BIODIVERSITAS
Volume 23, Number 5, May 2022
Pages: 2630-2639

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230546

Genetic diversity of rosewood (*Dalbergia latifolia*) in Yogyakarta, Indonesia for plus trees selection

INDIRA RIASTIWI[1,2], WITJAKSONO[1], DIAH RATNADEWI[2], ULFAH JUNIARTI SIREGAR[3,♥]

[1]Research Center for Genetic Engineering, Research Organization for Life Sciences and Environment, National Research and Innovation Agency. Jl. Raya Jakarta-Bogor Km 46, Cibinong, Bogor 16911, West Java, Indonesia

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Meranti, IPB University Campus Dramaga, Bogor 16680, West Java, Indonesia

[3]Department of Silviculture, Faculty of Forestry and Environment, Institut Pertanian Bogor. Jl. Agatis, IPB University Campus Dramaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8626806, Fax.: +62-251-8626886, ♥email: ulfahjs@apps.ipb.ac.id

Manuscript received: 29 March 2022. Revision accepted: 27 April 2022.

Abstract. *Riastiwi I, Witjaksono, Ratnadewi D, Siregar UJ. 2022. Genetic diversity of rosewood (*Dalbergia latifolia*) in Yogyakarta, Indonesia for plus trees selection. Biodiversitas 23: 2630-2639.* Rosewood (*Dalbergia latifolia* Roxb.) or *sonokeling* is an important woody plant species with a high selling value. However, due to overexploitation, this species has been listed in Appendix II of CITES and its trade is strictly regulated. Therefore, the cultivation of this plant needs encouragement, and superior planting materials from selected plus trees are needed. This study aimed to examine the genetic diversity of the rosewood population and select plus trees in Yogyakarta Province, Indonesia. The plus tree selection was performed by comparing plus tree candidates with five other trees nearby in four districts, i.e. Bantul, Gunungkidul, Kulon Progo, and Sleman. As many as 61 plus tree candidates have been identified. Genetic diversity was assessed using 10 selected ISSR primers, resulting in 101 ISSR loci with average Polymorphic Loci values of 64.71% and Nei Heterozygosity of 0.23. The highest gene diversity (He=0.29) was found in the Kulon Progo population, and the lowest He=0.17 was in the Bantul population. The dendogram and PCA analysis put Gunungkidul and Bantul populations into one group, separated from the Sleman and Kulon Progo populations. Based on morphological and molecular analysis, six superior plus trees were obtained.

Keywords: Genetic diversity, ISSR, plus trees, rosewood

Abbreviations: ISSR: Inter Simple Sequence Repeat, He: Expected Heterozygosity, PPL: Percentage of Polymorphic Loci, KL: Kulon Progo, BL: Bantul, SL: Sleman, GK: Gunungkidul, CITES: Convention of International Trade of Endangered Species

INTRODUCTION

Rosewood (*Dalbergia latifolia* Roxb.) or *sonokeling* of Fabaceae (Papilionoideae) was introduced to Indonesia, originating from India (Adema et al. 2016). Rosewood is distributed in Java, West Nusa Tenggara, South Sumatra, Sulawesi, Timor Island (Yulita et al. 2020). One of the rosewood production centers in Indonesia is D. I. Yogyakarta (DIY), where trees are cultivated by local communities on a small scale in their yard and field as community forests. However, large trees are hardly found in Java (Dwianto et al. 2019). The global rosewood population is currently facing the threat of extinction due to overharvesting (Hanifah 2022), that since 2015 the tree species have been listed in Appendix II of CITES (Convention on International Trade in Endangered Species).

In nature, rosewood reproduces by seed or by adventitious shoots growing from roots. Rosewood seeds have a low germination rate, only around 30-40% (Prawirohatmodjo et al. 1994). In Yogyakarta, Central Java-Indonesia, rosewood trees were reported to flower but not produce fruits and seeds. In those areas, people propagate rosewood using root cuttings. Rosewood roots of 5-20 cm in length were used as cuttings materials (Vasudevan et al. 2020). This reproductive system would affect the distribution pattern of the genetic diversity of a population.

Knowledge of the genetic diversity of a plant population is needed for tree breeding, especially to select the parent trees or plus trees. The selection of plus trees is vital for seed and clonal seed sources and as parents in plant breeding. The plus trees were selected based on their best phenotypic appearance compared to surrounding trees, while considering their diverse genetic background. Therefore greater genetic diversity of the population is preferable in order to include as many as possible different genetic background in the selected plus trees. This is important aspect as monoculture systems with narrow genetic diversity made trees more susceptible to pests and diseases (Liu et al. 2018; Crowther et al. 2020). Research on plus trees has previously been carried out in *Paraserianthes falcataria* resulting from mutation by gamma irradiation (Zakiyah et al. 2017).

The genetic diversity of a plant population can be studied using molecular markers. The molecular marker Inter Simple Sequence Repeat (ISSR) has become a popular tool in the study of plant population genetics (Abdelaziz et al. 2020; Li et al. 2020, Sheikh et al. 2021; Shakoor et al. 2022; Samarina et al. 2022). The genetic diversity of *Dalbergia* was studied using several molecular markers, for example ISSR (Hien and Phong 2012; Javaid

et al. 2014; Fatima et al. 2018a; Fatima et al. 2018b; Bal and Panda 2018; Ijaz et al. 2019; Junior et al. 2020), Random Amplified Polymorphic DNA (RAPD) (Bal and Panda 2018; Dobhal et al. 2019; Tewari et al. 2022), and Sequence Related Amplified Polymorphism (SRAP) (Yulita et al. 2020). This research aimed to study genetic diversity of four rosewood population in Yogyakarta using ISSR marker, and identify plus trees based on their morphological traits as sources of propagation and for their breeding program.

MATERIALS AND METHODS

This research was carried out in 2 stages. Firstly, the exploration and identification of plus tree candidates. Secondly, the genetic diversity analysis among the populations of plus tree candidates using ISSR to determine the best plus trees with high genetic diversity.

Plus tree selection

Exploration of plus trees was carried out in four districts in Yogyakarta Province, Indonesia, i.e., Bantul, Gunungkidul, Kulon Progo, and Sleman (Figure 1), by walking along the roads in the villages and visiting the community forests where rosewood trees were planted among the other trees (Figure 2). When a rosewood tree was identified, the tree was marked. After finding six or more trees, the one that visually has the best morphology was identified as a plus tree candidate and was compared with five other surrounding rosewood trees. Plus tree candidate was determined through an assessment using a scoring system modified from the method developed by Hidayat (2010) for *Toona sinensis.*

The complete scoring system is shown in Table 1. The score of a plus tree for height and diameter was calculated from the average score of the five comparison trees. The total score of a plus tree was the numerical sum of tree growth variables which included tree height, branch-free plant height (TBBC), trunk diameter at breast height, tree height score, trunk diameter score, straightness score, canopy condition score, and tree health score. The candidate plus trees frequency distribution was calculated using Phyton Software (Liu et al. 2021).

