Efficiency of Improved RAPD Marker in Assessing Genetic Diversity Kayu Kuku (*Pericopsis mooniana* THW)

Muh Restu[1], Gusmiaty[1], Siti Halimah Larekeng[1], Yuni Fitri Cahyaningsih[1], C.Andriyani Prasetyawati[2]

1) Tree Biotechnology and Breeding Laboratory Fac. Of Forestry, Hasanuddin University, Jl. Perintis Kemerdekaan Makassar, Indonesia 90245
2) Forestry Research Institute Makassar, Jl. Perintis Kemerdekaan Km 16 Makassar, Indonesia 90245

Corresponding author:
Muhammad Restu
Phone: 0811443515; Email: tueid@yahoo.com

ABSTRACT

Randomly amplified polymorphic DNA (RAPD) markers were used to analyze genetic diversity on Kayu Kuku (*Pericopsis mooniana* THW). The findings of this study provides important information for genetic diversity of this highly valuable but rare species of trees. The research was done from August to September 2015 in the Tree Biotechnology and Breeding Laboratory, Faculty of Forestry, Hasanuddin University. DNA from ten genotype *P.mooniana* was used to amplify with 9 highly polymorphic primers. Genetic diversity analysis was done using Darwin 6.00 software with PCoA and *Unweighted Pair Group Method with Aritmatic (UPGMA) method.* Results of the investigations indicated the presence of at least 10 alleles in the ten Kayu kuku's evaluated. Mean number of alleles per locus was 7.1. Results of genetic dissimilarity coefficient calculation and dendogram construction using DARwin 6.00 indicated that the Kayu kuku's were clustered into three groups. This study genetically characterized the Kayu Kuku species, which might have a significant contribution to the genetic and ecological conservation of this important rare species of trees. Also, this study indicates that the improved RAPD analyses is potential molecular tools for the study of genetic diversity.

Key words : Kayu Kuku, *Pericopsis mooniana* THW, genetic diversity, RAPD marker, Darwin 6.00 software

INTRODUCTION

Southeast and South Asia are natural distribution of Kayu Kuku (*Pericopsis mooniana* THW). Kayu Kuku is found in Sri Lanka (South Asia), Indonesia, Malaysia, Philipine and Papua Nugini (Southeast Asia). In Indonesia, natural distributions of this spesies are in Java, Kalimantan, Maluku, Papua, Sumatera and Sulawesi. Kayu Kuku belongs to Fabacea (Leguminosae) family and reaches 30-40 m in height with a diameter of about 0.8-1 m (Asian Regional Workshop, 1998).

Kayu Kuku is one of ornamental tree species, as Dahu (*Dracontomelon* spp.) and Linggua (*Pterocarpus* spp.). Kayu Kukus' durability level is classified to class II ,which means, it has ability to withstand in humid soil up to 5 years (Departemen Kehutanan, 2013; Akbar and Rusmana, 2013). Thus it has high demand and economical value.

Due to high price and quality of the timber, the exploitation of Kayu Kuku become uncontrolled. In other hands, natural regeneration of Kayu Kuku is limited (Asian Regional Workshop, 1998).According to UNEP-WCMC in 2014, Kayu Kuku is categorized as rare and endangered leguminosae type from Southeast Asia that need to be protected. The massive exploitation that is not accompanied by rehabilitation as well as limited natural regeneration level cause scarcity in Kayu Kuku. The scarcity of a species can reduce genetic diversity in the species. Loss of genetic diversity due to illegal logging can lower gene flow and cause evolution limitation from the species (Upadhyay*et al*., 2004).

Rehabilitation of Kayu Kuku needs to be done to prevent extinction. Explorations of genetic diversity are also necessary to investigate genetic sources that can be used in Kayu Kukus' breeding programs. One of methods for determining genetic diversity is through molecular marker.

RAPD (*Random Amplified Polymorphic* DNA) is one of molecular marker based on PCR that used for detemining genetic diversity in a population. RAPD primer forms in short nucleobase strand (10 pb) which randomly binds to DNA segment. Because of randomly binding of primer, this primer can be used for analysing genetic diversity with less genetic information, such as Kayu Kuku.

