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Genetic diversity and kinship of ebony population (*Diospyros celebica* Bakh.) in its natural population in Central Sulawesi

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Abstract

Diospyros Celebica Bakh. is a type of Sulawesi endemic that has a high economic value, but its existance becomes decreased due to excessive exploitation and no proper replanting. Conservation and plant development is urgently done to avoid extinction of *Diospyros Celebica* Bakh. Genetic diversity becomes a source of important information in conservation program and breeding for such development. This study aims to analyze genetic diversity of population and interpopulation as well as kinship among ebony population in Central Sulawesi. Leaf sample of ebony is taken from natural population in Donggala, Parigi-Moutong and Poso Regencies. Each population consisted of thirty samples (sample tree), and genetic analysis used RAPD genetic marker employing 10 primary RAPDs already selected. The results of this research showed that the average of genetic diversity of Ebony population was high (0.383), but genetic diversity among the populations was very small (0.063). The biggest genetic diversity occured in the population of Parigi Moutong, and the smallest occured in the population of Poso. The population of Ebony in Donggala and that of Ebony in Parigi Moutong are more closely related compared to that in Poso. The result also indicated that the species will be survived and exist providing the genetic diversity are well maintained, therefore in-situ and ex-situ coservation program are required.

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Introduction

Ebony (Diospyros Celebica Bakh) is endemic to Sulawesi island, where its natural population can only be found in Central Sulawesi, West Sulawesi and South Sulawesi. In Central Sulawesi, the natural population of Ebony is mainly in Poso, Parigi Moutong, Donggala, Morowali and Tojo Una-una, although it can also be found in other places. Ebony produces luxurious wood which is valued highly economical and legalized as the maskot of Central Sulawesi through Decree of Governor of Central Sulawesi No. 660/78//1995 dated 27 February 1995. Ebony has great motives/patterns with smooth grain. It is classified as durable and hardwood class 1 when it weighs around 1.05. Ebony has streaky pattern, black in color with combination of smooth straight grain, corrugated forming strip and beautiful reddish brown. Because of its beauty and strength, Ebony is made expensive souvenier and luxurious meubel that has been traded since the Dutch era either for fulfiling in country's need or for export.

Due to the decrease in its population, the IUCN (2010) categorizes Ebony as an endemic tree (vulnerable). While based on the criteria of scarce tree (Anonim, 2006), it belongs to the status of risky conservation (VU, Alc), that is a tree with high extinction risk from the nature in near future (in the medium-term future) as a result of habitat damaging and excessive exploitation. In spite of this, information on Ebony genetic diversity in its natural population in Central Sulawesi has not been much revealed.

Genetic diversity is a fundamental requirement for a long-term stability of an ecosystem. High genetic diversity is needed for a plant to survive from pest attact, health and adaptation to environment. There are some factors affecting genetic diversity value such as reproduction habit and habitat in the nature (Hamrick *et al.*, 1992).

Genetic diversity is an important source of information in conservation program and breeding for plant development.

Thus, information about genetic diversity is really needed as an initial step in breeding program and plant development. Information of population diversity value of a plant becomes one of the important considerations in deciding both conservation strategy and breeding program.

Molecular genetic approach is one of the effective and suitable ways to find out genetic diversity of a plant. Molecular genetic marker method which is often used to analyze, such a diversity of forest trees, is RAPD (Random Amplified Polymorphyc DNA) marker. RAPD is a PCR (Polymerase Chain Reaction)-based marker using short primer sized 10 bp. The advantage of this marker among others is that it is relatively easy to use by employing considerably simple and cheap equipment. Genetic diversity research on some different plants, such as *Instia biyuga, Santalum album, Eusideroxylon zwageri, Alstonia scholaris, Gyrinops versteegii* and *Araucaria cunninghamii*, employing RAPD marker have been reported (Widyatmoko *et al.*, 2011). This research aims to study intra and inter genetic diversity and kinship among Ebony populations in its natural population in Central Sulawesi.

The material and the methode

The material

The material studied was leaf sample taken from Ebony sample tree in three natural populations in Central Sulawesi, namely Nature reserve of Pangi Binangga, in Parigi Moutong Regency; Siweli Village, in Distric of Dampelas in Donggala Regency, and Nature reserve of Tambaro Village in Poso Regency (Picture 1).

