Genetic diversity of *Pericopsis mooniana* from South Kalimantan based on Random Amplified Polymorphism DNA (RAPD) Markers

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Abstract. The genetic diversity and relationship of *Pericopsis mooniana* collected from Pulau Laut, South Kalimantan, was analyzed using RAPD markers. Currently, the natural distribution of *P. mooniana* in Pulau Laut no longer exists. Instead, PT Inhutani II, as the concession holder in the area, had collected the remaining *P. mooniana* and planted it in the seed orchard, arboretum, or around PT Inhutani’s mess/office. Forty-two samples from four locations: mess/residential house (3), seed orchard (33), arboretum (3), and PT Inhutani II Unit Stagen (3) were analyzed using seven RAPD primers. Our study showed that the total genetic diversity of all populations was low (*He* = 0.191 ± 0.013). *P. mooniana* collected from seed orchards had the highest diversity (*He* = 0.289 ± 0.021), while the lowest was from mess/residential houses (*He* = 0.134 ± 0.025). The genetic relationship data indicated the possibility that *P. mooniana* from mess/residential house, arboretum, and seed orchard may come from the same origin. These results can be used to support the development of *ex-situ* conservation plots to avoid inbreeding depression. At the same time, the genetic diversity will be helpful in its conservation and further utilization, such as establishing the plantations to reduce the pressure of the species in nature and provide valuable timber production in Indonesia.

1. Introduction
Molecular markers such as Random Amplified Polymorphism DNA (RAPD), Internal transcribed spacer (ITS), Simple Sequence Repeat (SSR), or Single Nucleotide Polymorphism (SNP) markers have available. However, RAPD is still preferred over other markers since it is simple, can be accomplished in a small-direct laboratory for most of its uses, is relevant to all genomes, does not require sequence information, and requires only a tiny amount of DNA. RAPD technique allows for a quick and efficient search for polymorphism based on a DNA sequence at a large number of loci. The technique's diagnostic power comes from the wide range of possible primers that can be utilized, and only reproducible RAPD bands may be detected through careful primer selection. PCR condition optimization for target species and replication to ensure that only reproducible bands are scored. The markers have been used in many genetic diversity studies such as in the natural populations of desert teak (*Tecomella undulata*), a medically important agroforestry tree in western Rajasthan [1], *Nectandra megapotamica*, a tree species in the Atlantic Forest, Brazil [2], and *Prunus mira*, a widely-cultivated fruit tree [3].
Pericopsis mooniana is a valuable timber species in Indonesia, but it is currently categorized as Vulnerable A1cd on the IUCN Red List of Threatened Species (https://www.iucnredlist.org/species/31312/9623986, accessed on May 3, 2021). The United Nations Environment Programme's World Conservation Monitoring Centre has also identified P. mooniana as a non-CITES timber species that potentially requires further protection [4]. Because of scarce natural regeneration and large-scale exploitation that was not followed by replanting, P. mooniana was proposed for inclusion in Appendix II of the CITES convention in 1992, which requires all trade in a species to be registered [5].

A series of activities have been carried out in Indonesia to conserve the species from the threat of extinction, one of which was developing ex-situ conservation plots. The natural distribution of P. mooniana in Indonesia was reported in Banyu Asin (South Sumatera), Sampit (Central Kalimantan), South Kalimantan, Minahasa (North Sulawesi), Kolaka (Southeast Sulawesi), Moluccas, and Irian Jaya (Papua) [6]. The genetic material was planned to be collected from the reported natural distribution. However, our communication with forestry officials in several provinces such as South Sumatra and Moluccas indicated that P. mooniana might no longer be found in this area. It may remain in Southeast Sulawesi, South Kalimantan and Papua [7]. Even though the initial plan of the collection was from three remaining provinces, eventually it could only be conducted in two provinces: Southeast Sulawesi (Lamedai Nature Reserve/Cagar Alam Lamedai in Kolaka, and Tanggetada Forest) and South Kalimantan (Pulau Laut) due to budget constraints [7].

