

## Allozyme variation of the endemic and vulnerable *Dyera lowii* Hook.f. in Central Kalimantan: Implications for genetic resources conservation

Tri Suwarni Wahyudiningsih<sup>1\*</sup>, Mohammad Naiem<sup>2</sup>, Sapto Indrioko<sup>2</sup>, and Issirep Sumardi<sup>3</sup>

<sup>1)</sup> Faculty of Agriculture, Universitas Palangka Raya, Indonesia

<sup>2)</sup> Faculty of Forestry, Universitas Gadjah Mada, Yogyakarta, Indonesia

<sup>3)</sup> Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

### Abstract

*Dyera lowii* is an endemic and vulnerable tree species of commercial value as chewing gum found in peat swamp forests, scatteredly distributed in Sumatra, Kalimantan, and Peninsular Malaysia. Their existence is now under severe threat due to habitat conversion. This study is aimed to assess genetic diversity within four natural populations (Hampangan, Parahangan, Sebangau, Selat Nusa) and one plantation in Central Kalimantan based on allozyme variation. Electrophoresis procedures were conducted with an isoelectric focusing polyacrylamide slab gel system. The result showed high genetic diversity ( $H_E=0.52$ ) and gene flow (3.402) seemed to be effective. A total of 14 alleles were found among all the analysed population. Mean number of alleles per locus ( $A_n$ ) was 3.206, and the effective number of alleles per locus ( $A_e$ ) was 2.21. Genetic differentiation between populations ( $F_{ST}$ ) was significant at the moderately level (0.0685). Most allozyme variation was found within population (93.2%). Special attention is essential to conserve a private allele of *Got-1-e* (9%) at Selat Nusa population. Sebangau population missed the alleles of *Est-2-b* and *Got-1-a*, as found in other populations. Selat Nusa population is expected to enhance the effective management for genetic resources conservation of this vulnerable species in the future.

**Keywords:** allozyme, genetic diversity, genetic differentiation, populations, *Dyera lowii*.

### Introduction

The global centre of biodiversity of tropical peat swamp forests lies in the Indo-Malayan region where these unique habitats impact the functioning of ecosystems far beyond their borders through their influences on climate and hydrology. These forests support a biological resource of global importance, yet on a daily basis they are being rapidly degraded and destroyed (Yule, 2010). Several efforts at rehabilitation of peat swamp forests that have been degraded by drainage, fire and logging or converted to agriculture are under way in Central

Kalimantan (Australian Orangutan Project 2008; Dohong 2008).

Rehabilitation of the Mega Rice Project in Borneo (described by Dohong 2008) is supported by Presidential Instruction No 2/2007, and it aims to restore and conserve 80% of the former Mega Rice area. Rehabilitation involves restoration of hydrology through dam construction, blocking of the canals that were built to drain the peatlands, followed by replanting with indigenous species of commercial value such as *Dyera lowii*, *Alstonia pneumatophora*, and *Garcinia* spp. Use of these commercial species such as these, encourages the local people to conserve the forests in perpetuity (Yule, 2010).

*Dyera lowii* Hook.f. is a common tree species in the peat swamp forests of Sumatra,

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#### \* Corresponding author:

Tri Suwarni Wahyudiningsih, Faculty of Agriculture, Jalan Yos Sudarso Kompleks Tunjung Nyaho, Palangka Raya, Kalimantan Tengah 73112.  
E-mail: tri.s.w.basuki@gmail.com

Kalimantan, and Malaysia. This species producing the first NTFPs (*Non Timber Forest Products*) based on Minister of Forestry Decree No. 21/2009. NTFPs are regarded as a means of subsistence and an income generation resource for people living in or near forests, and are thought to reduce the depletion of natural tropical forests by humans (Donovan and Puri, 2004). NTFPs are obtained from forest resources, including resins, latex, bark, roots, seeds, flowers, fruits, leaves, mushrooms and other nonwood plant parts. Valuable latex is harvested from the stem of *D. lowii*, that is used in chewing gum. These wood are preferred for making all component acoustic instruments of violin and guitar except for the top plate of violin (Sedik *et al.*, 2010). In addition, this species is categorized as vulnerable (VU A1cd) by IUCN or *International Union for Conservation of Nature* (2010). Despite its great economic value, its ecological functions and conservation in peat swamp forest, environmental importance of the species, the level of risk, this endemic tree species has been poorly studied in regards genetic diversity across its distribution.

The existence of diversity is the main prerequisite of the capability of a living system to adapt the changes of environment on all hierarchical levels of biological organization from a population through a community to an ecosystem (Vandergast *et al.*, 2008). However, in consideration of the future restoration and long-term survival of this vulnerable species in the wild, conservation strategies and policies are further required to help effectively management on remaining and transplanted individuals in order to

maintain their genetic basis and evolutionary potentials.