Thirty (30) individual seed trees were randomly selected from each population under the criteria that tree diameter must be ≥20cm at breast-high (DBH) and that distance among trees was ≥50m. Name of population, geographical position, altitude and average altitude and diameter of the seed trees were presented in Table 1 below.

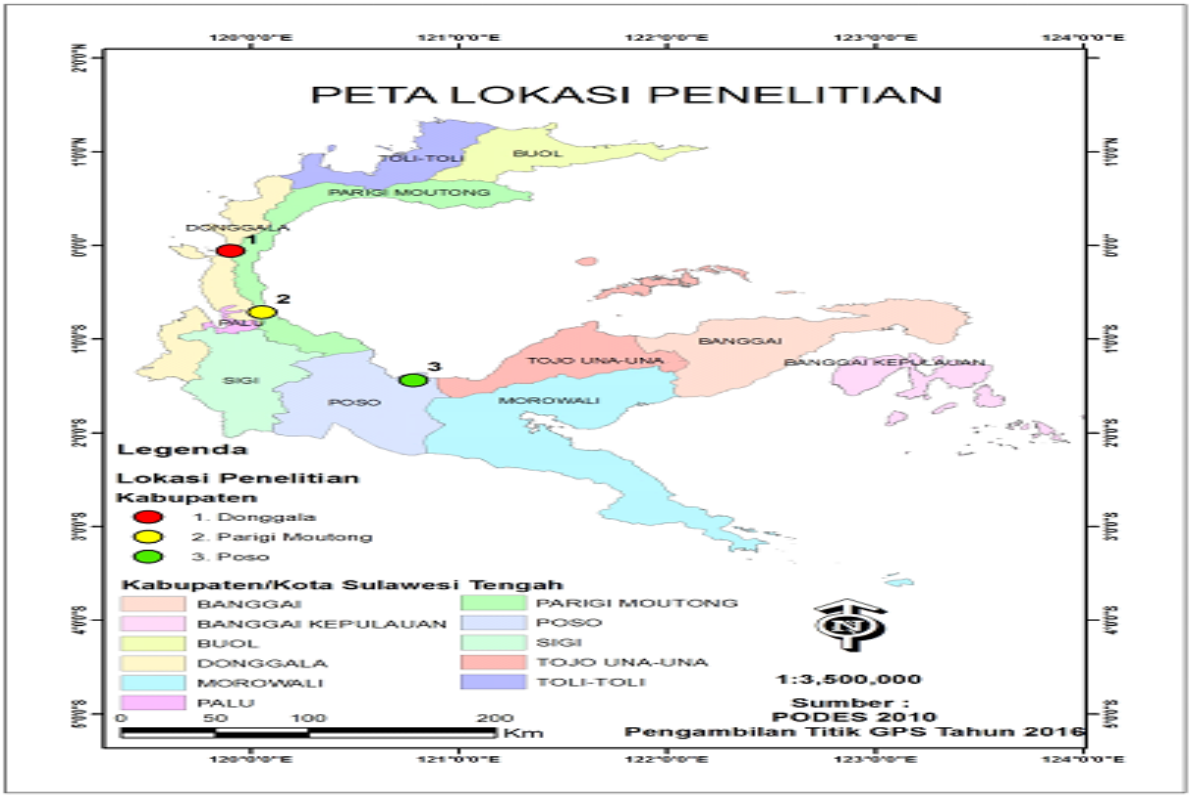

Fig. 1. Location where the sampled leaves were taken.

DNA Extraction and rapd analysis

DNA Extraction and RAPD Analysis were used as technical guide for Molecular Genetics Laboratory (RAPD Marker) in P3BPTH Yogyakarta (Anonymous, 2010). Sampled Leaf weighing 50 mgs that had been dried using silica gel was extracted using Cetyl Trimethyl Ammonium Bromide (CTAB) method.

Primer selection of RAPD was done before Polymerase Chain Reaction (PCR) process to choose primer that was going to be used in the process of PCR. Proses of PCR utilizing machine of Thermal Cycler GeneAmp PCR System 9700 (Applied Biosystem) involving 45 cycles for ± 3 hours. Result of PCR amplification was electrophoresed at 1.5 percent of agarose gel, 20 times of TBE buffer and 0.5 percent of ethidium bromide for 2 hours in 120 voltage. Then, the result of electroforesis was documented using Fotodyne Bio Rad Analyzer.