RAPD is widely used for genetic diversity analysis in various forest tree species. Ruas *et al.* (2011) analyzed genetic diversity of two *Schinus terebinthifolius* populations in Tibagi river area, Brazil. In their study, the genetic diversity between population and

in population were as many as 13,7% and 86,3%, respectively. RAPD was also used for genetic diversity analysis in Moringa (*Moringa oleifera* Lam). As many as 16 Moringa accessions were tested using 17 RAPD primer pairs showed lower genetic diversity (Silva *et al.*, 2012). Larekeng *et al.* (2015) reported preliminary study of RAPD primer selection and determinated annealing temperatures of Kayu Kuku.

MATERIALS AND METHODS

a. Sample Collection

Samples were collected from Pomalaa, Kolaka District, Southeast Sulawesi. Young leaves of Kayu Kuku from ten individuals were used as samples. The samples were analyzed at Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Hasanuddin University, Makassar in September 2015.

b. DNA Extraction

DNA extraction was conducted using *Qiagen Miniprep Plant DNA extraction kit* method (Qiagen, Chatsworth, CA). DNA quality was analyzed by horizontal electrophoresis on 2% of agarose at 100 volt.

c. DNA Amplification

DNAs were amplificated using RAPD primers from *Operon technologies.* Nine RAPD primers which their annealing temperatures and polymorphisms reported by Larekeng *et al.*(2015) were used to determine genetic diversity of Kayu Kuku (table 1).

Table 1. Primer names, sequences and annealing temperatures for genetic diversity analysis of Kayu Kuku

No	Nama Primer	Sekuen Primer	Suhu Annealing
1	OPQ-07	$^{5'}$CCCCGATGGT$^{3'}$	40,5°C
2	OPP-08	$^{5'}$ACATCGCCCA$^{3'}$	36°C
3	OPG-06	$^{5'}$GTGCCTAACC$^{3'}$	34,5°C
4	OPD-03	$^{5'}$GTCGCCGTCA$^{3'}$	45,5°C
5	OPA-05	$^{5'}$AGGGGTCTT$^{3'}$	35,5°C
6	OPAE-11	$^{5'}$AAGACCGGA$^{3'}$	39,2°C
7	OPAD-11	$^{5'}$CAATCGGGT$^{3'}$	34,9°C
8	OPG-19	$^{5'}$GTCAGGGCA$^{3'}$	38,4°C
9	OPZ-05	$^{5'}$TCCCATGCTG$^{3'}$	37,1°C

Total volume for one DNA amplification reaction was 12,5 µL. PCR amplifications were conducted using the following reaction mixtures: 2µl of DNA, 1,25 µl of primers RAPD, 6.25 µl PCR mix (KAPA Biosystem), and 3 µl ddH20. Amplifications were conducted by PCR Sensoquest mechine using the following steps: 1) pre-denaturation temperature at 94°C for 60 seconds, 2) denaturation termperature at 94°C for 30 seconds, 3) specific annealing temperature of each primer for 50 seconds, 4) extension temperature at 72°C for 60 seconds, 5) final extension temperature at 72°C for 300 seconds and 6) storage temperature at 4°C. Step 2 and 3 were repeated in 35 cycles.

d. Agarose Gel Electrophoresis

DNA amplification products were then separated using horizontal electrophoresis 2% of agarose and 1x TAE buffer at 80 volt for 60 minutes. Staining was done by adding 0.5µL Gel Red into hot agarose solution. The electrophoregrams were visualized using *gell doc* Biostep.

e. Data Analysis

The data obtained by specific primer DNA amplification were bands and then scored as binary characters for their presence (1) or absence (0). The resulting data were tabulated and analyzed using Darwin 6 software (Perrier dan Jacquemoud-Collet, 2006). Genetic diversity analysis was calculated using two methods: PcoA and UPGMA method.

RESULTS AND DISCUSSION

A set of nine RAPD primers was used in PCR reaction to amplify DNA fragments from ten individuls. Amplification products using RAPD primers in ten individuals Kayu Kuku showed different band pattern (figure 1). Figure 1 shows the individual 5 and 6 had different band pattern to the others. Individual 9 and 10 showed no band. A total of 65 bands were detected, of which 2% (1 band) was monomorphic and 64 bands (98%) were polymorphic (average of 12.8 bands per primer). All primers resulted different number of bands, between five to ten bands. The primer OPG-19produced maximum number of band with 10 bands. The primersOPG-06 and OPA-05 produced minimum number of bands with 5 bands (Table 2). This findings showed better result than in rosewood (*Dalbergia nigra* Vell) (Juchum *et al* 2007) andAcacia(Nanda *et al* 2004) which observed at most 22 polymorphic bands.