Allozyme variations, which have been widely applied to examine population genetic variations since the late 1960s, are still powerful in current population genetic analyses. They are almost invariably codominant, simple and despite being traditionally recognized as markers of neutral differences. Thus, the variants are potentially useful for plant and animal conservation and management applications to understand the genetic diversity of populations that are locally adapted (Ridgway, 2005). The amount and pattern of genetic diversity is needed to develop conservation strategies. In the present study, allozyme variations were used to detect the genetic diversity and differentiation of five *D. lowii* populations in Central Kalimantan. With the genetic information obtained, the possible management of these populations could enhance the effective management of the transplanted populations and individuals of *D. lowii*.

## Material and Methods

### *Plant material and extraction.*

Geographical locations of *D. lowii* sampled population (Table 1). Leaf samples of *D. lowii* were collected from five populations representing to Central Kalimantan *i.e.* four populations of natural peat swamp forest of Hampangen, Parahangan, Sebangau, Selat Nusa and one plantation population on Palangka Raya (Fig. 1). Sample trees were chosen at random and separated by a minimum distance of 15 m. The strategy for sampling trees involved the collection of single individuals for each population

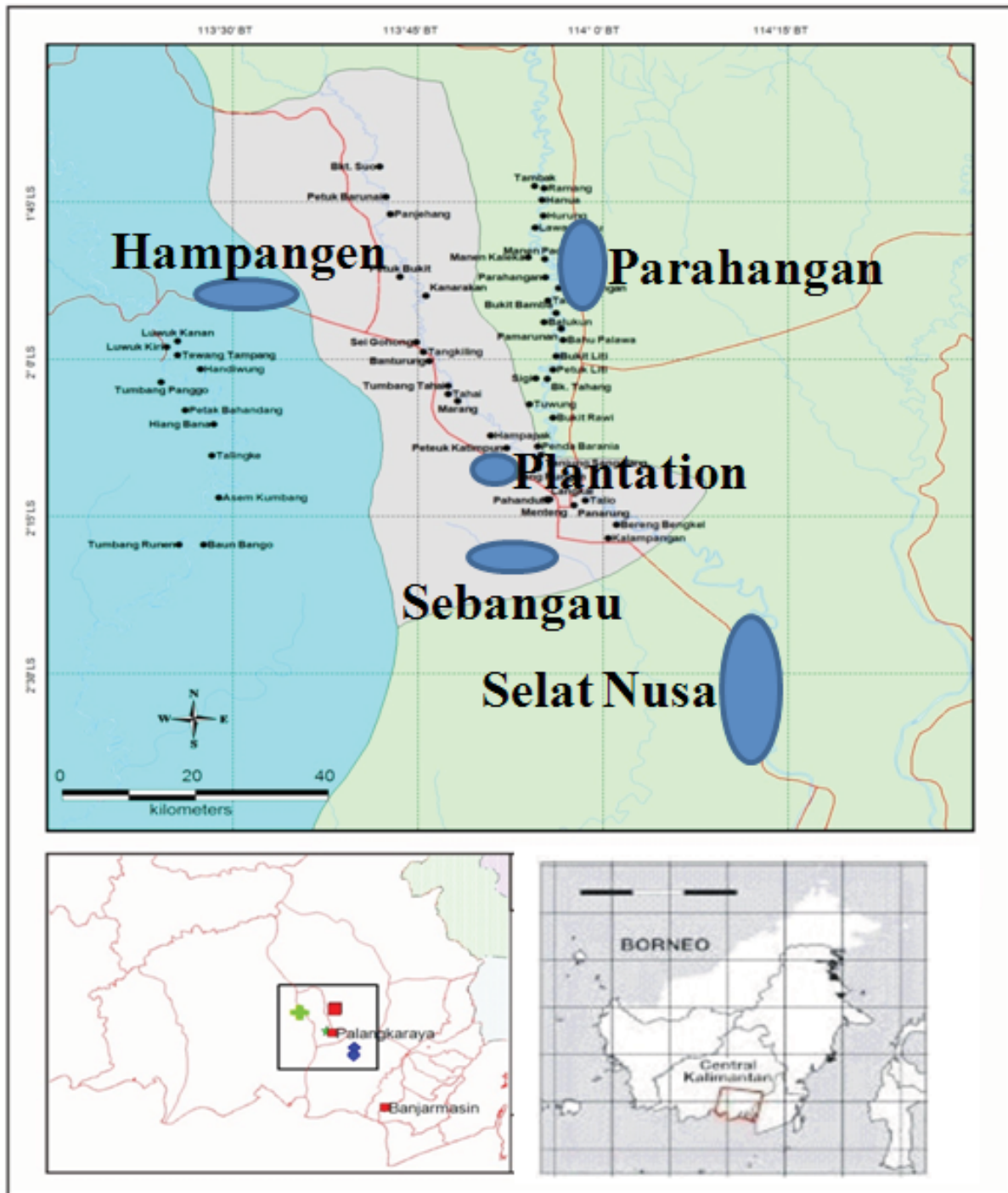
Table 1. Geographical location of *D. lowii* sampled populations