Data analysis

The result of PCR was assessed by scoring the result of amplification; score one (1) was given when Pita (amplification) existed and score zero (0) was given when Pita did not exist. Then, statistical analysis was done using GenAlex 6.41 (Peakall and Smouse, 2012). The amount of detected allele (Na), effective allele (Ne), and genetic diversity (He) were counted in each population.

Inter population genetic diversity was measured based on Nei's Genetic distance and Nei's Genetic Identity. Principal Coordinates Analysis (PCoA) was done to classify the population based on genetic distance. Analysis of Molecular Variance (AMOVA) was used to analyze variation of genetic diversity of intra and inter population within or among the groups.

Result and discussion

Primer Selection

Result of the 10 selected primers of RAPD revealed varied number of polymorphic loca ranging from 1-8. Primer of OPG-7 produced the most polymorphic locus, that was 8 loca, while primer of OPA-13 produced one polymorphic locus.

Number of polymorphic loca of the entire selected primers of RAPD were 39 or an average of 3.9 polymorphic loca per primer. Details of primer name, sequence and polymorphic loca produced are presented in Table 2.

Widyatmoko *et al* (2011) have revealed primers A-4, 7, and G-7, 12, when analyzing genetic of Ebony (Diospyros Celebica Bakh.) using RAPD method and produced clear and thick picture of polymorphic band. Primers of OPA-1 and OPA-4 have also been used to analyze RAPD of *Anthocephalus cadamba* and produced five (5) polymorphic loca each (Nurtjahyaningsih, 2014), while Widyatmoko and Aprianti (2013) using primer of OPG-7 to analyze RAPD on ramin *(Gonystylus bancanus)* produced five (5) polymorphic loca.

Table 1. Name of population, Geographical position, Altitude, Average Height and Diameter of sample trees of Ebony

Name of Population	Geographical Position	Altitude (m dpl)	Height Ave. (m)	Diameter Ave. (cm)
Donggala	S 00°00,636'-00°04,891' E 119°53,882'-119°56,977'	36 – 209	18,47	33,8
Parigi Moutong	S 00°43,839'-00°47,392' E 120°03,669-120°04,677'	128 – 471	21,10	35,3
Poso	S 01°27,880'-01°28,087' E 120°43,801'-120°44,135	85 – 276	22,9	32,3

In this research, primers of OPG-7 produced 7 polymorphic loca, of OPA-4 produced six (6) polymorphic loca and of OPA-1 produced only two (2) polymorphic loca.

Difference in the number of polymorphic locus produced by the same primer might have been due to different plants, since each plant has different genetic makeup.

Genetic diversity

The results of analysis on the number of allel (Na) and the number of effective allel (Ne) and the expected heterosigosity (He) along with the statistical analysis of GenAlex 6.41 are thoroughly displayed in Table 3 below.

Table 2. Primer name, sequence and polymorphic loca produced.

No	Primer Name	Sequence (5'-3')	Number of polym.	Polymorphic Loca
1.	OPA-01	CAGGCCCTC	2	1000; 1200
2.	OPA-04	AATCGGGCTG	6	600; 700; 750; 800; 850; 900
3.	OPA-05	AGGGGTCTTG	4	700; 900
4.	OPA-06	GGTCCCTGAC	2	800; 1300
5.	OPA-07	GAAACGGGTG	2	650; 700
6.	0PA-13	CAGCACCCAC	1	750
7.	OPG-07	CAACCTGCGG	7	600; 700; 750; 900; 1000; 1150; 1200
8.	OPG-12	CAGCTCACGA	8	550; 700; 750; 850; 900; 1000; 1250; 1300 600; 650; 700; 800; 900 700; 900
9.	OPG-15	ACTGGGACTC	5	
10.	OPG-18	GTCAGGGCAA	2	
		Total	39	

The number of allel (Na) on the analysis of RAPD is 2, that is with and without DNA band. Effective allel (Ne) is the allel producing polymorphic loca. The number of effective allel (Ne) influences value of expected heterosigosity (He). The bigger the number of effective allel (Ne) is the bigger the value of expected heterosigosity (He) or the value of genetic diversity will be.

