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Primer Screening and Genetic Diversity Analysis of Jabon putih (*Anthocephalus cadamba* (Roxb) Miq.) based on *Random Amplified Polymorphic* DNA (RAPD) Markers

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ABSTRACT

Introduction: Jabon putih population has currently decreased because of intensive exploitation and land use conversion. Genetic diversity is an essential factor in maintaining the existence of a species.

Objective: The objective of this study was to determine the genetic relationship in Jabon putih from the genetic resource area of the second region of Seed/Seedling Forest Office in Bellabori, Parangloe, Gowa, based on RAPD markers.

Methods: The analysis was carried out using 145 DNA samples from seven populations. Primer screening using randomly selected twelve DNA observed three polymorphic primers out of 21 screened RAPD primers.

Result: The polymorphic primers were OPP-08, OPY-09, and OPD-20. The mean of heterozygosity was 0.46, and that of the highest was detected in Luwu Population and Wajo Population (0.48, respectively).

Conclusion: The individuals tended to randomly group (did not group according to their provenances), and consequently, the genetic diversity among populations is high.

Key Words: Genetic diversity, *Jabon putih*, RAPD, Primer Screening

INTRODUCTION

Anthocephalus cadamba Miq., also known as Jabon putih, is a species originated from Southeast Asia like N. macrophylla and distributed throughout Indonesia having a high economic value in carpentry and health, specifically for cancer treatment.[1-3] In Indonesia, Jabon putih has been planted in a large scale since the 1930s. Besides, due to it is easily vegetative propagated, the distribution is quite extensive, namely in Java Island, Kalimantan Island, Sumatra Island, Sulawesi Island, Sumbawa Island, and Papua Island.[2]

Jabon putih population has currently decreased because of intensive exploitation and land use conversion. It generally grows associated with other species and groups of 3-6 individual trees because of the intense competition between stands. The most severe threat to fragmented populations is a decrease in genetic diversity. Jabon putih conservation strategy has begun with the construction of ex situ conservation plots to overcome this problem. In addition to being used as a genetic conservation plot, the plot is also expected to be used as genetic material for basic population development in breeding strategies.[4]

Genetic diversity is an essential factor in maintaining the existence of a species. A population with high genetic diversity has the ability to defend itself from disease and extreme climate change. Thus, it can live in sustainable conditions for generations. The level of genetic diversity is one of the determining factors in the success of breeding and conservation programs.[5]

The genetic diversity of a population depends on the success of the reproductive system in that population. It can be maintained if there is no selfing pollination or inbreeding. The rate of the reproductive system also depends on the synchronization of flowering phenology and environmental factors, for instance, tree density and height.[6] Analysis of

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genetic diversity is more accurate using molecular markers. Molecular markers consist of Isozim, Ran*dom Amplified polymorphic* DNA (RAPD), and *Simple Sequence Repeats* (SSR) or Microsatellite. A DNA marker that is widely used is RAPD.[7] This marker can be applied to almost all species of the plants. Its advantages are rapid results, do not require much DNA, and the process is relatively faster and cheaper.[8]

Genetic diversity studies using RAPD markers had been conducted on *Angelica sinensis*, Teak, cocoa.[9-11] Based on this, it is crucial to conduct this study to determine the genetic diversity of Jabon putih on based on Random Amplified Polymorphic DNA marker.

MATERIALS AND METHODS

Plant materials

The plant materials used in this study were 145 young leaves from Jabon putih's seedlings. The initial sample collection step was the selection of 29 adult trees. The adult trees consisted of 14 trees from North Luwu provenance, a tree from Jeneponto provenance, a tree from Maro's provenance, four trees from Pangkep provenance, four trees from Wajo provenance, two trees from Konawe provenance, and three trees from Gowa provenance. Five seedlings of each adult tree were selected and collected leaf from each seedling as the samples, thus the total samples were 145 leaves.

