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GENETIC VARIATION OF *GYRINOPS VERSTEGII* ORIGINATED FROM PAPUA BASED ON RAPD

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Abstract - *Gyrinops verstegii* is one of the potential tree species for production of agarwood in the eastern part of Indonesia. In its natural habitat, this species is found to be morphologically variable. This morpho-variation may reflect also the degree of genetic diversity as usually observed in many plant species. However, genetic information of *Gyrinops verstegii* is still lacking. A genetic inventory was therefore conducted, aiming at estimating the genetic variation using population samples from low and high altitudes, i.e. Kebar and Manokwari populations. Genetic analyses were performed using DNA marker RAPD following standard procedures. Results showed that the genetic variability of Kebar population (He= 0,2944) was higher than that of Manokwari (He= 0.2357). AMOVA analysis showed that most of the variation is stored in individual level contributed to 89 % of the total variation. Dendrogram analysis showed that the reproductive populations, i.e mother tree and its progenies of each location, were grouped together. The molecular information can be used for scientific consideration in developing strategies for conservation and breeding.

INTRODUCTION

Plants producing agarwood, called gaharu in Indonesia, have been identified and distribute widely in Papua are *Aquilaria filarial, Aquilaria secundana, Aquilaria tomentosa, Aetoxylon sympethalum, Enkleia malacensis, Wikstroemia poliantha, Wikstroemia androsaemofilia, Girynops cumingiana, Girynops salicifolia, Girynops audate* and *Girynops podocarpus* (Sumarna,2005). Previously Girynops verstegii according to Sumarna distributes widely in two provinces: NTT and NTB, but lately it was known from Waroy (2006), *Gyrinops verstegii* distributes widely at Bird's Head region, Papua.

Girynops verstegii in Papua has different morphology in leaf form in areas with high latitude. This variation according to Emrani *et al.*, (2011) is due to geographical (inter provenancy), local diversity and also due to variation within and inter tree.

Genetic information on the level and diversity distribution in one species is essential in relation with developing culture strategy and its conservation. The genetic diversity is an important resource in order to provide opportunity to do recombination, evaluation and selection. The genetic information can be gathered by doing genetic inventory either by field trial or by genetic analysis using genetic marker.

Genetic analysis using genetic marker is accurate and efficient primarily because the analysis is taken placed at DNA level, which is not affected by the environment and can be done at the initial stage of growing plant. DNA marker RAPD have been used widely to characterize genotype of plant. The DNA marker is capable to distinguish genotypes among individuals accurately either at the level of inter and intra species and also with distance relatives.

This research aims to determine genetic variation of *Gyrinops verstegii* by using DNA marker RAPD either within and inter population.

MATERIALS AND METHODS

Plant material used was the leaves of *Gyrinops*

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verstegii taken from two locations of natural forest which is Kebar and Asai Manokwari, Papua. Space difference growing Gyrinops verstegii as shown in Table 1 .The total sample is 114 observed sample primary with 5 polymorphic RAPD.

Genetic analysis conducted in Silvikultur of forestry laboratories, Biological The University Laboratories between the central university (Bogor Agricultural University) and Naval Medical Researh Unit 2 (Namru) Jakarta.

Methods

Sample collection and DNA isolation

Sample collection leaves to isolation dna done with the procedures standart , by adding five grams silica gel into plastic clip with sample of 1 g leaves (Yunanto, 2006). To isolate DNA done with the methods CTAB (Cetyl Trimethyl Ammonium Bromide) that has been modified (Eurlings *et al.*, 2009).

RAPD reaction and Microsatellith

Reaction of PCR amplification RAPD protocol based on Qiagen 2001. But formerly carried the purification of DNA concentration with reference on a procedure measurements Sambrook et al (1989). Primer that used to the method RAPD to products obtained from the selection results operon technology (Azwin 2007).

Data Analisis

Data analysis using software programs Popgene 1.32 , version of NTsys 1.80 (Rohlf 1993 in Hannum S et al, 2003), GSDE (Gillet, EM 1998) and the table AMOVA based on Arlequin version 3.1 (Schneider *et al*., 2000).

RESULTS

RAPD Polimorphisme

This study using a primer of operon technology, where than 10 random primary were selected, a primer that produce these as bands amplification varying to the method RAPD is OPO 10 primary, OPO-14, OPO-19, OPY-09 and OPY-13 as seen in Figure 1.

Skoring conducted against strand DNA results show the differences electrophoresis the locus of each primary. The primary OPO-09, 10 loci polymorphic found while in primary OPO-10 found 13 loci for primary OPY-13 nine and OPO-14 found fifteen locus polymorphic resemblance to the length of 200 bp to 1500 bp.