Table 1. The complete plus tree candidate scoring system developed for rosewood tree*

Morphological characters and tree health	Evaluation system	Score
Tree height	<105%	4
	105%-110%	5
	111-115%	12
	116-120%	16
	>121%	20
Diameter at chest height	<105%	5
	105%-110%	7
	111-115%	17
	116-120%	23
	>121%	30
Stand straightness	Not straight	0
	Straightness < 20%	1
	Straightness 25%	2
	Straightness 50%	3
	Straightness 75 %	4
	Straightness	5
Canopy condition	Damage >80%)	0
	Damage >60%	1
	Damage <50%	2
	Damage <40%	3
	Damage <30%	4
	Damage<20% (healthy and balance)	5
Tree health	Damage >80%)	0
	Damage >60%	1
	Damage <50%	2
	Damage <40%	3
	Damage <30%	4
	Damage <20% (healthy and balance)	5

Note: *modified from Hidayat (2010)

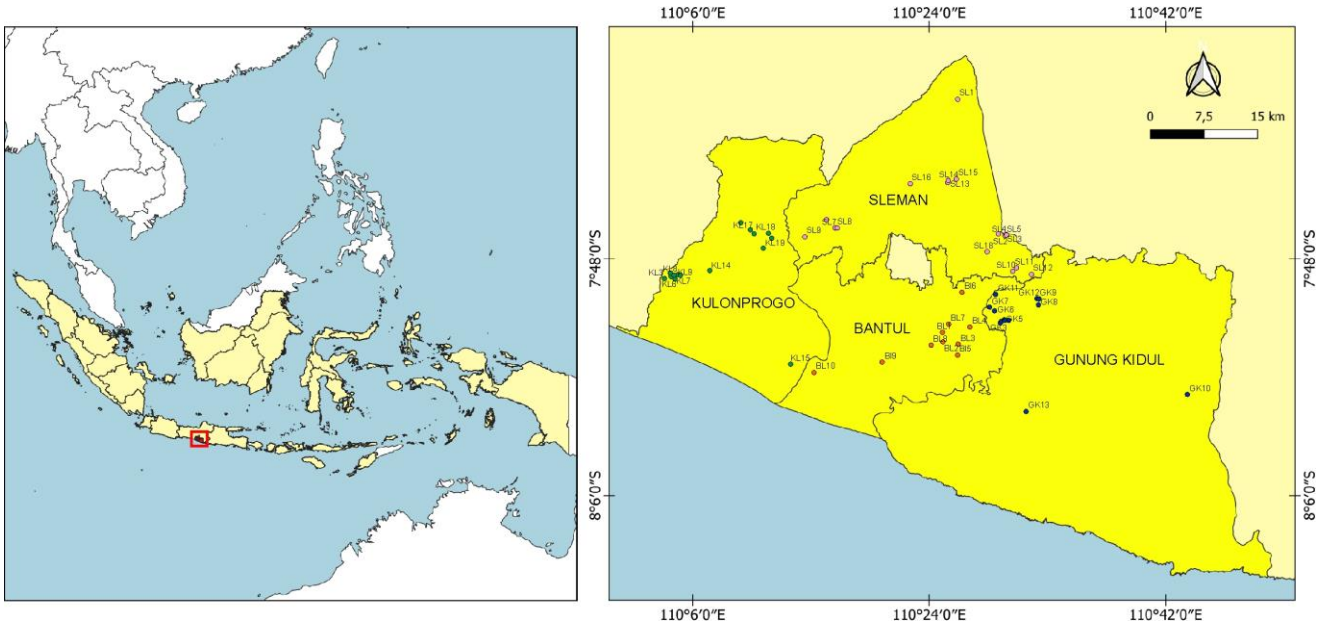

Figure 1. Location map of the study areas in Yogyakarta Province, Indonesia for plus trees selection and genetic diversity of rosewood

Figure 2. The habitat of the sampling tree of rosewood. A. Dryfield, B. Homegarden

Molecular work

Leaf samples were taken for molecular analysis from the selected plus tree candidates. Samples of 3-5 young leaves were stored in a tightly closed plastic box containing silica gel. Genomic DNA was isolated by grinding the 0.2 g leaf sample in a sterilized mortar using liquid nitrogen and Genomic DNA Mini Kit (Plant, Gene Aid) according to protocol from Geneaid, 2022. For ISSR analysis, out of 23 ISSR primers examined, only 10 primers gave polymorphic amplifications. Those 10 primers (Table 2) were subsequently used for genetic diversity analyzing of the tree populations studied. The PCR reaction mixture consisted of 1 x PCR master mix (Promega), 10 ng template DNA, 1 µL for each primer to get a total volume of 10 µL. The PCR reaction was run under the following optimum conditions (Poerba and Ahmad 2013): 94°C for 5 min, 94°C for 1 min, 50°C, for 45 seconds and 72°C for 2 min. The reaction was stopped by extension at 72°C for 5 min. PCR products (amplicons) were stained with GelRed (Biotium). Then the stained samples were electrophoresed on 1.5% agarose gel (Vivatis) in 1x TBE buffer and were run at 100 Volts for 120 min. The electrophoresis results were then photographed using a gel documentation system (Atto Bioinstrument).

Data analysis

The ISSR band profile of each ISSR primer was observed from the electrophoresis gel photographs. The clear and observable band patterns were scored according to the presence or absence of the bands in the existing band rows. Score 1 was for the presence of a band and 0 for the absence of a band. The score data matrix was compiled in Excel software and used for further analysis.

Percentage of polymorphic loci (PPL), Nei gene diversity index (H), and Shannon information index (I) are the key parameters to measure genetic diversity. The Shannon index can vary from 0 to 1, and lower genetic diversity is represented by values close to zero (Silva et al. 2015). The assessment of genetic diversity was based mainly on the polymorphisms found at loci, which were represented by PPL values. The molecular variance (AMOVA) and population genetics analysis were performed using the AIEx 6.3 Gene software (Peakall and Smouse 2006) with an individual binary model. Cluster analyses were executed using PAUP software (Zhang et al. 2019). Principal Component Analysis (PCA) was executed using the Metaboanalyst 5.0 software (Xia and Wishart 2016).