Research Methods

Leaf samples collected were put into plastic and coded according to the place of collection. The samples were then stored into a coolbox containing ice gel. Its function is to maintain the quality of the leaves until DNA analysis at the laboratory.

DNA extraction applies three steps, i.e., cell lysis, separation of DNA from solid materials such as cellulose and protein, and DNA purification. Through this process, DNAs are separated from other cellular components, such as protein, RNA, as well as fat. DNA isolation was conducted using CTAB methods with modification.[12,13]

Primer screening was performed by amplifying random chosen 12 DNA samples using 20 RAPD primers (Table 1). The amplification process was carried out by using ± 5^0C temperature gradient of the annealing temperature given on the primer label. It was done to obtain primers that were polymorphic and easily amplify and determine the right annealing temperature. The primers produced clear and easy to score bands will be considered as the specific primers.[8] In this study, the c*ross-amplification* method was used for amplifying the DNA.[14]

Table 1: RAPD primer name and sequence (Operon Technology)

Primer name	Nucleotide sequence	Tm(°C)
OPQ-07	5'-CCC CGA TGG T-3'	38.5
OPA-15	5'-TTC CGA ACC C-3'	34.2
OPZ-05	5'-TCC CAT GCT G-3'	34.3
OPD-03	5'-GTC GCC GTC A-3'	40.8
OPA-02	5'-TGC CGA GCT G-3'	40.7
OPP-08	5'-ACA TCG CCC A-3'	37.5
OPA-09	5'-GGG TAA CGC C-3'	37.5
OPAE-11	5'-AAG ACC GGG A-3'	35.6
OPAA-20	5'-TTG CCT TCG G-3'	35.6
OPA-05	5'-AGG GGT CCT G-3'	32.6
OPC-11	5'-AAA GCT GCG G-3'	36.9
OPG-19	5'-GTC AGG GCA A-3'	34.7
OPP-08	5'-ACA TCG CCC A-3'	37.5
OPAC-12	5'-GGC GAG TGT G-3'	38.1
OPA-11	5'-CAA TCG CCG T-3'	32
OPG-09	5'-CTG ACG TCA G-3'	32
PLC-14	5'-TGC GTG CTT G-3'	32
OPD-20	5'-ACC CGG TCA C-3'	39.1
PLR-13	5GGA CGA CAA G-3'	32
PLW-04	5' CAG AAG CGG A-3'	32
M29	5' CCG GCC TTA C-3'	32

A PCR reaction consisted of 2 µl DNA template, 1.25 µl RAPD primer, 6.25 µl Kappa 2G PCR Mix, and 3 µl ddH_2O. Thus the total PCR reaction was 12.5 µl. PCR process was conducted using PCR *sensoquest* machine. The PCR process was performed using the following steps: an initial denaturation for 3 minutes at 95 ^{0}C, 35 cycles of denaturation for 30 seconds at 95 ^{0}C, primer annealing for 50 seconds at each specific temperature, primer elongation for 60 seconds at 72^{0}C, and a final elongation for 5 minutes for 72 ^{0}C.

Data analysis

The results obtained were in the form of PCR product bands that appeared on agarose. The bands represent the alleles that are located at a specific locus. Each primer used presents a certain locus. The bands produced were scored according to the size of the bands. The bands with the longest base pair size would be signed as "1" and no band would be given as "0". The presence of the bands was done manually by observing the electropherogram.

The data were then tabulated and analyzed using Darwin 6.0 software to determine the genetic relationship and variation. Heterozygosis were calculated using the formula below in equation (1)[15]

$$\text{Heterozygote: } qi = \frac{\textit{Individual with absent band}}{\textit{Number of total samples}} \quad (1)$$

$$pi = 1 - q$$

$$He = 1 - pi^2 - qi^2$$

Notes: qi = Frequency of null allele

Pi = Frequency of dominant allele

Polymorphic Information Content (PIC) was calculated using the following formula in equation (2)[16]

$$PIC = 2\ fi\ (1\text{-}fi) \quad (2)$$

Notes: **PIC** = Polymorphic Information Content

Fi = Frequency

RESULTS

Primer Screening

The primer screening of 21 RAPD primers selected three primers that were able to generate polymorphic bands. Those primers were OPP0-08, OPY-09, and OPD-20. The selected primers are presented in Figure 1.