Table 3. The Number of Allel (Na), Effective allel (Ne) and the Expected heterosigosity (He).

population	Total Samples	Number of Allel (Na)	Number of Efct. allel (Ne)	He (SE)
Poso	30	2,00	1,598	0,358 (0,017)
Donggala	30	2,00	1,694	0,389 (0,020)
Parigi Moutong	30	2,00	1,716	0,403 (0,016)
Average	30	2,00	1,669	0,383 (0,010)

The value of genetic diversity in three (3) Ebony populations ranged from 0.358 – 0.403 with an average of 0.383. Ebony population of Parigi Moutong revealed the highest genetic diversity 0.403 and that of Poso was the lowest, which was 0.358. Meanwhile, Widiatmoko *et al.* (2011) found that value of genetic diversity of Ebony population in Parigi, Central Sulawesi was 0.3217, that in Mangkutana, South Sulawesi, was 0.2966 and that in Wasupoda, South Sulawesi, was 0.2476. The total samples used from each population in this research was eight. The difference in findings between these two research might had been due to individual sample and different number of samples.

Table 4. Value of genetic diversity (below line) and genetic identity (above line) among three Ebony populations in Central Sulawesi.

Population	Poso	Donggala	Parigi Moutong
Poso	-	0,907	0,927
Donggala	0,098	-	0,984
Parigi Moutong	0,076	0,016	-

Remarks:
Black = Genetic Diversity among populations
Red= Genetic Identity among populations

This research used 30 individual tree of each population. According to Nybom & Bartish (2000), genetic marker of RAPD was a fairly sensitive method in detecting genetic structure of population, so that method of collecting samples can significantly affect the value of genetic diversity gained. Individual tree has different genetic, so the more number of sample trees have the bigger possibility of genetic diversity. Hale *et al.* (2012) state that the number of samples for allele estimation can be accurate when number of sample per population is 25-30. Since the sample of this research was 30, the result could have been accurate.

The average of genetic diversity of other trees that have been studied including *Anthocepalus cadamba* was 0.204 (Nurtjahjaningsih *et al.*, 2014), *Gonystylus bancanus* was 0.329 (Widyatmoko and Aprianto, 2013), and *Aquilaria spp* was 0.225 (Widyatmoko *et al.*, 2011).

The genetic diversity of Ebony population in its natural population in Poso Regency, Donggala Regency and Parigi-Moutong Regency was ≥ 2.5 and was categorized very big (Harti and Clark, 1997).

Although the number of individual ebony tree in each habitat decreased, its genetic diversity value was still very high. It was probably caused by cross-breeding among invidual trees within the population.

As a result, individual genetic diversity of inter population was big, and tree exploitation in the recent years was decreasing (Widiatmoko *et al.*, 2011).

Table 5. The result of AMOVA analysis on Ebony genetic diversity.

Source	df	SS	MS	East.Var	P
Among Regions	1	66,068	66,068	11%	0,01**
Amg. Pops. In Reg.	1	17,597	17,597	3%	0,01*
Within Pops.	87	808,423	9,153	86%	0,01**
Total	89	889,088		100%	

Genetic diversity shown by the three Ebony populations in the three different regencies was categorized very big, though its population in its habitat and conservation status was prone to vulnerable (VU Alc) (IUCN, 2006; Anonim, 2006). Genetic materials used in this study were leaves taken from Ebony tree in each population with diameter ≥ 20 cms, so that the result of genetic diversity obtained was the remainings of genetic diversity in the population. Therefore, it can be concluded that Ebony population in Sulawesi was a large genetic diversity that should be preserved.

The population of Ebony tree in its habitat should be maintained, so that the cross-breeding occurs among individuals will be a large genetic diversity tiller as well.

The result of analysis of Nei's genetic distance and Nei's genetic identity among populations was presented in table 4 below.

The highest genetic diversity between Ebony population in Poso and Donggala Regencies was 0.098, while the lowest (0.016) was that of between Ebony population in Donggala and Parigi Moutong Regencies. The average genetic distance among populations was 0.063.