Population	Number of samples	Latitude	Longitude	Altitude (m)
Hampangen	55	1° 52'0" - 1°54'0"	113°28'0" - 113°32'0"	34-60
Parahangan	54	1° 50'40" - 1°50'50"	113°57'25" - 113°57'25"	31-52
Sebangau	55	2° 18'15" - 2°19'02"	113°50'0" - 113°54'35"	12-51
Selat Nusa	54	2° 25'41" - 2°32'55"	114°11'31" - 114°12'09"	10-25
Plantation	54	2° 9'44" - 2°9'51"	113°51'16" - 113°51'20"	60-61

categories. In total, leaf material from 266 individuals were assessed, 211 individuals from natural forest and 55 individuals from plantation.

*Allozyme electrophoresis*

Electrophoresis procedures were conducted with an isoelectric focusing polyacrylamide slab gel system following



**Figure 1.** Location of *D. lowii* Hook.f. sampled populations within the distribution of the species in Central Kalimantan.

the procedure of Wendel and Weeden (1989). Electrophoresis of enzymes extracted from young leaf of individual plants was carried out in vertical plates of 7.5% *polyacrilamide* gel. The conditions of extraction, electrophoretic fractioning, histochemical staining of enzymes, allele identification and nomenclature. The following seven enzymatic systems were used as markers in the given investigations: *Shikimate dehydrogenase* (SHD; EC 1.1.1.25), *NAD(P)H-Diaphorase* (DIA; EC 1.6.2.2), *Glutamate oxaloacetate transaminase* (GOT; EC 1.11.1.7), *Acid phosphatase* (ACP; EC 3.1.3.2), *6-Phosphogluconate dehydrogenase* (6-PG; EC 1.1.1.44), *Esterase* (EST; EC 3.1.1), dan *Peroxidase* (POD; EC 1.11.1.7).

Enzymes were extracted from the young leaves of each plant on an ice plate. Each enzyme, gene loci and alleles were inferred and labeled following numerical and alphabetical sequence, respectively. Electrophoretic isozyme phenotypes (hereafter zymograms) were genetically interpreted as one-banded homozygotes or multiple-banded heterozygote.

### Data analysis

Every enzyme system in each gel was observed to determine the related allele based on the banding pattern and to calculate the relative value to bromophenol blue front ( $R_f$  value). The alleles were inferred from the observed banding patterns and constructed as diploid genotypes.

POPGENE version 1.31 (Yeh *et al.*, 1999) was used to calculate the standard measure of genetic diversity, including allele frequency of each locus, number of alleles per locus ( $A_n$ ), number of effective alleles per locus ( $A_e$ ), percentage of polymorphic loci ( $PPL$ ), observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ).

The measure of population genetic differentiation was analyzed using the standard method of the estimation of Nei's (Nei, 1987),  $F_{ST}$ , using POPGENE program.  $F_{ST}$  is often designated as the latter for ease of discussion. UPGMA dendrogram of

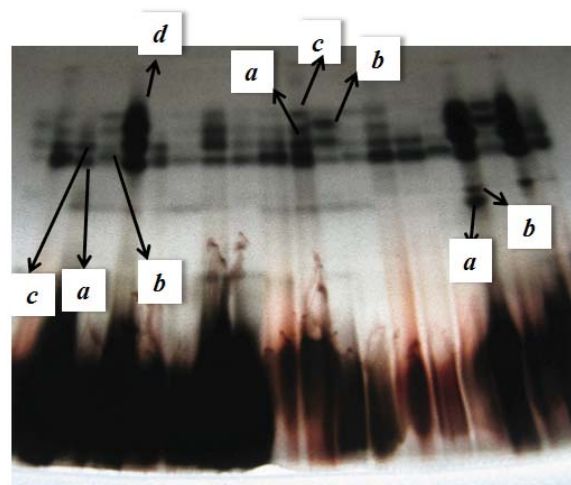
relationships between populations based on the allele frequency data and Nei's standard genetic distances (Nei, 1972) was constructed with POPGENE program.