Figure 1: Electropherogram of DNA amplification products using OPP-08. Notes: 1-12 = Jabon putih DNA samples

Three annealing temperatures that able to amplify the Jabon putih DNAs were OPP-08, OPY-09, and OPD-20. OPD-20 had the highest temperature, while OPP-08 used the lowest one. Overall, the annealing temperatures of OPP-08, OPY-09, and OPD-20 were 41.2 ^{0}C, 41.9 ^{0}C, and 47.0 ^{0}C, respectively. It could be seen that average temperatures of polymorphic primers had reached 40.0 ^{0}C or more (Table 2).

Table 2: Polymorphic primer and annealing temperature

Primer	Annealing temperature (°C)
OPP-08	41.2
OPY-09	41.9
OPD-20	47.0

Primer screening is performed to determine the right annealing temperatures and select polymorphic primers. It is conducted by making some PCR reactions amplified using different primers and different DNA samples under the same condition.[17] Thus, the optimum condition and variation level of the bands will be obtained for each primer.[8] The success of genomic DNA amplification using the RAPD technique is to being determined by primer sequence, the quantity of primer contained in the PCR reaction, the suitability of PCR condition, including primer annealing temperature and extension.[18] The number of primers produced polymorphic, clear, and bright bands were three out of 21 RAPD primers, consisting of OPP-08, OPY-09, and OPD-20.

The Polymorphic Information Content (PIC) showed that OPD-20 had the highest PIC out of the three evaluated RAPD which was 0.21 or close to 0.5, thus it was indicated as an effective primer used for distinguishing individuals.[19] stated that a PIC closed to 0.5 is very effective in distinguishing between individuals. The PIC of the RAPD primers can be seen in Table 3.

Table 3: Polymorphic primer and PIC

Primer	PIC
OPP-08	0.140
OPZ-05	0.158
OPA-05	0.212

OPD-20 was the best primer in detecting the genetic diversity of Jabon putih using seven populations from various provenances. PIC of OPD-20 was 0.21 so that it has high polymorphism compared to the others. This study obtained an average PIC in each provenance ranging from 0.21 to 0.14, which means that the primer has a moderate polymorphism. The PIC is lower than the one observed by [20] which detected a PIC of 0.31 on the Bamboo Parring (*Gigantochloa atter*) with RAPD markers. Guo, *et al*., (2014) stated that the dominant markers had a maximum PIC of 0.5.

Genetic Diversity/Variation

Genetic diversity is an identical relationship of a plant population. A population having a high genetic diversity will increase the level of population adaptation. The total number of bands produced by the three RAPD primers was 91 polymorphic bands. OPY-09 produced the highest amplified bands (39 polymorphic bands), OPP-08 generated 37 polymorphic bands, whereas the least amplified bands were using OPD-20 primer (15 bands).

Table 4 shows the heterozygosity of the samples in the entire population. Population I and V had the highest number of heterozygosity (0.48) with 34 polymorphic bands. Population VII had heterozygosity of 0.47 with 14 polymorphic

bands. Population II and population VI showed the same heterozygosity, which was 0.46 with 31 polymorphic bands. Population III had heterozygosity of 0.44 with four polymorphic bands.

Population IV had the lowest heterozygosity (0.43) with 14 polymorphic bands. The average heterozygosity was 0.14, which means that the genetic diversity of Jabon putih population is high. Thus the maximum He is 0.5. The heterozygosity of the entire population are depicted in Table 4.

