have higher adaptability to survive and more fitness than other populations. Our data on trade and harvest system showed that wood produced from Central Java has the best quality among other regions in Java and West Nusa Tenggara. Most of the samples of _D. latifolia_ tree stands in Central Java in this study were collected from various sites from the protected forest, production forest, and farmers’ land. With such conditions, the trees from the Central Java may have been experienced intensive domestication through vegetative propagation, but in some parts particularly when the trees existed in protected forests they experienced less human interference and therefore contributed more diverse genes/alleles as regeneration of _D. latifolia_ trees in Central Java may come from the mixed source between naturally reproduced/outcrossed and clonal.

### 4.3. Genetic relationship and genetic structure

In this study, the PCoA (Figure 3), _Nei_ genetic distance (Table 5), and STRUCTURE _K_ = 4 (Figure 4) suggested the close genetic distance within Java populations, Lombok populations, and Sumbawa populations. This genetic and geographic distance relationship pattern was similar to the _D. conchichinensis_ case in Vietnam, where populations growing 300 km apart were separated into different groups (Hien and Phong 2012). In addition, mixing of West-East Java populations and Lombok populations were also found in the Central Java population in this study indicating a past admixture of clusters that allowed some gene flow. This is possible if the seedling used for planting activities in the Central Java region come from mother trees that grow in West Java, East Java, and Lombok regions. However, since there is no clear information on the history of _D. latifolia_ plantation in Indonesia, it is essential to know the origin of _D. latifolia_ that presently occurs in many areas of Indonesia. A study on phylogenetics using single-copy gene regions with single nucleotide polymorphisms is worth carrying out to clarify the origin of _D. latifolia_ populations.

### 5. Conclusions

In general, the _D. latifolia_ population in this study has a low genetic diversity and variation. Vegetative propagation through roots that have been intensively carried out in the past has contributed to this low level of genetic variation. Central Java populations as the highest genetic diversity can be utilized as a source of germplasm for _D. latifolia_ from Indonesia. The PCoA indicated that three groups of _D. latifolia_ (West-East-Central Java, Central Java-Lombok, and Sumbawa populations) were grouped according to their bioregion. STRUCTURE analysis also revealed that West-East Java, Lombok, and Sumbawa formed their respective clusters and Central Java contains a mixture of West-East Java and Lombok populations. These patterns indicated complex/multiple origins of _D. latifolia_ in Indonesia needing further study to clarify.

It is suggested that the plantation should include populations or tree stands containing different genetic variations to allow cross-pollination to occur, thus enhancing higher genetic variation. In addition, the establishment of clonal seed orchard with selected genotypes with different genetic backgrounds is recommended as one of the conservation efforts to produce genetically diverse seeds.

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**Author contributions**

K.Y., F.G.D., H.H.R., T.D.A., B.P., and I.K. are the main authors of this manuscript and work equally in conceptualization, methodology, field work, sample collection, data collection, data curation, data validation, software analysis, writing – original draft preparation, writing – review and editing. S. A., S.T.S., W.W., R.A.F., and N.A. conduct field work, sample collection, and writing – review and editing. All authors have read and agreed to the published version of the manuscript.

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**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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