The plus tree was then selected based on the high score and genetic diversity within the population and between the populations according to the results of molecular marker analysis, namely genetic distance.

Table 2. Sequences of ISSR primer pairs used for genetic diversity analysis in this study

Primers pairs	Sequences	Primers pairs	Sequences
UBC807	AGAGAGAGAGAGAGAGT	UBC822	TCTCTCTCTCTCTCTCA
UBC825	ACACACACACACACACT	UBC826	ACACACACACACACACC
UBC817	CACACACACACACACAA	UBC819	CACACACACACACACAG
UBC809	AGAGAGAGAGAGAGAGG	UBC808	CTCTCTCTCTCTCTCTA
UBC811	GAGAGAGAGAGAGAGAC	UBC842	GAGAGAGAGAGAGAG AYG

RESULTS AND DISCUSSION

Characterization and determination of the plus tree candidates

The exploration and comparison of plus tree candidates with five surrounding trees resulted in 61 plus tree candidates. Those 61 identified candidates had been compared with 305 other individual trees. All candidates from the four regencies in Yogyakarta are distributed unevenly and form several clusters (Figure 1). It can be seen that there is one large cluster in the middle of Yogyakarta covering Gunungkidul, Sleman, and Bantul regencies (GSB cluster). One medium cluster is situated around the border of Kulon Progo and Sleman (KLS cluster), consisting of two small clusters in the middle of Sleman district (cluster S) and one on the eastern edge of Kulon Progo district cluster KL). However, those geographical clusters required molecular genetic studies to determine plus tree diversity.

The total scoring results of the 61 plus tree candidates in the four districts in Yogyakarta range from 43-147 with an average of 97.5 (median 96.5 and mode 97) (Figure 3). The total scores for morphological traits have a normal distribution, showing that 95% of the highest score falls on a score of at least 131. There are three plus tree candidates within that total score limit with a score of 132, 134.5, and 147, which are the accession no KL06, SL04, and GK05, respectively. These plus tree candidates are located in three districts, i.e., Kulon Progo, Sleman and Gunungkidul. Referring to the geographical map of the study area (Figure 1), the plus tree candidates with the 5% highest score were distributed in two different geographic clusters. The KL cluster on the eastern edge of Yogyakarta for accession no KL06, and the middle GSB cluster of Yogyakarta for the accession SL04 and GK06.

If the plus tree were selected at a lower percentile, 90%, then the limiting score drops to 124, and the plus tree candidates that can be selected increase to eight trees, i.e., accession no GK05, GK12, KL01, KL02, KL06, BL10, SL04, and SL12. These plus tree candidates are distributed throughout the entire districts being explored. The ten plus trees with the highest scores are presented in Table 3, including the plus trees of the 95% and 90% percentiles.

Genetic diversity analysis

DNA amplification using ten pairs of ISSR primers of the plus tree candidates from four rosewood populations resulted in 101 amplicon fragments. Different primers produced different polymorphism patterns. The UBC 825 primer was one of the primers that produced the most abundant amplicons (Figure 4.A) while the UBC 811 primer produced the fewest amplicons (Figure 4.B). Nevertheless, it can be said that all primer pairs produce high polymorphism. The fragment size also showed a high variation, ranging from 200-3000 base pairs (bp) (Table 4).

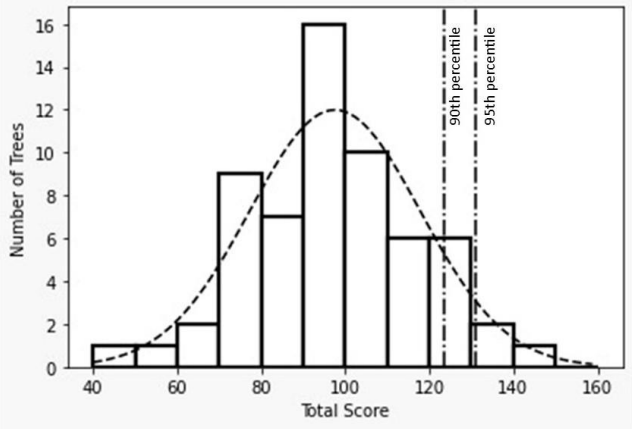

Figure 3. The frequency distribution of the plus tree candidate measurements was calculated using Phyton software

Table 3. The top ten rosewood plus tree candidates in Yogyakarta based on scoring method

Population	Plus tree accession no.	Measured variable			Score value of the measured variables and other qualitative traits					Total
		Height (m)	Diameter (cm)	Height of branch free trunk (m)	Height	Diameter	Canopy	Straightness	Tree health	
Gunungkidul	GK05	11	56	4	20	30	4	3	4	132**
	GK12	20	41	5	20	30	4	4	4	128*
Kulon Progo	KL01	20	40	5	20	30	2	3	4	124*
	KL02	20	40	6	20	30	2	2	4	124*
	KL06	20	56	6.5	12	30	3	3	4	134.5**
	KL07	20	32	7	20	30	2	4	4	119
Bantul	BL10	20	37	10	20	30	2	4	4	127*
Sleman	SL03	12	45	5	20	30	4	3	3	122
	SL04	20	62	4	20	30	5	3	3	147**
	SL12	20	35	10	20	30	4	4	4	127*