Many studies on P.mooniana have been conducted, such as the diversity of arbuscular mycorrhizal fungi in the growth habitat of P.mooniana [8], its growth performance in Luwu Timur Regency [9], and in-vitro germination growth [10, 11]. However, only two studies using molecular markers have been found for P. mooniana in Indonesia, i.e., ITS markers for DNA barcoding of two populations of P. mooniana [12] and RAPD markers for genetic diversity and structure of P. mooniana from Lamedai forest in Southeast Sulawesi [13]. A recent study using a barcoding marker rDNA ITS showed that P. mooniana from Southeast Sulawesi and South Kalimantan were clustered together. In contrast, P. mooniana from Purwodadi Botanic Garden was separated with only one base difference from those two populations [12]. However, the DNA barcoding study used only nine samples from South Kalimantan and has not focused on the genetic diversity and relationship of P. mooniana from several locations in South Kalimantan yet. Therefore, our current study will specifically examine P. mooniana grown in Pulau Laut, South Kalimantan. As the only representing of P. mooniana from South Kalimantan, a genetic study of the remaining P. mooniana in Pulau Laut, especially those planted in arboretum and seed sources, provides a valuable genetic resource for P. mooniana in Indonesia to support its conservation and further utilization.

2. Materials and Methods

2.1. Description of sampling

Our personal communication and field trip showed that P. mooniana could no longer be found in its natural distribution in Pulau Laut. Instead, PT Inhutani II, the concession permit holder in the area, has collected the remaining P. mooniana in its natural distribution and planted it in several locations such as seed orchard, arboretum, and around PT Inhutani II's mess/office. The location of planted P. mooniana in the seed orchard, arboretum, and mess/residential house was located in one village, namely PT Inhutani II Unit Samaras in the Samaras village, Pulau Laut Barat sub-district, Kotabaru district, South Kalimantan province, while the P. mooniana in PT Inhutani II Unit Stagen was located in the PT Inhutani II Unit Stagen office in the Gunung Sari village, Pulau Laut Utara sub-district, Kotabaru district, South Kalimantan province (Figure 1).
Figure 1. Sampling location of *P. mooniana* in Pulau Laut, South Kalimantan. P1-P5 (mother trees number 1-5) = mess/residential house, P6-P7 = seed orchard, P8 = arboretum, and P9 = PT Inhutani II Unit Stagen.

A total of two or three leaves from each of forty-two mother trees of *P. mooniana* were collected from four locations in Pulau Laut. The majority of the samples were conducted in the seed orchard due to the high number of trees in one area, while at other locations, the number of trees found was only a few. The differences in the number of samples used may influence the result of the study. However, as this is a preliminary study, the result is expected to bring valuable information on the genetic diversity and genetic relationship of the remaining *P. mooniana* in Pulau Laut, South Kalimantan, especially for the arboretum and seed orchard populations. The seed orchard is a valuable seed source, so any information related to the genetic diversity of the orchards is critical to support its conservation and further utilization in the future. Unfortunately, the origins of all *P. mooniana* of the four locations were not known in detail. Our communication with the head of PT Inhutani II Unit Samaras indicated that all *P. mooniana* were initially from Pulau Laut areas and not from other locations in South Kalimantan. Detailed collected sampling locations are shown in Table 1.

Table 1. List of locations and number of samples collected per location.

| No | Name                        | No of samples | Coordinate                  | Collected locations                                                                                     |
|----|------------------------------|---------------|-----------------------------|---------------------------------------------------------------------------------------------------------|
| 1  | Mess/Residential house       | 3             | S 03° 47' 32.04"E 116° 05' 48.10" | PT Inhutani II Unit Samaras in the Samaras village, Pulau Laut Barat sub-district, Kotabaru district, South Kalimantan |
| 2  | Seed Orchard                 | 33            | S 03° 46' 51.01"E 116° 05' 49.28" | PT Inhutani II Unit Samaras in the Samaras village, Pulau Laut Barat sub-district, Kotabaru district, South Kalimantan |
| 3  | Arboretum                    | 3             | S 03° 47' 03.78"E 116° 05' 30.80" | PT Inhutani II Unit Samaras in the Samaras village, Pulau Laut Barat sub-district, Kotabaru district, South Kalimantan |
| 4  | Inhutani II Unit Stagen      | 3             | S 03° 18' 33.58"E 116° 10' 33.61" | PT Inhutani II Unit Stagen in the Gunung Sari village, Pulau Laut Utara sub-district, Kotabaru district, South Kalimantan |
2.2. DNA extraction, PCR and gel electrophoresis

Total DNA was extracted from each sample using a modified CTAB method [14] and then precipitated two times using isopropanol and NAOAc. The concentration and ratio of the extracted DNA were measured, and then based on the concentration value obtained, the DNA was diluted into 2.5 mg µl⁻¹. Next, screening of RAPD primers was conducted using a set of 40 primers. The criteria used for the screening are based on its polymorphic and quality band produced. After screening, seven RAPD primers were finally selected and used for analysis (Table 2).