## Results and Discussion

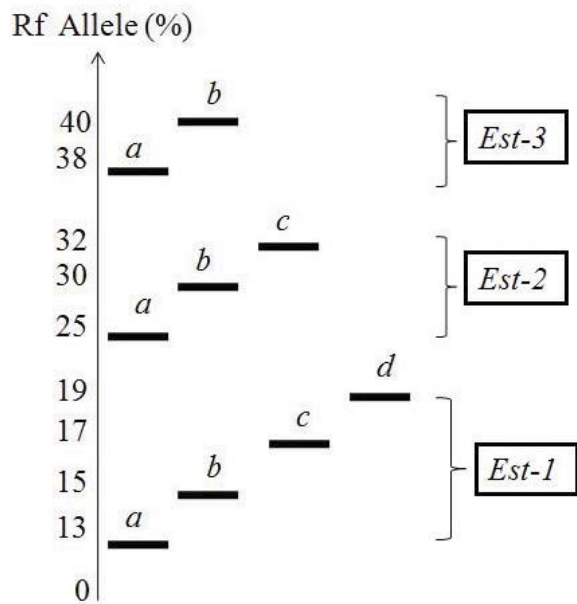
### Genetic variation

Seven enzymatic systems were used as markers in the given investigations: EST, GOT, SHD, DIA, 6-PG, ACP, and POD. The banding pattern of DIA, SHD, and POD showed monomorphisms. Enzyme systems of 6-PG and ACP were polymorphic, resolved into no clear and no consistent while the banding pattern of EST and GOT showed polymorphisms, resolved into clear and consistent (Fig. 2 and 4). The seven enzyme systems prescreened, two resolved into clear and consistent banding patterns and were used for genetic analysis are were EST and GOT.

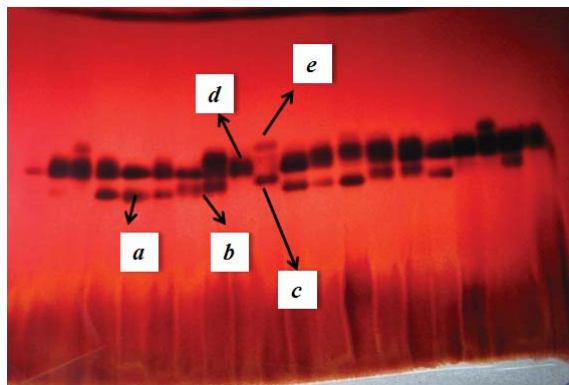
The examples of banding patterns EST can be shown in Fig. 2 and GOT in Fig. 4. A total of 14 alleles were found in all population, except Sebangau population. Based on  $R_f$  length percentage banding pattern of EST were distributed into three loci as follows locus *Est-1* (four alleles), locus *Est-2* (three alleles), and locus *Est-3* (two alleles) (Fig. 3). The  $R_f$  length percentage banding pattern of GOT are showed only one locus (*Got-1*)



**Figure 2.** Examples of banding patterns of EST resulted by Electrophoresis procedures on *D. lowii*.



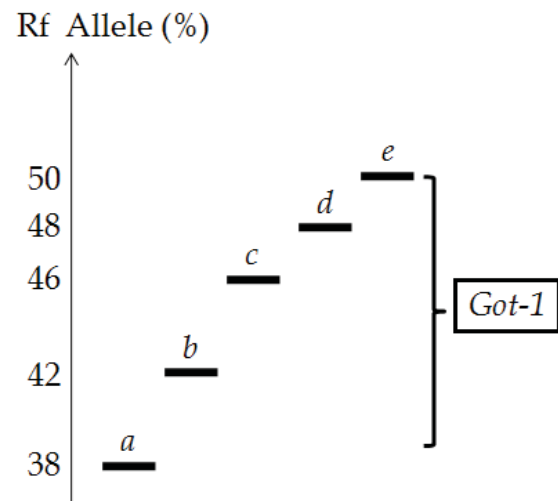
**Figure 3.** Allele distribution of locus of *Est-1*, *Est-2*, and *Est-3*.



**Figure 4.** Examples of banding patterns of GOT resulted by Electrophoresis procedures on *D. lowii*.

consisted of five alleles (Fig. 5).

Distribution of allele frequencies can be shown in Table 2. The population of Sebangau exhibited the rare allele *Est-1-a* (0.011) and *Est-1-d* (0.011). Populations of Selat Nusa, Sebangau and plantation have different common allele of locus *Est-3* (*Est-3-a* allele) in comparison with Hampangen population (*Est-3-b* allele). The alleles of *Est-2-b* and *Got-1-a* were found in all populations except of Sebangau population. Population of Selat Nusa was exhibited the presence of



**Figure 5.** Allele distribution of locus of *Got-1*.

14 allele. Populations exhibited the presence of the private allele *Got-1-e* was Selat Nusa population (0.093).

The value percentage of locus polymorphic shown 100% in all populations. Mean value of  $A_e$ ,  $H_o$  and  $H_E$  were 2.202 (1.784-2.503), 0.634 (0.532-0.739) and 0.521 (0.422-0.596), respectively (Table 3). Mean observed heterozygosity ( $H_o$ ) was higher than mean expected heterozygosity ( $H_E$ ) in all populations. The Parahangan population showed greater genetic diversity ( $A_e=2.503$ ,  $H_E=0.739$ ,  $H_o=0.596$ ) than Selat Nusa population ( $A_e=2.439$ ,  $H_E=0.721$ ,  $H_o=0.573$ ). Nevertheless, Parahangan population exhibited lower number allelic per loci (3.250) than Selat Nusa population (3.500), but Selat Nusa population has the private allele *Got-1-e*. So that, Selat Nusa was the center genetic diversity of *D. lowii*.