Note: *percentile 90%, **percentile 95%

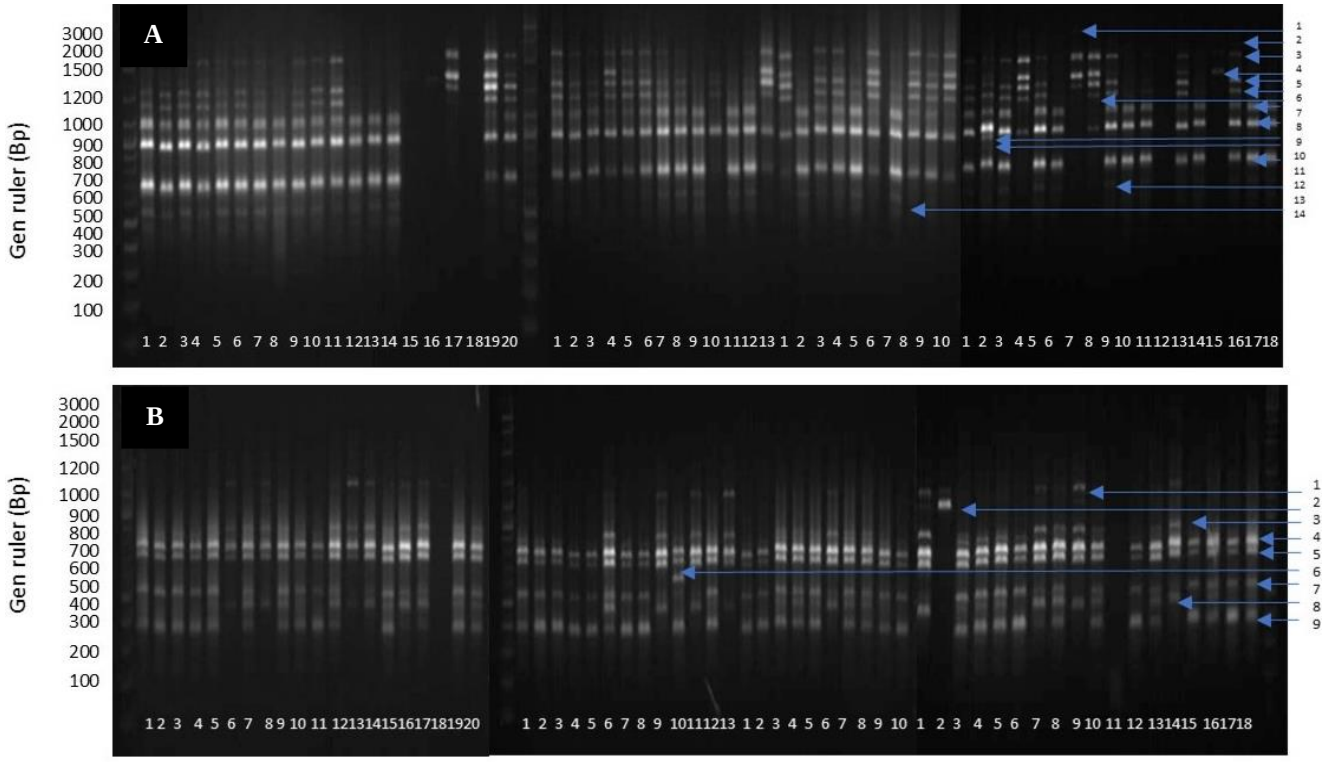

Figure 4. ISSR profile of 61 rosewood samples from four Yogyakarta populations using two different primers. A. primer UBC 825. B. primer UBC 811. Each row shows the banding pattern of one sample with a particular primer. The band pattern from left to right shows 20 samples from Kulon Progo, 13 samples from Gunungkidul, 10 samples from Bantul and 18 samples from Sleman. Blue arrows: polymorphic band

Table 4. Number of amplified and polymorphic fragments generated by ten selected ISSR primer pairs on the samples of four different populations of rosewood in Yogyakarta

Primers pairs	Number of amplified fragments	Number of polymorhic fragments	Size range (bp)
UBC 807	8	8	200-1500
UBC 825	14	14	500-3000
UBC 817	13	13	400-3000
UBC 809	8	8	400-3000
UBC 811	9	9	400-1400
UBC 822	8	8	200-1800
UBC 826	10	10	400-2500
UBC 819	9	9	400-2500
UBC 808	10	10	200-1800
UBC 842	12	12	300-2500

The genetic variation parameters (Table 5) show a fairly significant variation for each population. The average PPL value of 64.71% indicated that the ISSR molecular marker could be relied upon to detect polymorphisms at both individual and population levels. The value of each population's genetic diversity (He) showed a significant difference. The highest diversity was obtained in the Kulon Progo population, followed by Sleman, Gunungkidul population, and the lowest was Bantul population. The difference in genetic diversity from the highest to the lowest reached 7.91%, indicating a significant difference. This value is higher than the previous study on rosewood by SRAP, which obtained 56% on rosewood (Yulita et al. 2020). A population is considered to have a high genetic diversity value if it has high polymorphisms and heterozygosity levels. A low heterozygosity value indicates an organism's lack of genetic variation in wild populations. This parameter is vital in determining strategies for certain plant conservation (Siburian et al. 2017).

Several studies of natural populations have demonstrated the percentage of polymorphic loci as a vital measure of genetic diversity. However, despite the commonly used, variation in these values is also observed (Soares et al. 2016). According to (Nei 1987), the proportion of polymorphic loci is not a significant measure of genetic variation; therefore, the parameter of genetic diversity (He) is still required because it can add more accurate data.

The genetic diversity level of plus tree candidates in Kulon Progo, represented by the value of He=0.29, showed the highest diversity compared to plus tree candidates in other districts studied. Kulon Progo is known to have a protected forest in which the rosewood population exists. There may be less human intervention in this rosewood population than populations in other areas, such as Gunungkidul. Farmers in their yards and fields manage the rosewood population in Gunungkidul. The regeneration of rosewood stands in the Kulon Progo protected forest can occur more naturally, leading to higher genetic diversity (Yulita et al. 2020). However, Sleman and Gunungkidul

were found to have slightly lower genetic diversity than Kulon Progo, with He=0.27 and He=0.21, respectively.

The average genetic diversity (H) of rosewood candidate trees plus in Yogyakarta is high compared to *Adesmia bijuga* with a value of 0.22 and a Shannon Information Index of 0.36 (Guerra et al. 2018). The diversity level of the plus tree in Kulon Progo with He=0.29 is comparable to that of *Pityrocarpa moniliformis* with He values of He=0.24 (Felix et al. 2020) and *D. sissoo* with He=0.21 (Dobhal et al. 2019) and *Caragana microphylla* (He=0.16 and I=0.24) (Huang et al. 2016). That level of genetic diversity is still higher than those of *Hedysarum sangilense* with He=0.105 (Selyutina et al. 2021), *Parkia biglobosa* with He=0.18 (Lompo et al. 2017). The genetic diversity level of rosewood in Kulon Progo was also higher than that of *Oxytropis exserta* with He=0.156, *O. kamtschatica* with He=0.108, *O. revoluta* with He=0.089 (Kholina et al. 2013).

Genetic distance among rosewood population and individuals in Yogyakarta

Genetic distance among populations from the four, i.e., Sleman, Gunungkidul, Kulon Progo, and Bantul, and the individuals within these populations are measured using genetic distance and then demonstrated by clustering. The genetic identity and diversity parameters of four rosewood populations in Yogyakarta are summarized in Table 6. The similarity among individual mother trees is presented in a dendrogram (Figure 5). The highest genetic identity (0.985) and the lowest genetic distance (0.015) were observed between Gunungkidul and Bantul, indicating very high similarities between these populations. Those values explain Gunungkidul and Bantul on the dendrogram and PCA (Figure 5 and Figure 6), which are grouped into one population. The genetic identity between Kulon Progo and Sleman is 0.921, indicating that the populations' differences are pretty far.