Table 4: Number of bands and heterozygosity of each population

Population	Primer	Number of band	Heterozygosity
Population I	OPP-08	10	0.48
	OPY-09	5	
	OPD-20	3	
Population II	OPP-08	8	0.46
	OPY-09	6	
	OPD-20	1	
Population III	OPP-08	2	0.44
	OPY-09	0	
	OPD-20	2	
Population IV	OPP-08	4	0.43
	OPY-09	5	
	OPD-20	2	
Population V	OPP-08	3	0.48
	OPY-09	7	
	OPD-20	3	
Populasi VI	OPP-08	6	0.46
	OPY-09	8	
	OPD-20	2	
Population VII	OPP-08	4	0.47
	OPY-09	8	
	OPD-20	2	
Mean of Heterozygosity			0.46

The heterozygosity (He) of each population was quite diverse, ranging from 0.48 to 0.43 with 0.46 of average He. This heterozygosity is higher than the results on the genetic diversity of sengon conducted by Olivia, et al., (2013). [21] The genetic diversity of the Sengon (*Paraserianthes falcataria* (L) NIELSEN) from Kediri provenance had a high He, which was 0.2946, while He of sengon from Garut provenance was 0.1602. Other studies on species showed high genetic variation, like in mixed *Nothofagus* forest, *Diospyros kaki*, *Nilgirianthus ciliates*, aromatic rice.[22-25]

The maximum Heterozygosity (He) is 0.5.[26] Therefore, this study depicts a relatively high genetic diversity in Jabon putih. The high genetic diversity can be utilized as a source of genetic material for the construction of Genetic Resources Areas, which will support Jabon putih breeding program.[27]

DISCUSSION

Genetic relationship of the individuals in the populations

The results showed that Jabon putih's individuals in seven populations tended to cluster randomly, and only a small number of individuals grouped according to their population, thus the genetic relationship among individuals in a population was low. The lower genetic relationship, the higher genetic diversity in the populations. This study is in line with the analysis of the clustering genetic similarity in Bamboo Parring.[20] Detected the accessions were distributed into three main clusters, which some individuals were partially grouped based on their population, and others were clustered but scattered.

The dendrogram between Jabon putihs' individuals showed that the individuals were divided into three main clusters. The individuals in Cluster I were W30.6 and L2.1 and in Cluster II were L32.3, J3, J2, J1, L23.1, L5.9, L5.10, W27.1, P3.4, L15.5, P3.1, L2.4, P2.3, and G2.4 which were in the same line or having a relatively close relationship. Individuals between populations in different clusters had 0.46 of average genetic diversity between individuals (Figure 2).

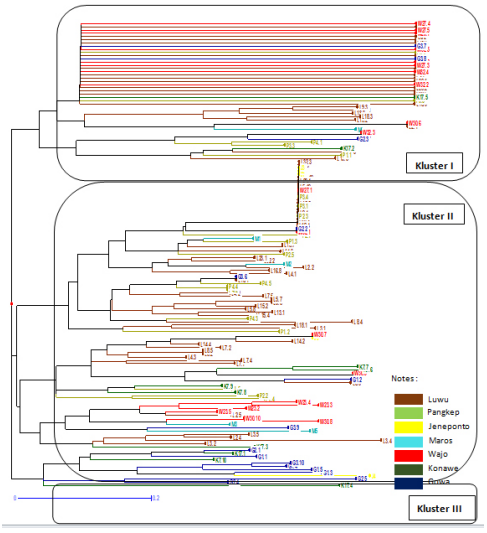

Figure 2: Dendrogram of genetic relationship in Jabon putihs' individuals from seven populations.

Strategy for future tree breeding requires increasing genetic variation within and between populations. The efforts were carried out by establishing seed orchards using plant material originated from different populations derived from the selected plus trees/superior trees. It aims to increase genetic variation in populations and eventually produced high genetic quality progenies.[28] High genetic variation will support individuals in populations to adapt to climate changes as well as conserve genetic resources.[24,29]

CONCLUSION

The RAPD primers for analyzing genetic diversity in Jabon Putih were OPP-08, OPY-09, and OPD-20 out of 21 screened primers. The genetic diversity in the evaluated provenances was high. The highest heterozygosity was observed in population I and V, which were 0.48, respectively, and had higher genetic diversity compared to population II, III, IV, VI, and VII. Seven populations of Jabon Putih were grouped into three main clusters. Each main cluster consisted of various populations, and the only cluster I and II had a close relationship.