It could be assumed from the smallest value of Nei's genetic distance (0.016 – 0.098) and quite large value of Nei's genetic identity (0.907 – 0.984) that the three populations were closely-related or formerly derived from the same population and widely spread enabling gen flow to occur and genetic identity among populations was large.

Based on Nei's genetic distance of the three populations, the researchers classified them into two groups. The first group was Ebony population in Poso Regency and the second group consisted of two populations (Ebony population in Donggala and Parigi Moutong Regencies). Then, they analyzed the coordinate using Principal Coordinate Analysis (PCA) with two coordinate axis. The result of PCA analysis showed that the percentage value of coordinate 1 was 88. 67% and coordinate 2 was 11.33%. This indicated that coordinate 1 was higher than that of coordinate 2.

The result of PCA above showed that Ebony population in Donggala Regency and that in Parigi Moutong Regency were in the same position in coordinate 1, while Ebony population in Poso Regency was in different position. Geographically, the distance between Ebony population in Poso Regency and that in Donggala Regency was the farest compared to the distance between Ebony population in Poso Regency and that in Parigi Moutong Regency.

The nearest was between the Ebony population in Donggala Regency and that in Parigi Moutong Regency.

Furthermore, the genetic identity value of Ebony population in Donggala-Moutong (0.984) was higher than that of Ebony population in Poso-Donggala (0.907) and that of Ebony population in Poso-Parigi Moutong (0.927). This indicated that the close relationship between geographical position and genetic makeup among the three populations occurred naturally or no human intervention in its distribution. Sampled trees were naturally distributed, not the result of cultivation or plants from other regions.

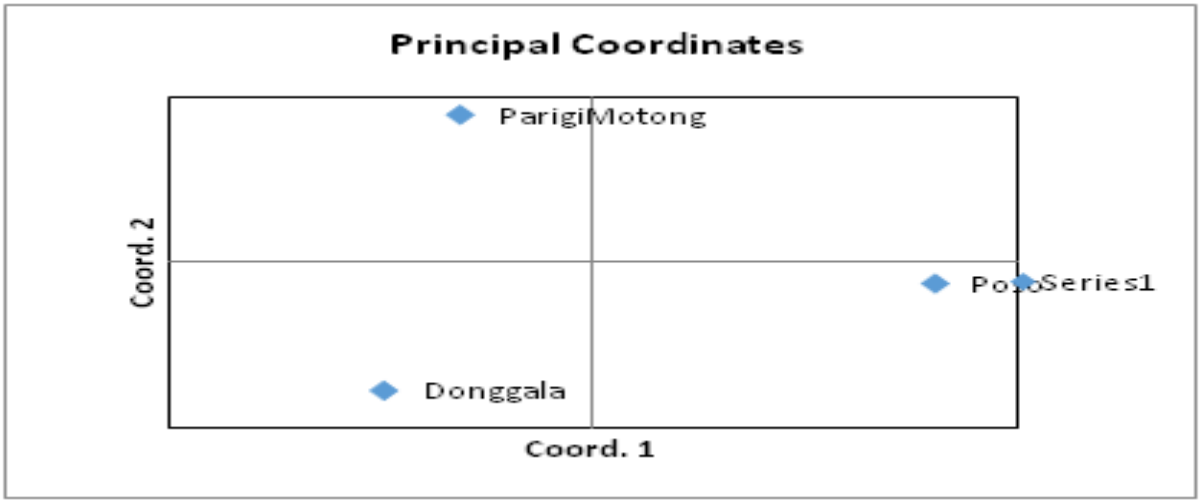

Fig. 2. Principal Coordinates Analysis of Three Ebony Populations.

Genetic diversity among individuals within the population of Ebony had the highest percentage, which was 86%, while that between Ebony population in Poso and Donggala-Parigi Moutong was 11%, and that between Ebony Population in Donggala and Parigi Moutong was 3%, which was the lowest, with the smallest value of Nei's genetic distance (0.016).

The result of AMOVA also indicated that the percentage of genetic diversity between Poso group and that of Parigi-Moutong was higher than genetic diversity between Donggala group and Parigi Moutong. These results strengthened the assumption that the population of Ebony in Donggala and that in Parigi Moutong were closely related; it was quite possible that in the past, they came from the same main population.