The AMOVA calculated to examine genetic variations between and within geographic populations was statistically significant ($p<0.001$). The results of the AMOVA analysis (Table 7) showed that the highest genetic variation within the population was 70%, rather than among the populations that were only 30%. This means there is not much genetic difference among the four geographical populations of rosewood in Yogyakarta, presumably due to the propagation history of rosewood in Yogyakarta by root cuttings. This analysis firmly indicates the low genetic differences among the populations.

Since among populations does not vastly different, it might be possible that the origin of vegetatively propagated rosewood populations studied here is similar. More significant genetic variation within the population (88.2%) was also observed in *Prosopis cineraria* compared to the variance between populations (11.8%) (Sharma et al. 2011). Similar results were also observed in the *Pseudotsuga menziesii* population, with diversity values of 72,2% within and 27.8% between populations (Castelan et al. 2019).

Table 5. Comparison of genetic variation of rosewood plus tree candidates from Kulon Progo, Gunungkidul, Bantul, Sleman in Yogyakarta, based on Nei's

Population area	N	PPL (%)	Na	Ne	He	I
Kulon Progo	20	75.49%	1.54	1.52	0.29	0.42
Gunungkidul	13	58.82%	1.32	1.38	0.21	0.32
Bantul	10	45.10%	1.09	1.30	0.17	0.25
Sleman	18	79.41%	1.58	1.48	0.27	0.40
Mean ± SD for all loci	15.25 (0.19)	64.71% (7.91%)	1.39 (0.043)	1.42 (0.020)	0.23 (0.011)	0.34 (0.015)

Note: N: number of individuals. PPL: percentage of polymorphic loci. Na: Observed number of alleles. Ne: Effective number of alleles. He: Nei's gene diversity (Nei 1973). I: Shannon's Information index

Table 6. Genetic identity (*above diagonal*) and genetic distance (*below diagonal*) based on Unbiased Measures Nei

Populations	Kulon Progo	Gunungkidul	Bantul	Sleman
Kulon Progo		0.880	0.866	0.921
Gunungkidul	0.127		0.985	0.864
Bantul	0.144	0.015		0.850
Sleman	0.082	0.146	0.163	

Table 7. AMOVA based on ISSR data for four rosewood populations in Yogyakarta

Source	Degree of freedom	Sum of square	Mean sum of square	Estimated variance	Proportion of total variance %
Among populations	3	270.464	90.155	5.245	30%
Within populations	57	682.257	11.969	11.969	70%
Total	60	952.721		17.214	100%

In contrast, in *Tecomella undulata* the diversity within populations was 64% and 36% among populations (Chhajer et al. 2018). AMOVA results that show more significant variation within the observed populations may be due to the clustering of Gunungkidul and Bantul populations into one group. This phenomenon also occurs in *Artocarpus annulatus*, in which the variation within the population is greater than between populations, with the PCA distribution intersecting between populations (Dickinson et al. 2020).

Cluster analysis using PCA supports the relatedness of plus tree candidates by genetic distances, generating three large clusters according to their geographic locations, namely the Sleman cluster, Kulon Progo cluster, and the Gunungkidul-Bantul cluster (Figure 6). This shows that the genetic diversity of plus trees originating from the same area or the same population cluster tends to be similar. The clustering analysis of plus tree candidates based on Nei genetic distance also showed the same clustering (Figure 5).

The dendrogram shows that the four rosewood populations in Yogyakarta were separated into three main groups (Figure 5). The first group is the Kulon Progo population. The second group is the Sleman population which is divided into four subgroups. The third group is the Bantul population together with the Gunungkidul population. The closeness of these two populations could result from human facilitated dispersal, for example, root cuttings exchange among farmers (personal communication). PCA analysis was performed to determine genetic relationships between individuals (Figure 6). Analysis of dendrogram clustering based on the genetic distance matrix among populations and PCA based on the ISSR data showed that the Sleman population was the most divergent compared to other populations (Figure 5). The three groups formed in this clustering clearly showed more significant genotypic differentiation, consistent with the dendrogram. Accession no SL 11 and SL 02 are separated and quite different from the other Sleman accessions, which indicated that SL 11 and SL 02 have different genetic constitutions than other samples. Allegedly, the SL 11 and SL02 grew from seeds and not from root cuttings that generated different properties.