Total PCR volume was 10 mL consisted of purified water, 10X buffer, 3 M MgCl₂, 200 uM dNTPs, primer and Biotaq DNA polymerase (Bioline). PCR was performed using a GeneAmp 9700 thermocycler (Applied Biosystems): preheating to 94°C for 5 minutes, then 45 cycles at each of three temperatures, 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 1.5 minutes. The PCR cycles were terminated at 72°C for 7 minutes. Agarose gel-based electrophoresis was then conducted using a 1.2% gel with ethidium bromide added and performed using 1X TBE buffer at 120 V for 2.5 hours. An image of each DNA band was taken using a GelDoc Image Analyser.

| Primer  | 5' to 3'        | No. of Bases | % GC Content |
|---------|-----------------|--------------|--------------|
| OPA-12  | TCGGCGATAG      | 10           | 60           |
| OPB-10  | CTGCTGGGAC      | 10           | 70           |
| OPC-20  | ACTTCGCCAC      | 10           | 60           |
| OPD-03  | GTGCCTGGTCA     | 10           | 70           |
| OPO-11  | GACAGGAGGT      | 10           | 60           |
| OPW-04  | CAGAAGCGGA      | 10           | 60           |
| OPY-20  | AGCCGTGGAA      | 10           | 60           |

2.3. Data analysis

The visualization of each DNA band was analyzed using the GenAlex 6.5 [15] and POPGENE 1.32 [16] and classified as "1" if there was amplification and "0" if there was no amplification. To determine the band's size produced from each primer, a DNA ladder (Vivantis) that marks every 100 bp was put in one of the gel lines to assess the size of a particular band. The DNA ladder is used to size PCR products and other double-stranded DNA fragments using molecular weight standards for electrophoresis on an agarose gel.

3. Results and Discussion

The seven RAPD markers generated a total of 54 polymorphic DNA bands of length between 400-1700 bp (Table 3).

| Primer  | Total number of polymorphic bands | (band size) (bp) |
|---------|----------------------------------|------------------|
| OPA-12  | 7                                | 1100, 1000, 900, 800, 680, 600, 590 |
| OPB-10  | 7                                | 1500, 1200, 1000, 900, 700, 650, 400 |
| OPC-20  | 8                                | 1100, 1000, 900, 850, 800, 650, 550, 500 |
| OPD-03  | 13                               | 1700, 1400, 1300, 1200, 1100, 1050, 1000, 900, 800, 700, 600, 500, 400 |
| OPO-11  | 4                                | 1050, 950, 850, 600 |
| OPW-04  | 6                                | 1200, 1000, 900, 850, 800, 700 |
| OPY-20  | 9                                | 1100, 900, 850, 800, 700, 600, 550, 500, 400 |
| Total   | 54                               |                  |
Primer OPD-03 produced the highest number of polymorphic bands (13), followed by OPY-20 (9), OPC-20 (8), OPB-10 and OPA-12 (7 each) (Table 3). The RAPD marker can identify and produce fine genetic markers at high levels of DNA variation. As a result, the number of polymorphic bands in a genetic diversity analysis is crucial to achieving the best results [17, 18]. The polymorphic DNA banding pattern is caused by differences in the arrangement of the bases in each DNA sample. Therefore, not all DNA samples can produce bands at a particular locus.

Table 4 presents the Ne value, which shows the number of effective alleles obtained from each locus. The higher the Ne value, the higher the number of heterozygous individuals in a population. Meanwhile, the proportion of polymorphic loci shows heterozygosity and heterogeneity between individuals in the population [19]. The higher the proportion of polymorphic loci, the higher the level of heterozygosity and heterogeneity. The low number of observed alleles (Na) in this study may be due to the limited number of sample populations. It tends to be more homogeneous, and there may still be a close kinship between each individual in the sample population used [20]. An additional number of samples from the same locations or other locations representing the Pulau Laut area is needed to obtain an accurate picture of the genetic diversity of P. mooniana in the region.