Implication for Ebony Conservation Program

Conservation strategy and breeding to develop a particular forest plant needs information of genetic diversity of the plant to enable efficient and effective implemention. The average of genetic diversity of Ebony was 0.383, which means genetic diversity of the plants in the existing population was still high. Although IUCN (2010) argues that Ebony is classified as a vulnerable plant, and Anonymous (2006) says that it is given the status of prone to conservation (VU, 1c), Ebony still has a very high genetic diversity.

This means that Ebony of this kind is genetically conservable and possible for future cultivation to get better individual tree. However, with high exploitation activities still taking place, intensive genetic conservation activities are urgently needed.

Genetic conservation can be carried out within original habitat (in-situ) and beyond original habitat (ex-situ) of Ebony. *In-situ* conservation is a preservation concept needing protection and preservation of a particular kind or even a genetic diversity within a particular plant based on its original habitat and ecosystem. This concept is needed to ensure genetic resource to be protected in line with its natural environment. Thus, genotype and phenotype characteristics of the plant remains original, biological and physical diversities remain potected, example of important natural area regarded as being representative is continually conserved.

In regard with *in-situ* conservation, population/area needed to be chosen is population with high genetic diversity. *In-situ* conservation area is chosen, big tree (main tree) needs to be conserved, and genetic enrichment may come from the population itself.

Conclusion

Genetic diversity of Ebony population in its natural population in Central Sulawesi is very high, with an average of diversity 0.383. Ebony Population in Parigi Moutong has the highest genetic diversity, which is 0.403. The examined population of Ebony has the smallest inter-genetic diversity. In other words, the tree population groups have considerable genetic similarity.

Ebony populations in Donggala and Parigi Moutong are closely related compared to that in Poso. Coordinate Principle Analysis (PCA) indicate that genetic distance and genetic geography is related to each other. This means that the distribution of the Ebony studied occured naturally without human intervention.

Ebony population in Central Sulawesi still have high genetic diversities indicating the species will be survived and exist providing the genetic diversity are well maintained, therefore in-situ and exitu conservation program are required.

References

Achmad A. 2002. Strategi Konservasi *In-situ* Eboni Bergaris/Kayu Hitam Makassar (*Diospyros celebica* Bakh.) di Sulawesi, Berita Biologi **6(2)**, 12-20.

Allo MK. 2011. Distribusi, Potensi dan Pengelolaan Eboni (*Diospyros celebica* Bakh.). Porsiding Lokakarya Nasional "Status Konservasi dan Formulasi Strategi Konservasi Jenis-jenis Pohon yang Terancam Punah (Ulin, Eboni dan Michellia)": 45-55. Badan Litbang Kehutanan bekerjasama dengan ITTO, Bogor, Indonesia.

Allo MK. 2012. Pembangunan Plot Konservasi Genetik Eboni (*Diospyros celebica* Bakh.) di Taman Nasional Bantimurung Bulusaraung, Sulawesi Selatan. Prosiding Lokakarya Nasional Plot Konservasi Genetik Untuk pelestarian Jenis-jjenis pohon terancam punah (ulin, Eboni dan Cempaka). Badan litbang kehutanan bekerjadama dengan ITTO Project PD 539/09 Rev. 1/F. Bogor. Hal : 45-65

Anonim, 2006, Data Base Jenis-jenis Prioritas untuk Konservasi Genetik dan Pemuliaan. Buku **2**: Kategori dan Kriteria Tumbuhan Langka. Pusat Penelitian dan Pengembangan Hutan Tanaman. Departemen Kehutanan. Jakarta.

Anonim, 2010, Petunjuk Teknis Kegiatan Laboratorium Genetika Molekuler (RAPD Marker) Pusat Penelitian dan Pengembangan Bioteknologi dan Pemuliaan Hutan (P3BPTH). Yogyakarta.

Hains R. 2010. Biotechnology in Forest Tree Improvement, With Special Reference to Developing Countries. FAO Forestry Paper **118**.

Harti DL, Clark AG. 1997, Principle of Population Genetics (Third Edition). Sinauer Associates, Inc. Publishers, Sunderland, Massachusetes.

Ismail B, Charomani MZ. 2011, Konservasi Sumberdaya Genetik Tanaman Hutan, Aspek Penelitian dan Cakupan Kegiatan, Balai Besar Penelitian Bioteknologi dan Pemuliaan Tanaman Hutan, Yogyakarta.