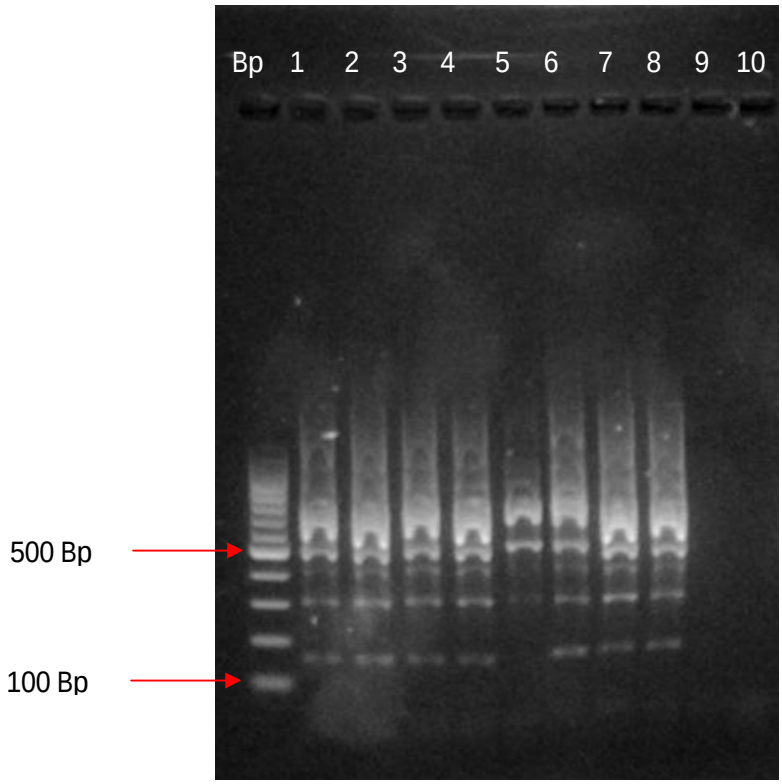

Figure 1. Band pattern of amplification products in ten individuals Kayu Kuku using RAPD OPP-08 primer. Lane 1: 100 bp DNA ladder markers, lane 2-11: different Kayu Kuku genotypes.

Table 2. Band number of each RAPD primer for genetic diversity detection in Kayu Kuku

No	Primers	Amplified bands	
		Scored Bands	Polymorphic band
1	OPQ-07	8	7
2	OPP-08	8	8
3	OPG-06	5	5
4	OPD-03	7	7
5	OPA-05	5	5
6	OPAE-11	7	7
7	OPAD-11	8	8
8	OPG-19	10	10
9	OPZ-05	7	7
	Total	65	64

PcoA analysis showed axis 1 and 2 are able to explain the diversity as many as 81,56% in ten induviduals Kayu Kuku based on nine RAPD primers (figure 2). As many as nine individuals were distributed in quadrant 2 and 3, whereas only five individuals were in quadrant 1. Similar result was presented in figure 3 and table 3, which individual five had furthest genetic distance and different cluster with other individuals. This result indicated that individual 5 had very distinct genetic than other individuals.