Table 4. Summary of effective alleles and expected heterozygosity by Population.

| Pop                  | Na     | Ne     | % Polymorphic Loci | He        |
|----------------------|--------|--------|--------------------|-----------|
| Mess/resident house  | 1.074±0.112 | 1.222±0.045 | 37.04%               | 0.134±0.025 |
| Seed Orchard         | 2.000±0.000 | 1.473±0.043 | 100.00%              | 0.289±0.021 |
| Arboretum            | 1.111±0.120 | 1.278±0.049 | 44.44%               | 0.165±0.027 |
| Inhutani II Unit Stagen | 1.074±0.121 | 1.318±0.056 | 42.59%               | 0.176±0.029 |
| Total                | 1.315±0.057 | 1.323±0.025 |                   | 0.191±0.013 |

Remarks:
Na = No. of Different Alleles
Ne = No. of Effective Alleles
I = Shannon’s Information Index
He = Expected Heterozygosity

The analysis using seven RAPD primers showed that the total genetic diversity of all populations from Pulau Laut, South Kalimantan was low (He = 0.191 ± 0.013). P. mooniana collected from seed orchards have the highest diversity (He = 0.289 ± 0.021), while the lowest was from mess/residential houses (He = 0.134 ± 0.025) (Table 4). The high genetic diversity in the seed orchard may be due to the larger number of samples collected so that the genetic diversity produced is also higher than in other locations. In addition, the results of this study also showed that the low level of genetic diversity from the arboretum was lower than other locations such as PT Inhutani II Stagen. The arboretum is expected to represent its diversity in nature and become a “show window” area of P. mooniana from Pulau Laut. Therefore, the selection of P. mooniana planted in the arboretum must be carefully considered to capture the diversity in its natural distribution.

The genetic diversity study of P. mooniana genetic material from Lamedai, South Sulawesi, has been previously conducted using 5 (five) RAPD primers. Of the 7 (seven) primers we used in this study, there were 4 (four) primers that were the same as the previous study, namely OPA-12, OPD-03, OPO-11, and OPW-04. Comparison of the resulting polymorphisms showed that the polymorphisms produced from samples from Pulau Laut in our study were more numerous and varied than in the previous study. The OPD-03 primer with samples from Pulau Laut has the highest polymorphism (13 loci) compared to Lamedai (4 loci), followed by OPA-12 (7 loci in Pulau Laut and three loci in Lamedai), OPW-04 (six loci in Pulau Laut and one locus in Lamedai), and finally OPO-11 (4 loci in Pulau Laut and five loci in Lamedai (Table 3 and [13]).
Our study found a lower level of genetic diversity than the previous study using genetic material from the Lamedai forest in Southeast Sulawesi. The RAPD study using *P. mooniana* from Lamedai forest in South East Sulawesi has a moderate level of genetic diversity for all populations (*He* = 0.361 ± 0.017), and it varied from *He* = 0.349 ± 0.030 (Balijaya) to *He* = 0.383 ± 0.031 (Cagar Alam Lamedai) [13]. The low level of genetic diversity in *P. mooniana* from Pulau Laut was presumed because apart from the smaller number of samples used, but also because the material used for the study were no longer from their natural distribution but from planted trees that might not fully capture all variations in its natural distributions. This condition is different from *P. mooniana* from South East Sulawesi. It was collected from the preserved natural distributions and established as a protected area of Lamedai Nature Reserve (Cagar Alam Lamedai) and gardens belonging to residents.

The highest value of the genetic distance between populations was 0.2271, namely between Inhutani II Stagen and Mess/residential house populations, while the lowest genetic distance between populations was between arboretum and seed orchard populations 0.0679 (Table 5). The results from PCoA analysis have also shown that the population from seed orchard, arboretum, and mess/residential house were grouped into one group. In contrast, the population from PT Inhutani Stagen was grouped into different groups (Figure 2).

**Table 5.** Genetic distance among the population.

| Population ID                  | Mess/residential house | Seed Orchard | Arboretum | Inhutani II Unit Stagen |
|--------------------------------|------------------------|--------------|-----------|-------------------------|
| Mess/residential house         | ****                   |              |           |                         |
| Seed Orchard                   | 0.0955                 | ****         |           |                         |
| Arboretum                      | 0.1002                 | 0.0679       | ****      |                         |
| Inhutani II Unit Stagen        | 0.2271                 | 0.1480       | 0.1170    | ****                   |

**Figure 2.** Principal coordinates analysis of four *P. mooniana* populations from Pulau Laut, South Kalimantan using Genalex 6.5.