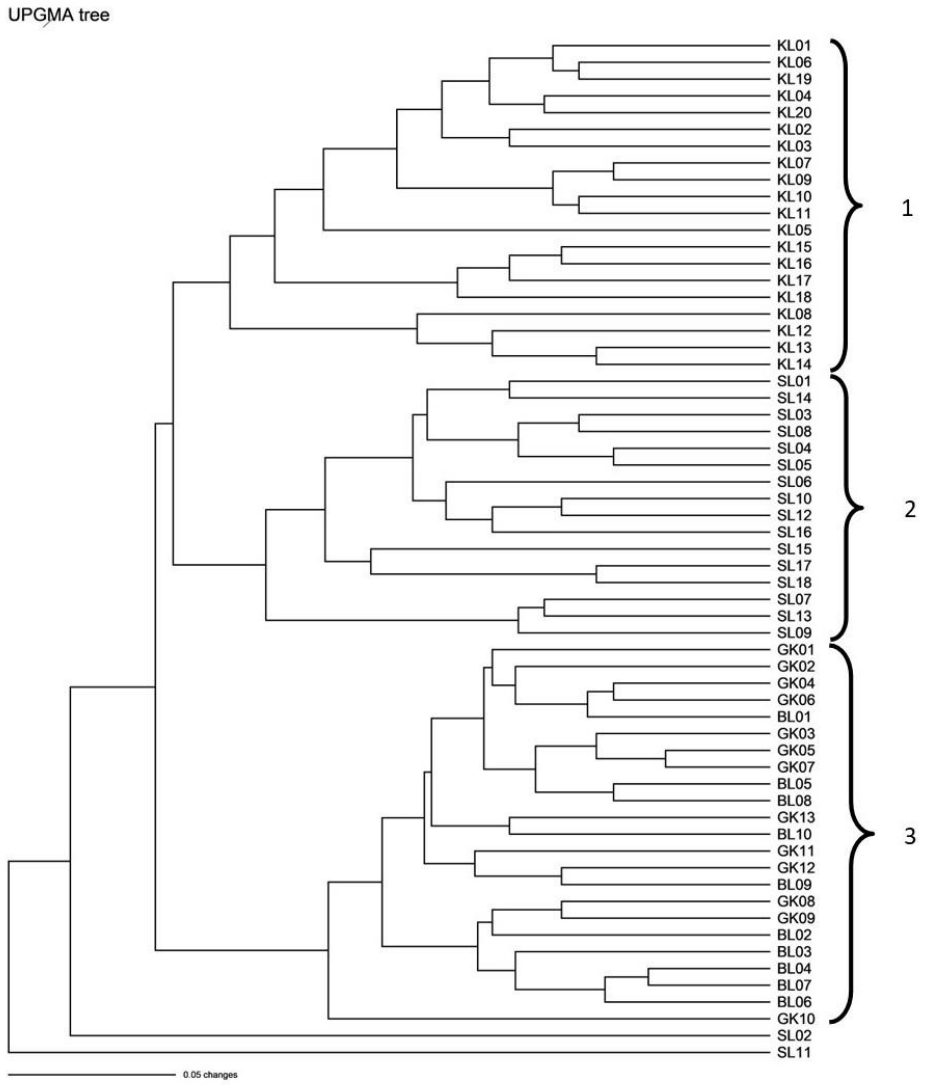

Figure 5. Dendrogram of four rosewood populations in Yogyakarta, Indonesia based on Ne i's genetic distance. Group 1 KL: Kulon Progo, Group 2 SL: Sleman, Group 3 are BL: Bantul and GK: Gunungkidul

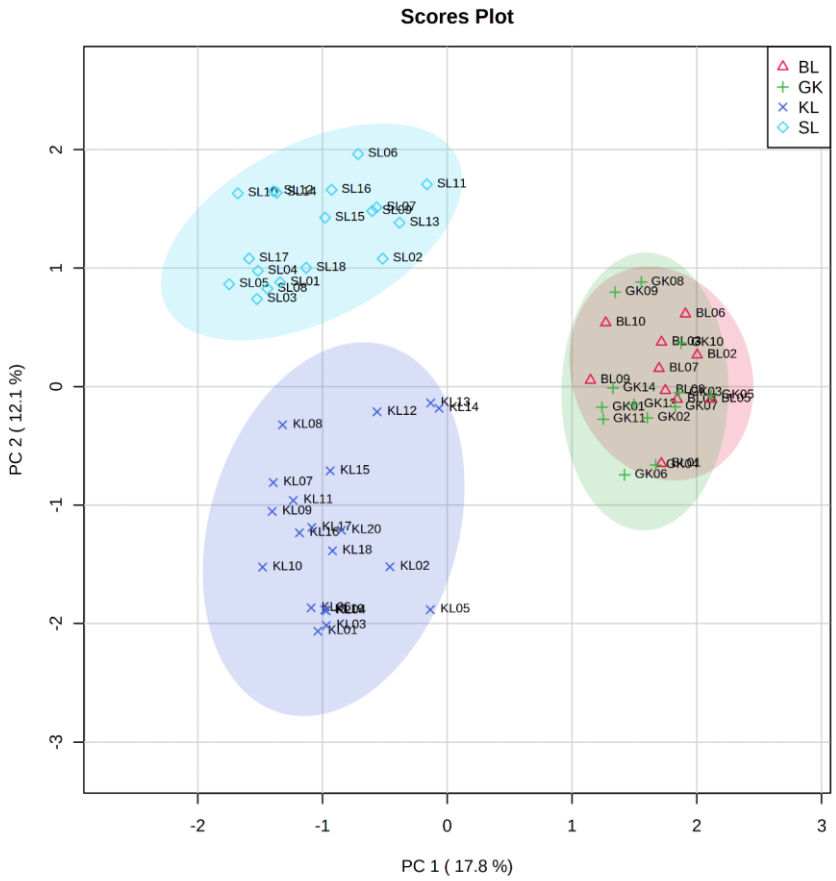

Figure 6. Cluster analysis using PCA based on ISSR divided population into three groups. Analysis using the MetaboAnalyst 5 program

The selection of plus trees with a 95% percentile resulted in three-plus trees, i.e., accession no GK05, KL06, and SL04, which came from different genetic clusters and thereby ensured their genetic diversity. A passing limit value that is too high will result in fewer plus trees being selected and eventually narrow genetic variation in the next generation. Conversely, a score limit value that is too low will give a low genetic gain (Zakiyah et al. 2017). Eight plus trees could be selected at the 90% percentile, namely the accession no GK05, GK 12, KL01, KL02, KL06, BL10, SL04, and SL12, which represent the four administrative regions of Gunungkidul, Kulon Progo, Bantul, and Sleman.

However, it is necessary to see whether the plus trees originating from the same administrative area show high diversity or uniformity because they might originate from clonal seedlings from the same parents. Comparison of plus trees from the same area, for example, accession no KL01 and KL06 were clustered with a narrow genetic distance. However, both were slightly different from accession no KL2 with Nei's ecludian (Figure 5). Considering the geographical proximity of the three trees of about 500 meters (Figure 1), the close relatedness of the three-plus trees could be implied that those trees might come from the same parent. In such a case, only one tree selected among those three-plus trees is sufficient to represent the diversity in that area. In the case of plus trees accession, no GK05 and GK12, both have genetic distances and geographical locations far apart, so it can be concluded that they might not come from the same parent. A similar case could be applied for the plus tree accession no SL04 and SL12, with large genetic distances and a far geographic location.

Therefore, out of eight-plus three candidates, it can be selected six accessions with the highest genetic diversity background, namely KL02, GK05, GK12, BL10, SL04, and SL12. The selection of plus trees using growth criteria with a scoring system combined with genetic distance analysis has also been carried out on *P. falcataria* (Zakiyah et al. 2017). In those plants, the main criteria in determining the plus tree are height, diameter, TBBC, straightness, permanent branches and trunk shape.

The selection of plus trees is an important step in any tree improvement and breeding program. Selection based on solely morphological characters, without considering the genetic background of the selected plus trees could lead to even more narrowing genetic diversity of the plus trees selected, and further the tree plantations generated. Unknown genetic background could lead to inter-bred of plus trees with closely related genetic background, which might be resulted in inbreeding. Inbreeding is disadvantageous to most tree species breeding programs, due to inbreeding depression. Therefore, this study which

incorporates both morphological characters with some genetic distance analysis using genetic markers should result in better improved tree plantations.