As an endemic species in peat swamp forest, *D. lowii* is characterized by high levels of allozyme diversity at both the species and population levels, expected heterozygosity ( $H_E=0.521$ ), number of alleles per locus ( $A_e=3.206$ ) and mean percentage of polymorphic loci ( $PP=100\%$ ) at the species level (Table 3) were all higher than those reported for other endemic plant species

**Table 2.** Distribution of allele frequencies of four polymorphic loci from five *D. lowii* populations.

Locus	allele	Population				
		Hampangen	Parahangan	Sebangau	Selat Nusa	Plantation
<i>Est-1</i>		n = 54	n = 53	n = 49	n = 53	n = 53
	<i>a</i>	0.075	0.047	0.011	0.217	0.217
	<i>b</i>	0.648	0.415	0.678	0.396	0.377
	<i>c</i>	0.204	0.377	0.3	0.274	0.274
<i>Est-2</i>	<i>d</i>	0.083	0.161	0.011	0.113	0.132
		n = 55	n = 54	n = 49	n = 54	n = 54
	<i>a</i>	0.546	0.417	0.582	0.417	0.417
	<i>b</i>	0.027	0.111	-	0.083	0.083
<i>Est-3</i>	<i>c</i>	0.427	0.472	0.418	0.5	0.5
		n = 53	n = 54	n = 49	n = 54	n = 54
	<i>a</i>	0.142	0.407	0.653	0.667	0.657
<i>Got-1</i>	<i>b</i>	0.858	0.593	0.347	0.333	0.343
		n = 55	n = 54	n = 55	n = 54	n = 54
<i>Got-1</i>	<i>a</i>	0.036	0.148	-	0.018	0.009
	<i>b</i>	0.082	0.222	0.163	0.176	0.074
	<i>c</i>	0.109	0.093	0.283	0.074	0.083
	<i>d</i>	0.773	0.537	0.554	0.639	0.834
	<i>e</i>	-	-	-	<b>0.093</b>	-

$H_E = 0.138$ ,  $A_a = 1.76$  and  $PP = 48.1\%$  (Hamrick and Godt, 1989, 1996a) and *Instia bijuga* ( $H_E = 0.409$ ,  $A_a = 2.49$ , and  $PP = 96.4\%$ ) using isozyme (Mahfudz, 2011).

The values of expected heterozygosity of *D. lowii* ( $H_E = 0.521$ ) was higher than the endemic species level in peat swamp forest of *Gonystylus bancanus* ( $H_E = 0.1894$ ) using RAPD (Kusumadewi *et al.*, 2010) and other tropical forest i.e *Alstonia* sp. ( $H_E = 0.247$ ) using RAPD (Hartati *et al.*, 2007), and *Shorea leprosula* ( $H_E = 0.406$ ) using isozyme (Lee *et al.*, 2000; Finkeldey, 2007). The  $H_E$  value of *D. lowii* was lower than *Hevea brasiliensis* ( $H_E = 0.742$ ) (Le Guen *et al.*, 2010) and a threatened tree *Dalbergia nigra* ( $H_E = 0.735$ ) using microsatellite (Resende *et al.*, 2011).

Therefore, contrary to conventional expectations of lower genetic diversity levels for endemic species, the present results support the hypothesis that endemic plant species do not always harbor less total allozyme variation than more widespread species, as concluded in some recent studies (e.g., Delgado *et al.*, 1999; Gonzales-Astorga and Castillo-Campos, 2004; Torres-Diaz *et al.*, 2007). High genetic diversity in endemic

species is commonly associated with unique species characteristics of life history, breeding system, and mode of reproduction (Hamrick and Godt, 1996a, 1996b). High levels of genetic variation are important because they provide the ability for tree species to adjust to new environments, such as the shifting climate and more variable weather conditions caused by elevated  $CO_2$  levels and global warming, allowing local adaptation and the migration of better-suited provenances along ecological gradients (Bawa and Dayanandan 1998; Atta-Krah *et al.*, 2004; Williams *et al.*, 2007; Aitken *et al.*, 2008; Dawson *et al.*, 2009).

*D. lowii* is a long-lived woody plant, high seed production, the seeds have a wing, all morphological adaptations to wind dispersal. How effective this can be when on many occasions having found seeds on the ground, we have subsequently been unable to find the parent plant, suggesting the plant might be quite far away (Middleton, 2007). All of these characteristics are probably responsible for the high genetic diversity retained in *D. lowii* populations. Therefore, long-term adaptive evolution in highly heterogeneous in peat swamp forest possibly contributes greatly

**Table 3.** Descriptive summary of the genetic statistics of five populations of *D. lowii* in Central Kalimantan.  $A_n$ , allele numbers per locus;  $A_e$ , effective number of allele per locus;  $P$ , percentage polymorphic loci;  $H_o$ , observed heterozygosity;  $H_e$ , expected heterozygosity.

