

Genetic diversity of *Pongamia pinnata* (*Millettia pinnata*, aka. malapari) populations in Java Island, Indonesia

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Abstract. Aminah A, Supriyanto, Suryani A, Siregar IZ. 2017. Genetic diversity of *Pongamia pinnata* (*Millettia pinnata*, aka. malapari) populations in Java Island, Indonesia. *Biodiversitas* 18: 677-681. One of the promising tree species to be utilized as biodiesel legume in Indonesia is malapari (*Pongamia pinnata* (L.) Pierre). The tree grows naturally along the North and South Coasts of Java Island. Malapari is now subjected to an initial breeding program, but its population diversity in Indonesia is not known yet. The objective of this study was to estimate genetic diversity in Java Island, namely populations of Carita, Batukaras, Kebumen, Alas Purwo National Park and Baluran National Park. RAPD markers were used to study the genetic diversity using the following DNA primers: OPL-11, OPAH-15, OPAO-01, OPAB-14 and OPAH-13. Results showed that DNA fragment lengths ranged from 250 to 1000 bp with a variable number of polymorphic bands, ranging between 13-24. Based on these RAPD markers, the population in Baluran was considered as a potential diversity center of malapari in Java, whereas the population in Kebumen was found to have the lowest genetic diversity. In general, the average genetic diversity within a population was lower than that among the populations. Cluster analysis of genetic similarity in five malapari population resulted in separation into two main clusters, the first group consisted of four populations (i.e., Carita, Batukaras, Kebumen and Alas Purwo). The other cluster was only one population only, namely Baluran.

Keywords: Genetic distance, malapari, *Pongamia pinnata*, population, RAPD

INTRODUCTION

Pongamia pinnata Merrill is one of potential trees for biodiesel raw material sources found in Indonesia that is distributed from the islands of Sumatra to Papua. In Java Island, the tree grows along the North and South Coasts. The tree has been used as biodiesel source in India, and is being developed in Australia and the USA (Dwivedi et al. 2011 and Halder et al. 2014). The plant also has many other uses from its roots, leaves, flowers, fruit to the timber. The roots can be used as fish poison, and the leaves are believed to cure cough and diarrhea (Oommen et al. 2000). Malapari seeds as a source of alternative fuel that can be updated as bioenergy containing 30-40% oil, which is rich in triglycerides. The wood is used for making furniture and firewood. Remnants from oil presses are used as feed for livestock and poultry as well as the production of biogas. The flowers are good source of honey bee production (Lakshmi et al. 1997). *Pongamia pinnata* is a hermaphrodite (Syamsuwida, 2012), as stamens and pistils are in one flower. The flowers have a chance to pollinate themselves (selfing), but generally, plants of tropical forests do cross-pollination (outcrossing) with the help of insect pollinators.

Although the reproduction system is already known, the information on the genetic diversity of malapari in Indonesia, especially in Java, is not available. There is, however, already information on genetic diversity status of malapari, but the information had resulted from populations

in India and Australia using different genetic markers. (Pavithra et al. 2014; Jiang et al. 2012). One of the techniques widely used to determine the genetic diversity of plants is RAPD (Random Amplified Polymorphic DNA). Despite many newly developed genetic markers, the use of RAPD as genetic marker is still being preferred to estimate quickly genetic diversity status.

RAPD technique is used for genetic diversity analysis, because it is easy to conduct, inexpensive and quick in obtaining the result, and DNA band polymorphism is produced in large quantities (Tingey et al. 1994). However, this technique has a disadvantage in amplification product consistency (Jones et al. 1997). It can be overcome by performing optimal extraction, conditioning PCR well and selecting the appropriate primer. RAPD can also identify the diversity within population and between populations (Qian et al. 2001, Adam et al. 2002, Jena and Das, 2006). The aim of this study was to estimate the genetic diversity of malapari population in Java based on RAPD markers.

MATERIALS AND METHODS

Study area

The study area covered five natural malapari population in Java Island, namely Banten Province (Carita Village), West Java Province (Batukaras Village), Central Java Province (Ambalresmi Village), and East Java Province (Baluran National Park and Alas Purwo National Park) as

presented in Figure 1. The number of tree samples, geographical location and altitude of malapari population are presented in Table 1.