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REFERENCES

1. Shi S, Larekeng SH, Lv P, Nie Y, Restu M, Shi S, et al. The complete chloroplast genome of Neolamarckia macrophylla (Rubiaceae). Mitochondrial DNA Part B. 2020;5(2):1611–2.
2. Krisnawati H, Kallio M, Kanninen M. Anthocephalus cadamba (Miq.) Ekologi, Silvikultur, dan Produktivitas. [Accessed June 2020]. http//www cifor org/publications/pdf_files/Books/Bkrisnawati1108 pdf. 2011.
3. Sari RK, Armilasari D, Nawawi DS, Darmawan W, Mariya S. Aktivitas antiproliferasi ekstrak jabon putih (Anthocephalus cadamba Miq) terhadap sel kanker payudara dan serviks. J Ilmu Teknol Kayu Trop 2014;12(1):91–100.
4. Nurtjahtjaningsih ILG, Qiptiyah M, Yudohartono TP, Widyatmoko A, Rimbawanto A. Karakterisasi keragaman genetik populasi jabon putih menggunakan penanda random amplified polymorphism DNA. J Pemuliaan Tanam Hutan 2014;8(2):81–92.
5. Arif A, Larekeng SH, Restu M, Cahyaningsih YF, Mukti J. A genetic diversity on Jabon Merah (Anthocephalus macrophyllus Roxb.) from three different provenances in South Sulawesi. IOP Conf Ser Earth Environ Sci 2019;270(1).
6. Larekeng SH, Gusmiaty, Cahyaningsih YF, Arsyad MA, Sari WM, Restu M, et al. Estimation of Pollination in Mahogany Revealed by Microsatellite Markers : Case in South Sulawesi, Indonesia. Syst Rev Pharm 2020;11(4):660–73.
7. Sharma R, Sharma S, Kumar S. Pair-wise combinations of RAPD primers for diversity analysis with reference to protein and single primer RAPD in soybean. Ann Agrar Sci 2018;16(3):243–9.
8. Larekeng SH, Dermawan R, Iswoyo H, Mustari K. RAPD primer screening for amplification on Katokkon pepper from Toraja, South Sulawesi, Indonesia. IOP Conf Ser Earth Environ Sci 2019;270:012023.
9. Mei Z, Zhang C, Khan MA, Zhu Y, Tania M, Luo P, et al. Efficiency of improved RAPD and ISSR markers in assessing genetic diversity and relationships in Angelica sinensis (Oliv.) Diels varieties of China. Electron J Biotechnol 2015;18(2):96–102.
10. Mohammad N, Mahesh S, Jain YK, Ansari SA. Effect of discrete (individual) and mixed (bulk) genomic DNA on genetic diversity estimates and population structure in Teak (Tectona grandis L.f.). 2017;55:44–8.
11. Syahri YF, Rauf M, Paembonan SA, Larekeng SH, Cahyaningsih YF. RAPD Amplification on Cocoa (Theobroma cacao L.) from East Kolaka, Southeast Sulawesi Province. IOP Conf Ser Earth Environ Sci 2019;270(1).
12. Russell DW, Sambrook J. Molecular cloning: a laboratory manual. Vol. 1. Cold Spring Harbor Laboratory Cold Spring Harbor, NY; 2001.
13. Larekeng SH, Purwito A, Mattjik NA, Sudarsono S. Microsatellite and SNAP markers used for evaluating pollen dispersal on Pati tall coconuts and Xenia effect on the production of "Kopyor" fruits. IOP Conf Ser Earth Environ Sci 2018;157(1).
14. Gusmiaty, Restu M, Pongtuluran. Seleksi Primer Untuk Analisis Keragaman Genetik Jenis Bitti (Vitex cofassus). J Perenn. 2012;8(1):25–9.
15. Wallace L. Methods Available for the Analysis of Data fromDominant Molecular Markers. Dep Biol Univ ofSouth Dakota. 2003;