Population	$A_n$	$A_e$	$PPL$	$H_o$	$H_e$
Hampangan	3.250	1.784	100	0.532	0.422
Parahangan	3.250	2.503	100	0.739	0.596
Sebangau	2.750	2.003	100	0.533	0.499
Selat Nusa	3.500	2.439	100	0.721	0.573
Plantation	3.250	2.276	100	0.642	0.513
Mean	3.206	2.202	100	0.634	0.521

to the high level of allozyme variations of *D. lowii* populations detected in the present study.

### Differentiation and Genetic Structure

Investigation of the fixation index ( $F_{IS}$ ) showed significantly negative values -0.228 (Table 4), suggesting significant excesses of heterozygotes in all populations. It might be due to assortative mating and selection (Yeh, 2000; Lowe *et al.*, 2004). The  $F_{IS}$  value is similar to *Instia bijuga* (-0.224) using isozyme (Mahfudz, 2011). The  $F_{IS}$  value was higher than of a Neotropical Forest Pioneer Tree *Vochysia ferruginea* in the secondary forest populations observed at Tirimbina ( $F_{IS} = -0.136$ ) and Ladrillera ( $F_{IS} = -0.157$ ) (Davies *et al.*, 2010).  $F_{IT}$  showed the relative individual inbreeding significantly negative value (-0.143) in all loci (Table 4). The  $F_{IT}$  value is similar to *Instia bijuga* (-0.136) using isozyme (Mahfudz, 2011).

$Nm = \text{Gene flow estimated from } F_{ST} = 0.25(1 - F_{ST})/F_{ST}$

Genetic differentiation between populations was evaluated using  $F_{ST}$  (Wright,

1951), and its significance of deviation from zero was tested based on 1,000 permutations. The value of  $F_{ST}$  was moderately differentiated ( $F_{ST} = 0.068$ ), shown in Table 4. It means, most allozyme diversity was found within population (93.2%) and among population (6.85%), indicating that high genetic diversity is maintained within rather than among populations.

Based on allozyme genetic data regarding seed plants, Hamrick and Godt (1996b) showed that wind-pollinated and outcrossing species usually have low values of  $G_{ST}$  values (approx. 0.094). In the present study, the population genetic differentiation of *D. lowii* indicated moderately differentiated ( $F_{ST} = 0.068$ ) shown in Table 4. A wind-pollinated and long-lived woody species usually have weak genetic differentiation. In wind-pollinated, long-lived woods, the average  $F_{ST}$  values tend to be low (Duminil *et al.*, 2009),  $F_{ST} = 0.028$  in *Cryptomeria Japonica* (Takahashi *et al.*, 2005), the average  $G_{ST} = 0.073$  in Gymnosperms (Hamrick *et al.*, 1992). The moderately level was similar to seedling population of *Vochysia Ferruginea* ( $F_{ST} = 0.069$ ) at Ladrillera (Davies *et al.*, 2010) and a threatened tree *Dalbergia nigra* ( $F_{ST} = 0.088$ ) in the Brazilian Atlantic Forest (Resende *et al.*, 2011). These values are within the range of differentiation found in other tropical tree populations, where low differentiation was found for *Dinizia excelsa* ( $F_{ST} = 0.00167$ ) (Dick *et al.*, 2003a) and high levels of differentiation have been found for *Caryocar brasiliense* ( $F_{ST} = 0.29$ ) (Collevatti *et al.*, 2001), *Taxodium distichum* shown strong genetic differentiation

**Table 4.** Value of  $F_{IS}$ ,  $F_{IT}$ ,  $F_{ST}$  and  $N_m$  on loci.

Locus	Sample Size	$F_{IS}$	$F_{IT}$	$F_{ST}$	$N_m$ *
EST-1	528	0.247	0.375	0.17	1.217
EST-2	532	-0.701	-0.676	0.014	16.682
EST-3	516	-0.106	-0.053	0.048	4.975
GOT	526	-0.252	-0.187	0.052	4.535
Mean	526	-0.227	-0.143	0.068	3.416

$N_m = \text{Gene flow estimated from } F_{ST} = 0.25(1 - F_{ST})/F_{ST}$

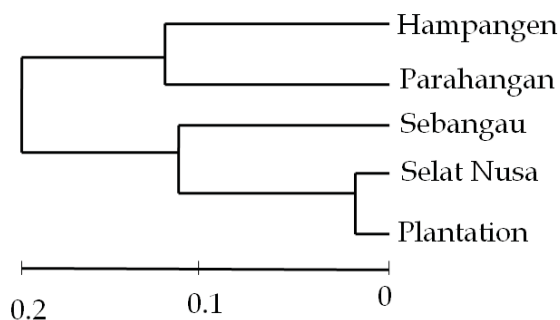
**Table 5.** Genetic distance Nei's Unbiased Measures Genetic Distance (Nei, 1987).