Procedures

Malapari trees with pods bearing the seeds were selected for leaves sample collection in each population. Two to five leaves of each tree were cut and stored in a zip plastic containing silica gel with a ratio of 1:5 (w/w). Leaf samples were collected from 20 trees per population. The collected leaves were dried in silica gel and stored at -20°C until DNA extraction was performed following CTAB (*Cetyl Trimethyl Ammonium Bromide*) method with slight modification (Aritonang et al, 2007). RAPD primers used in this study were based on the study conducted by Kesari

et al. (2011) where 10 primers were used with details as presented in Table 2.

Data analysis

Analysis of PCR results was scored and translated into binary data based on the presence of the band where value of 0 (zero) is for no band and value of 1 (one) is for band in a similar position of each compared. Data resulted were processed using POPGENE 32 software version 1.31 (Yeh and Yang 1999). Dendrograms were created using UPGMA (Unweighted-Pair Group Method Arithmetic) method with NTSYS (Numerical Taxonomy and Multivariate System) program version 2.0 (Rohlf, 2008) and STRUCTURE version 2.3.4 (Pritchard et al. 2011).



Figure 1. Location of sampled materials for genetic analysis from western to eastern Java, Indonesia. 1. Carita, 2. Batukaras, 3. Kebumen, 4. Alas Purwo, 5. Baluran

Table 1. Number of tree samples, geographical location of population and altitude

Location of population	No. of samples	Latitude	Longitude	Altitude (m asl.)
Carita, Pandeglang, Banten	20	6°16'19" S	105°49'45" T	0-5
Batukaras, Pangandaran, West Java	20	7°44'55" S	108°29'51" T	0-22
Ambalresmi, Kebumen, Central Java	20	7°47'58" S	109°43'39" T	8
Alas Purwo, Banyuwangi, East Java	20	8°39'26" S	114°21'39" T	15
Baluran, Situbondo, East Java	20	7°55'07" S	114°25'18" T	0 - 1

Note: asl. = above sea level

Table 2. RAPD primers used in this study

Locus	Primer sequence (5'-3')	T Annealing (°C)	Length (bp)	No. of detected polymorphic bands
OPL 11	ACGATGAGCC	33 (29-39)	250-1000	14
OPAH 15	CTACAGCGAG	33 (26-36)	300-850	13
OPAO 01	AAGACGACGG	33 (29-39)	300-800	20
OPAB 14	AAGTGCGACC	37 (30-40)	350-900	18
OPAH 13	TGAGTCCGCA	37 (32-42)	450-1000	24
OPC 07	GTCCCGACGA	33.9 - 43.9	n.a	n.a
OPAN 01	ACTCCAGGTC	27.2 - 37.2	n.a	n.a
OPAP 20	CCCGGATACA	27.6 - 37.6	n.a	n.a
OPAP10	TGGGTGATCC	27.8 - 37.8	n.a	n.a
OPAB01	CCGTCGGTAG	31.0 - 41.0	n.a	n.a

Remarks: n.a = no stable amplification products

RESULTS AND DISCUSSION

Molecular characterization was conducted using ten primers to quantify the genetic diversity of malapari in Java Island. However, among the primers, only five gave good amplification and stable band pattern. Suitable temperatures for those primers were at 33°C and 37°C. Basepair length ranged from 250 to 1000 bp with the number of polymorphic bands varied, ranging between 13-24. Different primers showed variations in the ability to detect the effective polymorphism between population malapari in Java Island.

The genetic diversity of malapari in Java Island varied between population (Table 3). In Table 4, the Baluran population had the highest value for all parameters, *i.e.*, PLP (84.27%), N_a (11.8427), N_e (1.5764), H_e (0.3183) or I (0.4647). It indicates that the Baluran population was possibly as one of the diversity centers of malapari in Java Island. In contrast, Kebumen population had the lowest value of all genetic diversity parameters, *i.e.*, PLP (59.55%), N_a (1.5955), N_e (1.3455), H_e (0.2024) and I (0.3038). It indicates that the Kebumen population may have originated from the same or limited malapari parent trees.

The dendrogram (Figure 2) shows the separation of the five malapari population into two main groups. The first group formed three subgroups, where the population of Carita and Batukaras were independent each other, while the population of Kebumen and Alas Purwo were clustered into one group. The other group consisted of one population only, *i.e.*, Baluran. This is possible, because only one Baluran population existed in the North Coast of Java Island, while the other four population were on the South Coast of Java Island. The Kebumen and Alas Purwo population had the closest level of relationship.