16. Guo Z-H, Fu K-X, Zhang X-Q, Bai S-Q, Fan Y, Peng Y, et al. Molecular insights into the genetic diversity of Hemarthria compressa germplasm collections native to southwest China. Molecules 2014;19(12):21541–59.
17. Larekeng S. Selection of Dominant and Co-dominant Markers for Red Wood (Pterocarpus indicus Willd) Polymorphism from Five Provenances in East Nusa Tenggara. In: 1st International Conference on Science and Technology, ICOST. 2019.
18. Gunawan. Red Wood DNA Amplification (Pterocarpus indicus Willd) on some Provenances in East Nusa Tenggara. Hasanuddin University; 2019.
19. Wang DY, Chen YJ, Zhu HM, Lv GS, Zhang XP, Shao JW. Highly differentiated populations of the narrow endemic and endangered species Primula cicutariifolia in China, revealed by ISSR and SSR. Biochem Syst Ecol 2014;53:59–68.
20. Larekeng SH, Restu M, Mis'al, Oktavina J, Cahyaningsih YF. Penggunaan Penanda RAPD untuk mengevaluasi keragaman genetik bambu parring (Giganthocloa atter) di Kabupaten Maros, Sulawesi Selatan. In: Prosiding seminar nasional silvikultur V. 2018; 404–13.
21. Olivia RD, Siregar UJ. Genetic Diversity of Sengon Population (Parasireanthes falcataria (L)) on Citizen Forest in Java Baso on RAPD Marker. J Silvikultur Trop 2013;3(2).
22. Sola G, El V, Tsuda Y, Giuseppe G, Gallo L. Forest Ecology and Management The effect of silvicultural management on the genetic diversity of a mixed Nothofagus forest in Lanín Natural Reserve, Argentina. Fores Ecol Mgmt 2016;363:11–20.
23. Liang Y, Han W, Sun P, Liang J, Wuyun T, Li F, et al. Genetic diversity among germplasms of Diospyros kaki based on SSR markers. Sci Hortic (Amsterdam) 2015;186:180–9.
24. Rameshkumar R, Pandian S, Rathinapriya P, Selvi CT, Satish L, Gowrishankar S, et al. Genetic diversity and phylogenetic relationship of Nilgirianthus ciliatus populations using ISSR and RAPD markers: Implications for conservation of an endemic and vulnerable medicinal plant. Biocatal Agric Biotechnol 2019;18:101072.
25. Zakiyah NM, Handoyo T, Kim K-M. Genetic Diversity Analysis of Indonesian Aromatic Rice Varieties (Oryza sativa L.) Using RAPD. J Crop Sci Biotechnol 2019;22(1):55–63.

26. Weising K, Nybom H, Pfenninger M, Wolff K, Kahl G. DNA fingerprinting in plants: principles, methods, and applications. CRC press; 2005.
27. Larekeng SH, Restu M, Millang S, Bachtiar B. Moderate Level of Genetic Diversity in Anthocephalus Macrophyllus Roxb, an Endemic Tree of Sulawesi and Its Implication in Conservation. Int J Agric Syst 2018;6.
28. Bessega C, Pometti C, Campos C, Saidman BO, Vilardi JC. Implications of mating system and pollen dispersal indices for management and conservation of the semi-arid species Prosopis flexuosa (Leguminosae). Fores Ecol Mgmt 2017;400:218–27.
29. Manurung J, Siregar IZ, Kusmana C, Dwiyanti FG. Genetic variation of the mangrove species Avicennia marina in heavy metal polluted estuaries of Cilegon Industrial Area, Indonesia. Biodiversitas 2017;18(3):1109–15.