Population	Hampangen	Parahangan	Sebangau	Selat Nusa	Plantation
Hampangen	****				
Parahangan	0.071	****			
Sebangau	0.149	0.081	****		
Selat Nusa	<b>0.175</b>	0.059	0.055	****	
Plantation	0.161	0.076	0.077	<b>0.008</b>	****

between populations  $F_{ST}=0.208$  (Tanaka *et al.*, 2012), and *Gonystylus bancanus* ( $G_{ST}= 0.9956$ ) (Kusumadewi *et al.*, 2010).

The moderately differentiated among population of *D. lowii* could be caused of gene flow process. Gene flow could be influenced by tree individual self, i.e mating system, landscape and habitat (Lowe *et al.*, 2004). The estimation of gene flow ( $N_m$ ) was 3.402 (Table 4), resulting from  $F_{ST}$  values suggested a high level of gene exchange among the four natural populations and one plantation.

The values of pairwise genetic distances shown in Table 5. The highest of genetic distances was observed between the populations of Hampangen and Selat Nusa (0.175) with the highest distance ( $\pm 92$ km). The smallest of genetic distances was observed between the populations of Selat Nusa and Forest plantation (0.008). Clustering of populations was mainly associated with their location. As shown in Figure 6, populations of Hampangen and Parahangan conformed a cluster by genetic distance of 0.071. Base on observations, the distance of both population 50 Km and continuous populations. The result found in Sebangau showed as the groupe of

**Figure 6.** Dendrogram based Nei's (1987) genetic distance

Selat Nusa and plantation because to find out higher of allele frequencies ( $A_e$ ) of *Est-3-a* than *Est-3-b*, and contrary the population of Hampangen and Parahangan (Table 2), the distance between Sebangau and Selat Nusa was 40 km and the genetic distance was 0.555. The populations of Parahangan and Selat Nusa have a same stream of Kahayan river by distance of 80 km between them. Parahangan and Selat Nusa populations have a genetic distance (0.059) which is lower than the genetic distance between Selat Nusa and Hampangen population (0.175) which is not the river flow. Population of Selat Nusa and plantation conformed a cluster. Nevertheless, seeds were planted as plantation were not from Selat Nusa population, but these were from Sampit population (Suyitno, 2013; personal communication).

This was further confirmed from the high gene flow ( $Nm$ ) of 3.402. Moreover if  $Nm < 1$ , then local populations tend to differentiate; if  $Nm \geq 1$ , then there will be little differentiation among populations and migration is more important than genetic drift (Wright, 1951). In accordance with genetic structure results, estimates of contemporary gene flow based on assignment tests suggested that genetic connectivity among populations is still high. Consequences of landscape alterations in plants are correlated with their life history characteristics (Sork and Smouse 2006; Aguilar *et al.* 2008), especially their mechanisms of seed and pollen dispersal. Seeds of *D. lowii* are wind-dispersed (Middleton, 2007) and it has been reported that native bees are the main pollinator (Wasis; personal communication). As expected by the species dispersal agents, pollen contribution to historical gene flow was much higher than



seed contribution. Parentage assignments in tropical trees have demonstrated that insect pollen dispersal range is often greater than traditional estimates have shown, with mean pollination distances of hundreds of meters and maximum distances reaching up to 14 km (Nason *et al.*, 1998; Hardesty *et al.*, 2006; Hanson *et al.*, 2008). In addition, some studies described an increase in recent gene flow among fragments in the insect pollinated trees of *Dinizia excelsa* (Dick, 2001), *Swietenia humilis* (White *et al.*, 2002) and *Dipteryx panamensis* (Hanson *et al.*, 2008). Increasing gene flow in *D. lowii* could also be determined by long-distance seed dispersal, as reported for a wind-dispersed species (Bacles *et al.*, 2006).

Hughes *et al.* (1994) concluded that seeds larger than 100 mg tend to be adapted for dispersal by vertebrates, while those smaller than 0.1 mg tend to be dispersed unassisted. Most seeds, however, are between 0.1 and 100 mg; in this range all dispersal modes are feasible. *D. lowii* have dehiscent fruits and wind blown seed with weight of about 1-10 mg. Nevertheless, the success of effective long-distance seed dispersal are variable depending on other ecological and physiological conditions. Typically, the wind-dispersal seeds of *D. lowii* individuals usually settle within 50 m or may be prevalent 300-400 m from the parent plants (Handadhari, 2004). These characteristics are important contributors to reduce gene flow among populations, as evidenced by the significant by distance pattern occurring in peat swamp forest.