Genetic variation of Gyrinops verstegii base on RAPD

Genetic variation within a population

The genetic variation within a population *Gyrinops verstegii* variabilitas shown by some of the parameters of genetics. The measure is the parameter of genetic variabilitas alel observed (na), the number of effective alel (ne), Persen lokus polimorfik (PLP), and heterozygosity (he). The analysis of genetic diversity lokus polimorfik in determining the very diversity of the population. The data result from skoring RAPD mixed with the DNA method of using software Popgene version of the genetic variabilitas 3.2 (Table 2).

Based on the data of genetic diversity within populations of stem Gyrinops verstegii originating from the region of high value Kebar, i.e. of 0,2944 with a polymorphic 92,65% percent, while the population of saplings of the Kebar of polymorphic

Table 1. Information about two different location

No.	Data Variable	Manokwari	Kebar
1.	Rainfall (mm/yr)	2.688	2.383
2.	Air humidity (%)	81	82,97
3.	The Air temperature (0C)	31,50	27,50
4.	The Height of place (m dpl)	100-300	>500
5.	Topography	Flat -Wavy	Flat - Wavy
6.	The slope	(0 – 25 %)	(>25%)
7.	Type of Soil	Podsolik red to yellow	Podsolik red to yellow
8.	Gap between mother plant (m)	460	255
9	Gap between parent and sapling (m)	2,5	1,8
10	The number of mother plat	11	20
11	The number of saplings	34	49

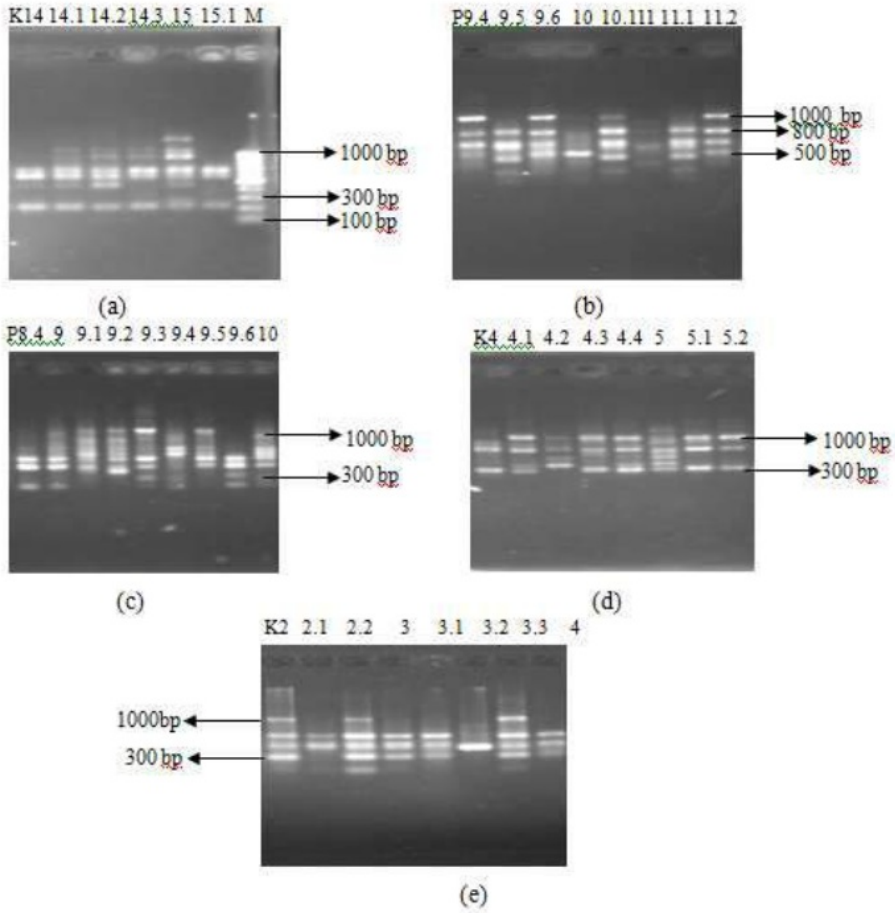

Fig. 1 Profil band DNA with primer OPO 09, OPO 10, OPY 13, OPY 9 and OPO 14 (a) Primer OPO 9, (b) Primer OPO 10, (c) Primer OPY 13, (d) Primer OPY 9, (e) Primer OPO 14, M=Marker; P= Manokwari parent, K= Kebar parent;