In conclusion, plus trees exploration in four d of Yogyakarta has identified 61 candidates, each of which has been compared with five other nearby trees. A modified scoring system used for selecting plus trees resulted in values that were normally distributed. Combination of selection using 90% percentile highest scores with genetic relatedness from genetic distance analysis and the geographic location of each plus tree candidates, from the eight selected plus trees from different administrative regions can be narrowed down to six, i.e., the accession no KL02, GK05, GK12, BL10, SL04, and SL12. They represent both the genetic diversity of the populations and their geographic locations. Thus, molecular genetic analysis supports the morphological parameters in selecting plus trees to ensure the genetic diversity of the selected plus trees.

ACKNOWLEDGEMENTS

This research was funded by the By Research BRIN program, No. 1457/H/2019 granted to the first author.

REFERENCES

Abdelaziz MS, Medraoui L, Alami M. 2020. Inter simple sequence repeat markers to assess genetic diversity of the desert date (*Balanites aegyptiaca* Del.) for Sahelian ecosystem restoration. Sci Rep 10: 1494. DOI: 10.1038/s41598-020-71835-9.

Adema F, Ohashi H, Sunarno B. 2016. Notes on Malesian Fabaceae (Leguminosae-Papilioideae) 17. The Genus *Dalbergia*. Blumea 61 (3): 186-206. DOI: 10.3767/000651916X693905.

Bal P, Panda PC. 2018. Molecular characterization and phylogenetic relationships of *Dalbergia* species of Eastern India Based on RAPD and ISSR analysis. Intl J Innov Sci Res Tech 3 (7): 417-425.

Castelan PM, Cruz MC, Castillo MCM, Izquierdo SC, Upton JL, Padilla IS, Cruz VG. 2019. Diversity and genetic structure inferred with microsatellites in natural populations of *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae) in the Central Region of Mexico. Forest 10: 101. DOI: 10.3390/f10020101.

Chhajer S, Jukanti AK, Bhatt RK, Kalia RK. 2018. Genetic diversity studies in endangered desert teak *Tecomella undulata* (Sm) Seem] using arbitrary (RAPD), semi-arbitrary (ISSR) and sequence based (nuclear rDNA) markers. Trees 32 (4): 1083-1101. DOI: 10.1007/s00468-018-1697-9.

Crowther J, Zimmer H, Le Thi H, Quang TL, Nichols JD. 2020. Forestry in Vietnam: The potential role for native timber species. For Policy Econ 116: 102182. DOI: 10.1016/j.forpol.2020.102182.

Dickinson L, Noble H, Gardner E, Puad ASA, Zakaria WNFW, Zerega NJC. 2020. Genetic diversity and structure of the critically endangered *Artocarpus annulatus*, a crop wild relative of jackfruit (*A. heterophyllus*). PeerJ 8: e9897. DOI: 10.7717/peerj.9897.

Dobhal S, Sharma S, Ahmed N, Kumar A. 2019. Genetic polymorphism in *Dalbergia sissoo* Roxb. using RAPD markers. Ind J Biotechnol 18 (2): 164-173.

Dwianto W, Bahanawan A, Kusumah SS, Darmawan T, Amin Y, Pramasari DA, Lestari E, Akbar F, Sudarmanto. 2019. Study on the existence and characteristics of Sonokeling (*Dalbergia latifolia* Roxb) as an Appendix II CITES Wood. IOP Conf Ser Earth Environ Sci 374: 012063. DOI: 10.1088/1755-1315/374/1/012063.

Fatima T, Srivastava A, Hanur VS, Rao MS. 2018a. An effective wood DNA extraction protocol for three economic important timber species of India. American J Plant Sci 9 (2): 139-149. DOI: 10.4236/ajps.2018.92012.

Fatima T, Srivastava A, Somashekar PV, Hanur VS. 2018b. Optimization of genomic DNA extraction routine from rosewood (*Dalbergia latifolia*) and ben teak (*Lagerstroemia lanceolata*). Intl J Gen 10 (6): 446-451.

Felix FC, Chagas KPTD, Ferrari CDS, Vieira FDA, Pacheco MV. 2020. Applications of ISSR markers in studies of genetic diversity of *Pityrocarpa moniliformis*. Rev Caatinga 33 (4): 1017-1024. DOI: 10.1590/1983-21252020V33N417RC.

Geneaid. 2022. Genomic DNA Mini Kit (Plant) Protocol. https://www.geneaid.com/data/download/attached/160274369346632 9086.pdf. [Access April 2022]

Guerra FP, Gomez P, Gutierrez A. 2018. Genetic diversity of *Adesmia bijuga* Phil., an endangered fabaceae species from Central Chile. Braz J Bot 41: 247-251. DOI: 10.1007/S40415-018-0440-5.

Hanifah N. 2022. Peraturan Terbaru Peredaran Kayu Sonokeling (*Dalbergia latifolia*) Dalam Negeri dan Luar Negeri. http://ksdae.menlhk.go.id/. [Access 12 April 2022 April] [Indonesian]

Hidayat Y. 2010. Morphological variation of surian (*Toona sinensis* Roem) candidate plus trees collected from communty forest populations in West Java and Central Java. Conference: Promoting Biodiversity, Rainforest Protection, and Economic Development in Indonesia.

Hien VTT, Phong DT. 2012. Genetic diversity among endangered rare *Dalbergia cochinchinensis* (Fabaceae) genotypes in Vietnam revealed by random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers. Afr J Biotechnol 11 (35): 8632-8644. DOI: 10.5897/AJB11.3598.

Huang WD, Zhao XY, Zhao X, Li YL, Pan CC. 2016. Environmental determinants of genetic diversity in *Caragana microphylla* (Fabaceae) in Northern China. Bot J Linn Soc 181 (2): 269-278. DOI: 10.1111/boj.12407.

Ijaz S, Ul Haq I, Razzaq HA, Nasir B, Babar M. 2019. ISSR based population genetics study for tagging a diverse population of shisham (*Dalbergia sissoo*) in Pakistan. App Eco Env Res 17 (3): 5851-5861. DOI: 10.15666/aeer/1703_58515861.

Javaid A, Akram W, Shoaib A, Haider AS, Ahmed A. 2014. ISSR analysis of genetic diversity in *Dalbergia sissoo* in Punjab, Pakistan. Pak J Bot 46 (5): 1573-1576.

Junior AS, Da L, Cabral RLR, Sartori L, Souza LC, Miranda FD, Caldeira MVW, Moreira SO, Godinho TO. 2020. Evaluation of diversity and genetic structure as strategies for conservation of natural populations of *Dalbergia nigra* (Vell.) Allemao Ex Benth. Cerne 26 (4): 435-443. DOI: 10.1590/01047760202026042754.