In general, the genetic distance between population did not correspond with geographical distance in some species; this depends on nature and artificial factors involved in shaping the genetic structure of species population (Stankiewicz et al. 2001). On the other hand, the ecological origin and geographic factors have limited role. Despite this, variation in genetic diversity within the species is typically associated with geographic range, reproduction system, mating systems, seed dispersal and fecundity (Loveless 1992). In this study, the shortest genetic distance was found for the Alas Purwo and Kebumen populations (0.0602), indicating that the two populations have close relationship; it is possible that the two populations originated from the same source. The longest genetic distance was between the Baluran and Carita populations (0.0995). It suggests that the Carita population had unlikely originated from the Baluran population. It also corresponds to the longest geographic distance between Carita and Baluran (1191 km), while the shortest distance was found in between Batukaras and Kebumen (90 km) (Table 4).

The results of this study do not support finding from the previous studies conducted by Schnabel and Hamrick (1990) and Alpert et al. (1993), which stated that the genetic distance is positively correlated with geographical distance. However, Stankiewicz et al. (2001) stated that in

general, the genetic distance between population may or may not correlate with geographical distance between population in some species, as it depends on the natural and artificial factors involved in shaping the genetic structure of population of these species. Julisaniah et al. (2008) stated that mating between individuals with a close genetic relationship has the effect on the increased homozygosity, and *vice versa* if the mating between individuals within a large genetic relationship that usually has the effect on the increased heterozygosity. It may occur in malapari which is a tropical plant species.

Table 3. Parameter values of genetic diversity in five malapari populations in Java Island, Indonesia

Populatio	N	PLP (%)	N_a	N_e	H_e	I
Carita	20	70.79	1.7079	1.3857	0.2302	0.3481
Batukaras	20	64.04	1.6404	1.3573	0.2080	0.3126
Kebumen	20	59.55	1.5955	1.3455	0.2024	0.3038
Alaspurwo	20	64.04	1.6404	1.3542	0.2075	0.3128
Baluran	20	84.27	1.8427	1.5764	0.3183	0.4647
Average	20	68.54	1.6854	1.4038	0.2333	0.3484

Note: N: number of individuals, PLP: Polymorphic locus percentage, N_a : number of alleles observed, N_e : average number of effective alleles, H_e : average gene diversity index, I : Shannon index

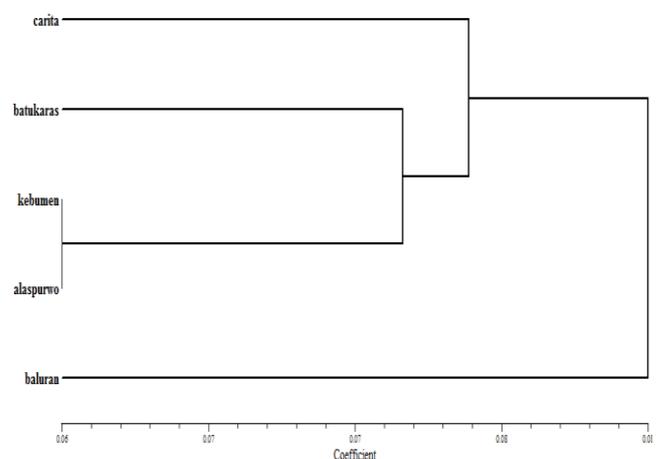


Figure 2. Dendrogram of five population of malapari in Java Island, Indonesia

Table 4. Genetic distance based on Nei's Unbiased Measures and geographical distance

Population	Carita	Batukaras	Kebumen	Alas Purwo	Baluran
Carita	****	423 ^G	474 ^G	1067 ^G	1191 ^G
Batukaras	0.0793 ^g	****	90 ^G	683 ^G	807 ^G
Kebumen	0.0617 ^g	0.0661 ^g	****	594 ^G	718 ^G
Alas Purwo	0.0865 ^g	0.0805 ^g	0.0602 ^g	****	124 ^G
Baluran	0.0995 ^g	0.0807 ^g	0.0781 ^g	0.0726 ^g	****

Note: (g) is value of genetic distance and (G) is value of geographical distance (km)