IUCN, 2010, Red List of Endangered Species. International Union for the Conservation of Nature and Natural Resources, Gland, Switzerland. www.iucnredlist.org.

Khanlou KM, Vanepitte K, Asl KL, Bockstaele E. 2011. Toward and Optimal Sampling Strategy for Assessing Genetic Variation Within and Among White Clover (*Trifolius repens,* L.) Cultifar using AFLP, Genetics and Molecukar Biology **34(2),** 252-258.

Mc Pherson MJ, Quirke P, Taylaor GR. 1994. PCR **1**. A Practical Approach. Oxford University Press. New York.

Muller-Starck G, Baradat PH, Bergman F. 1992. Genetic Variation within European Tree Species, Proceeding International Symposium of Population Genetics of Forest Trees, Corvalis, Oregon, USA.

Nurtjahyaningsih ILG, Widyatmoko dan AYPBC, Rimbawanto A. 2013, Karakterisasi dan Aplikasi Penanda Mikrosatelit pada Beberapa *Species Eucalyptus*, Jurnal Pemuliaan Tanaman Hutan **7** No. **2**. September 2013.

Nurtjahjaningsih ILG, Qiptiyah M, Pamungkas T, Widyatmoko AYPBC, dan Rimbawanto A, 2014, Karakterisasi Keragaman Genetik Populasi Jabon Putih Menggunakan Penanda Random Amplified Polymorphism DNA. Jurnal Pemuliaan Tanaman Hutan Vol. **8(2)**, September 2014, 8-92.

Nybom H, Bartish IV. 2000, Effects of the History Traits and Sampling Strategies on Genetic Diversity Estimates Obtained with RAPD Markers in plants. Perspectives in Plant Ecology, Evolution and Systematics **3(2),** 93-114.

Peakall R, Smouse PE. 2012. GenAlEx 6.41 : genetic Analysis in Excel. Population Genetic Software for teaching and Research an Update. Bioinformatics Applications Note, **28(19),** 2537-2537.

Prajadinata S, Effendi R, Murniati. 2011, Tinjauan Pengelolaan dan Konservasi Jenis Pohon Eboni (*Diospyros celebica* Bakh.) dan Cempaka (*Michelia champaca* Linn.) di Indonesia dalam Porsiding Lokakarya Nasional "Status Konservasi dan Formulasi Strategi Konservasi Jenis-jenis Pohon yang Terancam Punah (Ulin, Eboni dan Michelia), Badan Litbang Kehutanan bekerjasama dengan ITTO, Bogor.

Prastyono dan Ismail B. 2014, Eksplorasi dan koleksi materi Genetik Eboni (*Diospyros celebica* Bakh.) untuk Pembangunan Plot Konservasi Sumberdaya Genetik. Wana Benih **15(1),** 41-60.

Restu M, Dan Mukrimin. 2007. Keragaman Genetik Eboni (*Diospyros celebica* Bakh.) Provenansi Amaro Kabupaten Barru. Jurnal Hutan dan Masyarakat **2(3),** 263-267.

Widyatmoko AYPBC, Ariningsih ED, Prasetyaningsih A. 2011a, Keragaman Genetik dan Hubungan Kekerabatan pada Tiga Jenis *Aquilaria* Menggunakan Penanda RAPD, Jurnal Pemuliaan Tanaman Hutan Vol. **5(3)**, 26-34.

Widyarmoko AYPBC, Nurtjahjaningsih ILG, Prasetyono. 2011b, Study on The Level of Genetic Diversity of *Diospyros celebica, Eusideroxylon zwageri and Michellia* spp, Using Rapid Marker, Technical Report No.**2**. ITTO project PD 539/09 Rev. 1(F) in Coorporatipn with Center for Conservarion and rehabilitation research and Development, Ministry of Forestry. Bogor. Indonesia.

Widyatmoko AYPBC, Aprianto. 2013. Keragaman Genetik *Gonistylus bancanus* (Mig.) Kurz, Berdasarkan Penanda RAPD (*Random Amplified Polymorphic DNA*). Jurnal Pemuliaan Tanaman Hutan **7**(1), 53 – 71.