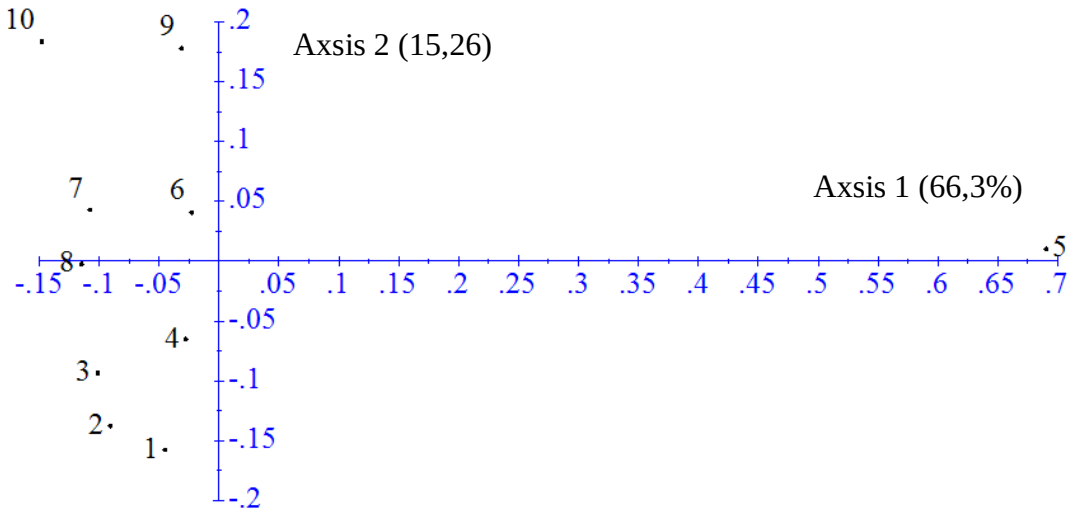

Figure 2. PcoA (Principal Coordinate Analysis) of Kayu Kuku based on nine RAPD markers

Table 3. Genetic distance of Kayu Kuku based on nine RAPD markers

individu	1	2	3	4	5	6	7	8	9	10
1	0									
2	0.18	0								
3	0.18	0.05	0							
4	0.18	0.10	0.10	0						
5	0.78	0.78	0.78	0.78	0					
6	0.24	0.24	0.24	0.24	0.78	0				
7	0.24	0.24	0.24	0.24	0.78	0.17	0			
8	0.24	0.24	0.24	0.24	0.78	0.17	0.13	0		
9	0.34	0.34	0.34	0.34	0.78	0.34	0.34	0.34	0	
10	0.34	0.34	0.34	0.34	0.78	0.34	0.34	0.34	0.30	0

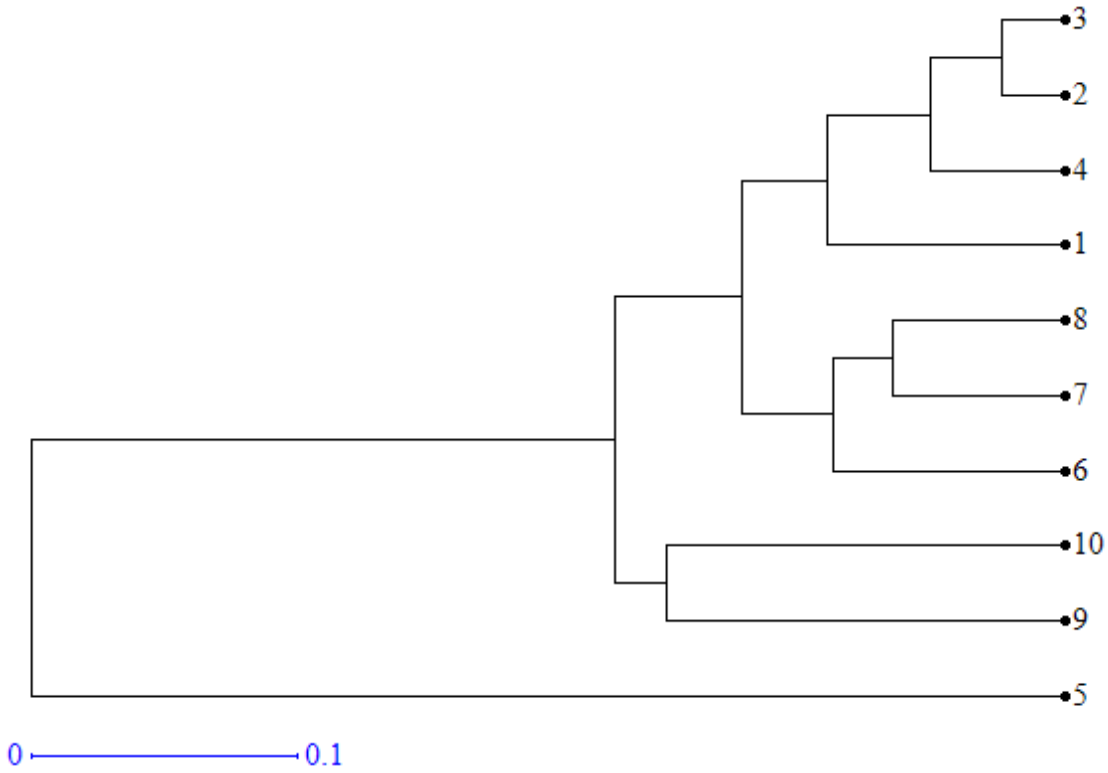

Figure 3. Dendrogram of Genetic similarity of Kayu Kuku based on nine RAPD markers