The tropical plant species such as malapari, is a cross-pollinated species. Cross-pollinated plants have the potential for gene flow as indicated by the high level of genetic diversity within population (Loveless and Hamrick, 1984; Hamrick and Godt, 1989; Pither et al. 2003). The results also showed the same finding that the genetic diversity of each population was much higher than the genetic diversity between populations (Table 5). The average genetic diversity of malapari was 0.2333. This value is higher compared with the same species at different locations, that is in North Guwahati, Assam, India by 0.024 (Kesari et al. 2010). The genetic differentiation between populations of malapari was 16.36%. It is lower compared with the same species of study results of Kesari et al. (2010) by 37.7%. The significant differences in genetic variabilities may be caused by differences in sample sources originating from seeds taken from limited number of candidate plus trees from one region (Kesari et al. 2010), whereas this study used sample sources from leaves of mature trees located in five populations. The genetic differentiation between population is possibly due to gene flow and local selection (Bawa, 1998). Gene dispersal of malapari is considered high ($N_m > 2$), as seed can disperse for long distances, aided by coastal water movement (currents, storm, etc.). In addition, gene flow by pollen highly depends on the pollinating agent. Gene flow of malapari in Indonesia may be larger than that in India, because Indonesia is an archipelago with high wind speeds.

Population genetic structure

Results of the structure analysis showed that the number of structure harvester dataset malapari best for the fifth population is $K = 2$ ($\Delta K = 124.928$). This shows that the five populations can be divided into two clusters, namely cluster 1 consisting of population from Carita, Batukaras, Kebumen and Alas Purwo (black) and cluster 2 with only one population from Baluran (gray) (Figure 3).

Irmayanti (2015) mentions that the same color or patterns on the chart indicates that the population has in common with respect to the genetic structure.

Implication for conservation and breeding programs

The findings of this research would imply the formulation of suitable strategy for genetic conservation and breeding programs. The collected materials from this research could be tested in the laboratory for seed quality analysis and also in the field for determining the growth performance of each malapari population. Later, based on the results, the future planning for genetic conservation (e.g. in situ conservation) of malapari should consider the combined results of genetic diversity and growth performance variability in the field including also the seed quality test.

In conclusion, the genetic diversity analysis of five population of malapari in Java showed that the Baluran ($H_e=0,3183$) was considered to be the potential diversity center of malapari in Jawa, because of its high genetic diversity, whereas the Kebumen population ($H_e=0,2024$) had the lowest genetic diversity. The average genetic diversity between populations is lower than that of within the population. Cluster and structure analysis showed population separation into two main groups in which Baluran population clustered differently in contrast to the other four remaining populations.

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Table 5. Comparison of the average value of genetic diversity between malapari in Indonesia and India based on Nei analysis (1987)

Species	Location	Ht	Hs	Gst	Dst	Nm	Source
<i>P. pinnata</i> (malapari)	Carita, Batukaras, Kebumen, Alas Purwo, Baluran	0.2789	0.2333	0.1636	0.0456	2.5565	This study
<i>P. pinnata</i>	North Guwahati, Assam, India	0.038	0.024	0.377	0.014	0.828	Kesari et al. 2010

Note: Ht: genetic diversity in all population; Hs: genetic diversity within the population; Gst: genetic differentiation; Dst: genetic diversity between population; Nm: gene flow

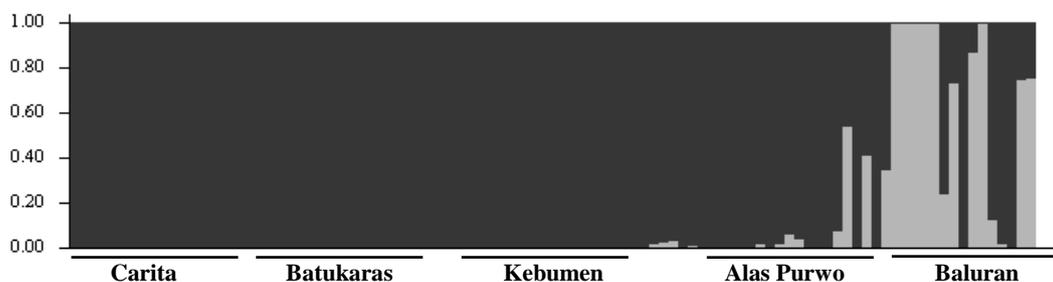


Figure 3. Structure of five malapari populations cluster 1 consisting of population from Carita, Batukaras, Kebumen and Alas Purwo (black) and cluster 2 with only one population from Baluran (gray)

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