### ***Implications for conservation***

The results obtained in this study appear to be encouraging regarding the survival of *D. lowii*. The species still harbors high genetic diversity and gene flow seems to be effective. Thus, the recent vulnerable of some populations because of human-mediated activities could be counterbalanced by gene exchange among remnant populations or oil-palm plantations. In addition, Indonesia

is the world's largest palm-oil producer, and it is predicted that a further 6Mha of (primarily forested) land will be converted to oil-palm plantations by 2020, with half of this on peatland (Hooijer *et al.*, 2006).

Conservation of *D. lowii* with rehabilitation of the peat swamp forest in Central Kalimantan is supported by Minister of Forestry Decree No. P. 55/Menhut-II/2008. It aimed to rehabilitate and to conserve the development peatland area in Central Kalimantan by 2007-2017, i.e. activity restoration community structure and forest vegetation, activity of ecosystem revitalisation and forest function as Enrichment planting i.e., *D. lowii*, *Shorea belangeran*, *Gonystylus bancanus* replanted along the edge primary trunk ex MRP (Mega Rice Project). In addition of the policies of Presidential Decree No. 3/2012: conservation of biodiversity of plants endemic areas and rehabilitation of degraded areas of protected works. On other hand, low-intensity logging which retains some proportion of reproductive trees, followed by activity that accelerate natural or silviculturally induced *D. lowii* regeneration in peat swamp forest and plantation, may represent the best strategy for combining conservation and economic use of *D. lowii* with increasing human pressure. These activities support sustainability of high genetic variation of *D. lowii*.

Nevertheless, land use in this region has intensified over the years, and the absence of adequate habitat for recolonization is potentially hazardous in the long term. Our allozyme analysis based on five populations have the moderately level coefficient of genetic differentiation among population and the fixation index ( $F_{IS}$ ) showed significantly negative values (-0.2288), suggesting significant excesses of heterozygosity in all populations. The value  $Nm$  (3.402)  $\geq 1$ , then there will be little differentiation among populations and migration is more important than genetic drift (Wright, 1951). Estimates from the  $F_{ST}$  value indicated that five populations would adequately represent

93.2% of the genetic diversity attributable to most common alleles. Based on genetic diversity of the others, and degree of threat, priority populations to monitor could be Sebangau population because the alleles absence of *Est-2-b* and *Got-1-a*, the rare allele *Est-1-a* (1%) and *Est-1-d* (1%). In addition, Hampangen population has the lowest level of genetic diversity.

The conservation efforts of *D. lowii* must be done so that no such species that already have low genetic diversity i.e  $H_E = 0.17$  of the threatened tree daisy *Olearia gardneri* (Barnoud and Housliston, 2010),  $H_E = 0.183$  of the endemic and vulnerable *Vellozia gigantea* (Lousada *et al.*, 2011),  $H_E = 0.12-0.14$  of *Polylepis multijuga* (Quinteros-Casaverde *et al.*, 2012) and *Polylepis incana* ( $H_E = 0.13-0.17$ ) currently classified as vulnerable (Hensen *et al.*, 2011),  $H_E = 0.023$  of the vulnerable Bankouale' Palm *Livistona carinensis* (Shapcott *et al.*, 2009), and  $H_E = 0.189$  of *Gonystylus bancanus* (Kusumadewi *et al.*, 2010).

To promote programs for the conservation of vulnerable species, the over all genetic basis of its natural populations must be examined when formulating correct management decisions. Given that (1) there is still substantial genetic diversity in *D. lowii*, (2) According to our results, special attention needs to be given to conserve a private allele *Got-1-e* at Selat Nusa. The Parahangan population of greater genetic diversity was found ( $A_e = 2.50$ ,  $H_E = 0.73$ ,  $H_O = 0.59$ ) than Selat Nusa population ( $A_e = 2.44$ ,  $H_E = 0.72$ ,  $H_O = 0.57$ ). Nevertheless, Parahangan population exhibited lower number allelic per loci (3.25) than Selat Nusa population (3.50). So that, Selat Nusa population was the center genetic diversity of *D. lowii*. This population expected to enhance the effective management for genetic resources conservation in the future.

In conclusion, *D. lowii* in Central Kalimantan showed high genetic diversity and ranged  $H_E$  0,422 (Hampangen population) up to 0,596 (Parahangan population). Most allozyme variation was found within population (93.2%). Special attention is

essential to conserve a private allele of *Got-1-e* (9%) at Selat Nusa population. Sebangau population missed the alleles of *Est-2-b* and *Got-1-a*, as found in other populations.

To forward for conservation of genetic resources from five populations studied were quite taken only one population is a Selat Nusa population. This population is expected to enhance the effective management for genetic resources conservation of this vulnerable species in the future.

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