Table 2. Variability of genetic in Gyrinops verstegii population

Populasi	N	PLP	na	ne	he
Parent Manokwari	11	73,53	1,7353	1,4077	0,2357
Sapling Manokwari	34	86,76	1,8676	1,4700	0,2744
Parent Kebar	20	92,65	1,9265	1,4952	0,2944
sapling Kebar	49	94,12	1,9412	1,4778	0,2839

PPL= Percentage of Polymorphic Loci ; na = Observed number of alleles ; ne = Effective number of alleles; h = Nei's (1973) gene diversity

loci with 0,2839 percent of 94,12%. The magnitude of the value of the observed population heterozygosity is indicates that the variation that occurs not only because of differences in genetic structure of each population but is also influenced by environmental factors. Heterozygosity is high expectations suggest that genetic factors are relatively more instrumental compared to the factors the environment. The value of heterozigositas very beneficial as the parameters in the process of selection. The trait of being used for selection should it has value heterozigosity which was not always high because of the nature of is to be easily bequeathed according to Namkoong *et al*.,

Tabel 3. Genetik distance of Gyrinops verstegii Manokwari and Kebar

	Manokwari	Anakan Manokwari	Kebar	Anakan Kebar
Manokwari	0.0000			
Anakan Manokwri	0.0309	0.0000		
Kebar	0.0805	0.0932	0.0000	
Anakan Kebar	0.0964	0.1024	0.0282	0.0000

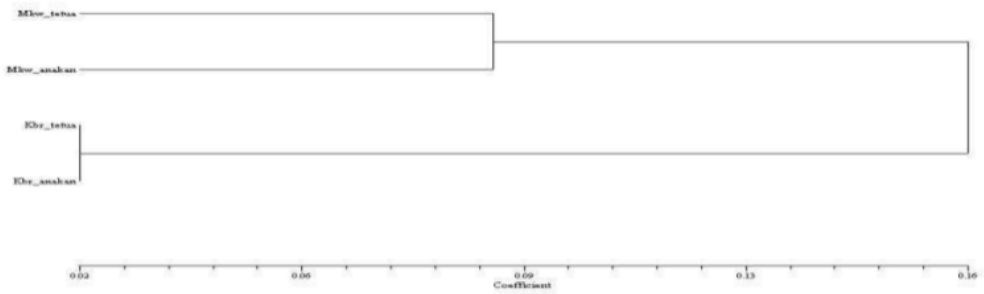

Fig. 2 Dendogram genetic diversity among the population *Gyrinops verstegii*

(1996), one sign in the practice of genetic of forest management a sustainable is the magnitude of the diversity of genetic. Genetic diversity its large deeply affecting another kind of the ability to adapt. Individual or a population by diversity narrow genetic will be vulnerable with the environment condition which heterogeneous. Basically the ability of a type of forest trees to adapt in a range of environmental conditions is very much dependent on the diversity of genetic and a multiplicity of individual tree in the population.

Variation between population

Genetic distance measuring the difference the genetic structures between two the population at a locus of a particular gene. Genetic distance of two or more of the population in general analyzed by a matrik where elements in the form of the distance and the genetic a combination of each population (Finkeldey, 2005). Data on genetic distance in this research as on a Table 3.

Based on an analysis of the value of genetic distance in Table 3 then dendogram that can be formed with methods of arithmetic the installation groups not weighted (unweighted pair-grouping method with aritmatic averaging, UPGMA) by using software version 2.02 ntsys hence produced dendrogram genetic distance between the population as seen in Figure 2. On this dendogram can look the kinship relation and pattern grouping population based on Dna that is owned by Gyrinops verstegii Papua.

The analysis of genetic distance and dendrogram above shows grouping a population that very clear according to the area in which each population growing. Grouping consists of two groups, where the host plant forms a group and saplings growing population is in accordance each location. Manokwari Parent population with saplings, at a distance of genetic parent and 0,0309 and population of tillers of Kebar of 0,0282 shows between that the genetic structures of saplings population and parent very close. But to value the distance between the two genetic groups in the population Kebar and Manokwari parent having considerable distances as much as 0,0805. Large genetic distance this indicates that the kinship relation both this population is quite a long way even separate and isolated. This is further strengthened with the results of the observation shows that saplings of each population there is no similar to parent other plants . In addition of both population different flowering time , so did not allow for the flow of gene.

CONCLUSIONS AND SUGGESTION

Genetic diversity in the population parent Kebar shows a high diversity of the population than others. Genetic diversity among the population in the same region shows the value of genetic variation Analysis based on genetic distance , the

population of tillers of parent and form one group at a distance of small genetic , being between the population parent Manokwari and Kebar having distances large genetic. What this demonstrates the two groups apart and it is this seems to be a barrier the occurrence of the flow of genes.

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