Further analysis using PopGene showed that *P. mooniana* from Pulau Laut was clustered into two clusters (Figure 3). The first cluster was the population from arboretum and seed orchard that was clustered together, then they are clustered with mess/residential house, while the second cluster was population from PT Inhutani II Unit Stagen. Based on the cluster analysis, it was indicated that the population of *P. mooniana* from mess/residential house, seed orchard and arboretum may have a close genetic relationship. It is supported by the results of principal coordinates analysis (Figure 2) and dendrogram (Figure 3), indicating that the three populations may come from the same origin. This presumed was made because they were planted in adjacent locations (Figure 1), so there is a possibility that the genetic material used for planting came from the same collection but was planted in different locations. This is different from *P. mooniana* planted at the PT Inhutani II Unit Stagen, which has
different clusters (Figure 3) and planted in a relatively distant location (Figure 1) from the others so that
it might be collected at different times and/or different parts of Pulau Laut areas.

Figure 3. Cluster analysis of four *P. mooniana* populations from Pulau Laut, South Kalimantan
using PopGene.

Analysis of molecular variance showed that 94% diversity was within-population while only 6% was
among the population (Table 6), showing that only 6% the diversity among the population.

Table 6. Analysis molecular variance of four *P. mooniana* populations from Pulau Laut, South
Kalimantan.

| Source          | df | SS     | MS     | Est. Var. | %       |
|-----------------|----|--------|--------|-----------|---------|
| Among Pops      | 3  | 40.245 | 13.415 | 0.655     | 6%      |
| Within Pops     | 37 | 372.219| 10.060 | 10.060    | 94%     |
| Total           | 40 | 412.463| 10.715 | 100%      |         |

An interesting result was obtained from the analysis of band patterns across four *P. mooniana*
populations. Of the four populations studied, only *P. mooniana* from the seed orchard has 8 (eight)
specific alleles (Table 7 and Figure 4). The specific alleles in seed orchards are important to conserve
as they show unique characters in the population

Table 7. Analysis of band patterns across the population of four *P. mooniana* populations.

| Population      | Mess | Seed orchard | Arboretum | Inhutani II Unit Stagen |
|-----------------|------|--------------|-----------|-------------------------|
| No. Bands       | 38   | 54           | 36        | 35                      |
| No. Bands Freq. >= 5% | 38   | 50           | 36        | 35                      |
| No. Private Bands | 0    | 8            | 0         | 0                       |
| No. LComm Bands (<=25%) | 0    | 0            | 0         | 0                       |
| No. LComm Bands (<=50%) | 3    | 9            | 2         | 4                       |

Remarks:
- No. Bands = No. of Different Bands
- No. Bands Freq. >= 5% = No. of Different Bands with a Frequency >= 5%
- No. Private Bands = No. of Bands Unique to a Single Population
- No. LComm Bands (<=25%) = No. of Locally Common Bands (Freq. >= 5%) Found in 25% or Fewer Populations
- No. LComm Bands (<=50%) = No. of Locally Common Bands (Freq. >= 5%) Found in 50% or Fewer Populations
Figure 4. Analysis of band patterns across the population of four *P. mooniana* populations from Pulau Laut, South Kalimantan using Genalex 6.5.

*P. mooniana* in Pulau Laut, South Kalimantan, has naturally been distributed on the island. However, the natural distribution nowadays is difficult to find and possibly has entirely lost due to land shifting and illegal logging. PT. Inhutani II, as a forest concession company in Pulau Laut, fortunately, collected the remaining *P. mooniana* in the area and planted it in a seed orchard located in Samaras Village, Pulau Laut Barat Sub-District, Kotabaru District, South Kalimantan province [7]. Thus, a 2.19 ha orchard was planted in 1987 using a 3 x 3 m spacing and all used genetic material from Pulau Laut (Figure 5).

Figure 5. *P. mooniana* seed orchard in Pulau Laut, South Kalimantan.

This seed orchard provides a valuable genetic resource of *P. mooniana* from Pulau Laut. The seed orchard proved to have better genetic material than others planted locations, as showed in its highest genetic diversity (*He* = 0.289 ± 0.021) (Table 4) and the presence of 8 (eight) specific alleles (Table 7 and Figure 4). Our personal communication had indicated that the natural distribution of *P. mooniana* in South Kalimantan could be found only in Pulau Laut Sub-District, not elsewhere in South Kalimantan. Pulau Laut island is located on a small island that is not integrated with the big island of Kalimantan (Figure 1). Thus, the presence of *P. mooniana* in the orchard is expected to be considered as the representing of *P. mooniana* from South Kalimantan. Even though the genetic diversity of the seed orchards is the highest than other locations in South Kalimantan, the value is much lower than others found in Southeast Sulawesi. Therefore, some approaches should be conducted to increase its genetic diversity, such as infusion by collecting other genetic materials from the remaining natural distributions in Pulau Laut or artificial mating to enrich its genetic variability.