Genetic similarity (cluster analysis) using UPGMA reported ten individuals Kayu Kuku were divided into 2 major clusters (figure 3). Cluster 1 consisted nine individuals, whereas cluster 2 consisted of 5 individuals. Cluster 1 was then divided into three sub-clusters. Sub-cluster 1 consisted of individual 1, 2, 3 and 4. Sub-cluster 2 consisted individual 6, 7 and 8. While sub-cluster 3 consisted individuals 9 and 10. Individual 2 and 3 had the highest genetic similarity and closest genetic distance, 0.05 (figure 2; table 3). This result indicated that both individuals grew on the same soil type, white soil. Indiviuals which grow on the same habitat commonly have high genetic similarity (Nanda *et al*, 2004). The high level of genetic similarity was assumed that, caused by individual 2 and 3 grew on the same soil type. These results showed genetic diversity of ten tested individuls was high with genetic variation level ranged between 5% - 85%. Genetic variation is high when genetic diversity level more than 70% (Nanda *et al*, 2004).

CONCLUSIONS

Results of genetic dissimilarity coefficient calculation and dendrogram construction using DARwin 6.00 showed that Kayu kuku were clustered into two clusters and cluster 1consist of three sub-clusters. This study genetically characterized the Kayu Kuku species, which might have a significant contribution to the genetic and ecological conservation of this important rare treespecies. This study also indicates that the improved RAPD analysisis potential molecular tool for genetic diversitystudies.

ACKNOWLEDGMENT

The authors are grateful to BalaiPenelitianTanamanKehutanan Makassar for providing genetic plant materials of Kayu Kuku from Southeast Sulawesi.

REFERENCE

Akbar A dan Rusmana. 2013. Membangkitkan Primadona yang Mulai Langka: Kayu Kuku (*Pericopsis mooniana* Thw). Bekantan 1(1): 4-6.

Asian Regional Workshop (Conservation & Sustainable Management of Trees, Vietnam, August 1996). 1998. *Pericopsis mooniana*. The IUCN Red List of Threatened Species 1998 :e.T31312A9623986.http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T31312A9623986.en. didownload tanggal 7 Oktober 2015.

Departemen Kehutanan. 2013. Profil Kehutanan 33 Provinsi. Jakarta, (ID). hal 605.

Larekeng, S,H., Muh Restu, Gusmiaty. 2015. The Use of RAPD Marker on Kayu Kuku's (*Pericoopsis mooniana* THW) Breeding Program. Oral Presentation at International Seminar on Challenges of Sustainable Forest Plantation Development On 26th November 2015 in Yogyakarta, Indonesia.

Nanda RM, Nayak S, Rout GR. 2004. Studies on Genetic Relatednes of Acacia Tree Species Using RAPD Markers. Biologia Bratislava 59(1): 115-120.

Juchum FS, Leal JB, Santos LM, Almeida MP, Ahnert D, and Corrêa RX. 2007. Evaluation of genetic diversity in a natural rosewood population (*Dalbergia nigra* Vell.Allemão ex Benth.) using RAPD markers. Genetics and Molecular Research. 6(3): 543-553.

Perrier X and Jacquemoud-Collet JP. 2006. DARwin Software. http://darwin.cirad.fr/.

Ruas EA, Ruas FC, Medri PS, Medri C, Medri ME, Bianchini E, Pimenta JA, Rodrigues LA, and Ruas PM. 2011. Anatomy and Genetic Diversity of Two Population of *Schinus terebinthifolius* (Anacardiaceae) from tha Tibagi River basin in Paraná, Brazil. Genetics and Molecular Research. 10 (1): 526-536.

Silva AVC, Santos ARF, Lédo AS, Feitosa RB, Almeida CS, Silva GM, Rangel SA. 2012.Moringa Genetic Diversity From Germplasm Bank Using RAPD Markers. Tropical and Subtropical Agroecosystems. 15: 31-39.

UNEP-WCMC. 2014. Non-CITES Timber Species from Southeast Asia (Leguminosae) Potentially Warranting Futher Protection. Cambridge (UK):UNEP_WCMC.

Upadhyay.,A, K. Jayadev, R. Manimekalai, V.A. Parthasarathy. 2004. Genetic relationship and diversity in Indian coconut accessions based on RAPD markers. Scientia Horticulturae 99 : 353–362.

*