Kholina AB, Nakonechnaya OV, Yakubov VV. 2013. Genetic variation in six species of the genus *Oxytropis* DC. (Fabaceae) from Kamchatka Peninsula. Russ J Genet 49: 1021-1029. DOI: 10.1134/S1022795413100049.

Korshikov II, Demkovich AE. 2010. Genetic polymorphism of plus tree clones and their seed progeny in the scotch pine clone plantation. Cytol Genet 44 (1): 28-36. DOI: 10.3103/S0095452710010068.

Li H, Chappell M, Zhang D. 2020. Assessing genetic diversity and population structure of *Kalmia latifolia* L. in the Eastern United States: An essential step towards breeding for adaptability to southeastern environmental conditions. Sustainability 12: 8284. DOI: 10.3390/su12198284.

Liu CL, Kuchma O, KonstantinV, Krutovsky. 2018. Mixed-species versus monocultures in plantation forestry: Development, benefits, ecosystem services and perspectives for the future. Global Ecol Conserv 15: e00419. DOI: 10.1016/J.GECCO.2018.E00419.

Liu Q, Qiao Z, Lv Y. 2021. Pyvt: A python-based open-source software for visualization and graphic analysis of fluid dynamics datasets. Aero Sci Technol 117: 106961. DOI: 10.1016/J.Ast.2021.106961.

Lompo D, Vinceti B, Gaisberger H, Konrad H, Duminil J, Ouedraogo M, Sina S, Geburek T. 2017. Genetic conservation in *Parkia biglobosa* (Fabaceae: Mimosoideae). Silvae Genetica 66: 1-8. DOI: 10.1515/sg-2017-0001.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proc Nat Acad Sci USA 70 (12): 3321-3323.

Nei M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research an update. Bioinformatics 28 (19): 2537-2539. DOI: 10.1093%2Fbioinformatics%2Fbts460.

Poerba YS, Ahmad F. 2013. Genetic variation analyses of *Musa balbisiana* Colla based on RAPD and ISSR markers. Berita Biologi 12 (2): 259-267. DOI: 10.14203/beritabiologi.v12i2.540. [Indonesian]

Prawirohatmodjo S, Suranto J, Martawijaya A, den Outer RW, Sosef MSM. 1994. Plant Resources of South-East Asia. Timber trees: Major commercial timbers. PROSEA. Bogor.

Samarina LS, Valentina I. Malyarovskaya, Rakhmangulov RS, Koninskaya NG, Matskiv AO, Shkhalakhova RM, Orlov YL, Tsaturyan GA, Shurkina ES, Gvasaliya MV, Kuleshov AS, Ryndin AV. 2022. Population analysis of *Diospyros lotus* in the Northwestern Caucasus based on leaf morphology and multilocus DNA markers. Intl J Mol Sci 23 (4): 2192. DOI: 10.3390/ijms23042192.

Selyutina IY, Konichenko ES, Zibzeev EG, Kuban IN. 2021. Genetic diversity of the endangered endemic species *Hedysarum sangilense* Krasnoborov et Timokhina (Fabaceae). J Plant Sci Cons 10 (1): 29-35. DOI: 10.17581/bp.2021.10109.

Shakoor A, Zaib G, Zhao F, Li W, Lan X, Bozchaloyi SE. 2022. ISSR markers and morphometry determine genetic diversity and population structure in *Hedera helix* L. Czech J Gen Plant Breed 58 (2): 73-82. DOI: 10.17221/93/2021-CJGPB.

Sharma SK, Kumar S, Rawat D, Kumaria S, Kumar A, Rama Rao S. 2011. Genetic diversity and gene flow estimation in *Prosopis cineraria* (L.) Druce: A key stone tree species of Indian Thar desert. Biochem Syst Ecol 39 (1): 9-13.DOI: 10.1016%2Fj.bse.2010.12.018.

Sheikh ZN, Sharma V, Shah RA, Sharma N, Summuna B, Al-Misned FA, El-Serehy HA, Mir JI. 2021. Genetic diversity analysis and population structure in apricot (*Prunus armeniaca* L.) grown under North-Western Himalayas using ISSR markers. Saudi J Bio Sci 28 (10): 5986-5992. DOI: 10.1016/J.Sjbs.2021.06.051.

Siburian RHS, Siregar UJ, Siregar IZ. 2017. Genetic variation of *Gyrinops versteegii* originated from Papua based on RAPD. Asian J Microbiol Biotechnol Env Sci 19 (3): 1-5.

Silva AVC, Muniz EN, Almeida CS, Vitoria MF. 2015. Genetic diversity and sex identification in *Genipa americana*. Trop Subtrop Agroecosyst 18 (1): 81-86.

Soares ANR, Vitoria MF, Nascimento ALS, Ledo AS. 2016. Genetic diversity in natural populations of mangaba in Sergipe, the largest producer State in Brazil. Genet Mol Res 15: 3. DOI: 10.4238/gmr.15038624.

Tewari SK, Tewari L, Dubey A, Kumar A, Kumar N, Kaushal R. 2022. Use of the RAPD marker to determine the genetic diversity of various *Dalbergia sissoo* Roxb. (Shisham) genotypes and their evolutionary relationship in Indian subcontinents. Vegetos. DOI:10.1007/s42535-021-00334-7

Vasudevan R, Sasidharan KR, Venkataramanan KS, Thangamani D, Sivanantham S. 2020. Standardization of macropropagation technique for *Dalbergia latifolia* Roxb. through root cuttings, its application in production of quality planting stock and conservation of genetic resources. Intl J Adv Res Rev 5 (11): 9-19.

Xia J, Wishart DS. 2016. Using MetaboAnalyst 3.0 for comprehensive metabolomics data analysis. Curr Protoc BioinForm 55: 14.10.1-14.10.91. DOI: 10.1002/cpbi.11.

Yulita SK, Atikah TD, Wardani W, Susila. 2020. Unraveling genetic variations of *Dalbergia latifolia* (Fabaceae) from Yogyakarta and Lombok Island, Indonesia. Biodiversitas 21 (2): 833-841. DOI: 10.13057/biodiv/d210251.

Zakiyah R, Siregar UJ, Hartati N. 2017. Morphological characterization of sengon (*Paraserianthes falcataria* L. Nielsen) gamma radiation mutation results. J Silv Trop 8 (1): 41-47. [Indonesian]

Zhang Y, Yuan Y, Pang Y, Yu F, Yuan C, Wang D, Hu X. 2019. Phylogenetic reconstruction and divergence time estimation of *Blumea* DC. (Asteraceae: Inuleae) in China based on nrDNA ITS and cpDNA trnL-F sequences. Plants 8 (7): 210. DOI: 10.3390/plants8070210.