Habitat loss, illegal logging, and low replanting are some of the causes of the threat of *P. mooniana* extinction in nature. This condition has prompted a series of activities to conserve *P. mooniana* in Indonesia through a collection of *P. mooniana* genetic material from its natural distributions (Southeast
Sulawesi and South Kalimantan), conducting supporting studies such as genetic, tissue culture, silviculture, and establishing ex-situ conservation plots in Gunung Kidul, DI Yogyakarta [7]. Information on the genetic diversity and genetic relationship of *P. mooniana* from Pulau Laut, South Kalimantan, can directly support ex-situ conservation plots. The presence information of the genetic relationships of some populations from Pulau Laut will determine the design of the ex-situ conservation plots. The genetic material from seed orchard, arboretum, and mess/residential house can be considered one population and planted in the same location, while *P. mooniana* from PT Inhutani II Stagen has a different cluster that can be planted separately from the other Pulau Laut populations. This careful design of the ex-situ conservation plots is important to avoid inbreeding depression which can reduce the quality of seeds produced.

The development of *P. mooniana* plantations is an alternative to reduce the pressure of this species in nature. Plantations are expected to fulfill the demand for *P. mooniana* wood while at the same time, it can also conserve the species in its natural distribution. The wood quality has proved can also be improved by carrying out a series of tree breeding activities or post-harvest processing. A study in the same location as our study in Pulau Laut to promote the development of *P. mooniana* plantations showed that the wood properties such as Young's modulus in *P. mooniana* could be improved by a tree breeding program for wood quality [21]. Establishing plantations of *P. mooniana* are expected to be able to reduce pressure on natural forests and provide valuable timber production in Indonesia.

4. Conclusion

The genetic diversity analysis in four populations of *P. mooniana* from Pulau Laut, South Kalimantan showed that the total diversity of all populations was low (*He* = 0.191 ± 0.013) with the lowest variation in mess/residential house (*He* = 0.134 ± 0.025) and the highest in the seed orchard (*He* = 0.289 ± 0.021). *P. mooniana* from mess/residential house, arboretum, and seed orchard had a close genetic relationship, indicating they may come from the same origin. The seed orchard population had the highest genetic diversity and had eight specific alleles, proving that this seed orchard is a valuable genetic resource and the main seed source for *P. mooniana* from Pulau Laut, South Kalimantan.

References

[1] Chhajer S, Jukanti A K, Bhatt R K and Kalia R K 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* August 2018

[2] Costa L S, Reiniger L R S, Helnzmann B M, Amaral L P and Serrota C M L 2015 Study of the genetic diversity and structure of a natural population of *Nectandra megapotamica* (Spreng.) Mez. Using RAPD markers *Genet. Mol. Res.* 14 18407-13

[3] Tian Y, Xing C, Cao Y, Wang C, Guan F, Li R and Meng F 2015 Evaluation of genetic diversity on *Prunus mira* Koehne by using ISSR and RAPD markers *Biotechnol. Equip.* 29 1053-61

[4] Soerianegara I and Lemmens R H M J 1994 Plant Resources of South-East Asia 5(1) Timber trees; Major Commercial Timbers Bogor

[5] UNEP-WCMC 2014 Non-CITES timber species from Southern Asia (Leguminosae) potential warranting further protection UNEP-WCMC Cambridge 30p

[6] Yuniarti N and Syamsuwida D 2011 Kayu Kuku (*Pericopsis mooniana* Thw.) in Atlas Benih Tanaman Hutan Indonesia Jiilid II (Eds. Buharman *et al*.) Balai Penelitian Teknologi Perbenihan Tanaman Hutan Bogor Publikasi Khusus 5 32-34

[7] Yuskianti V 2017 Genetic conservation of a threatened species, kayu kuku (*Pericopsis mooniana*) in Indonesia In. Proceedings of IUFRO-INAFOR Joint International Conference 2017 24-27 July 2017 in Yogyakarta Indonesia Promoting sustainable resources from plantations for economic growth and community benefits (Eds. Rimbawanto A *et al*.) 403-10

[8] Husna, Budi S W, Mansur I and Kusmana C 2015 Diversity of arbuscular mycorrhizal fungi in the growth habitat of kayu kuku (*Pericopsis mooniana* Thw.) in Southeast Sulawesi *Pakistan J. Bio. Sci* 18 1-10
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Authors' contributions
The authors contributed equally to this work as the main contributor.