

Ecological aspects and genetic diversity of *Shorea balangeran* in two forest types of Muara Kendawangan Nature Reserve, West Kalimantan, Indonesia

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Abstract. Indriani F, Siregar U, Matra DD, Siregar IZ. 2019. Ecological aspects and genetic diversity of *Shorea balangeran* in two forest types of Muara Kendawangan Nature Reserve, West Kalimantan, Indonesia. *Biodiversitas* 20: 482-488. *Shorea balangeran* (balangeran) belongs to the Dipterocarpaceae family that is distributed naturally in Indonesia with a wide range of ecological conditions including peat swamp and heath forests. Good adaptability of *S. balangeran* remains unknown concerning molecular (genetic) responses that are important to provide scientific knowledge for supporting forest restoration, especially for peatland restoration efforts. The main objective of this research was to analyze the ecological aspects and genetic diversity of *S. balangeran* in two types of forests, namely: peat forests (wetland) and heath forests (dry land). Field research was conducted in Muara Kendawangan Nature Reserve, West Kalimantan following standard vegetation analysis by establishing purposively sample plots (50 m x 50 m). Genetic diversity was assessed by RAPD analysis using ten random polymorphic primers. Results showed that both in heath and peat forests, *S. balangeran* was dominant, but only at pole and tree stages indicating that poor regeneration capacity of *S. balangeran*. This finding also confirmed the critically endangered status of *S. balangeran* as listed in the IUCN Red List of Threatened Species. Genetic analysis using RAPD did not show a clustering of *S. balangeran* between peat forest and heath forest populations, indicating that *S. balangeran* has good adaptability to grow in both forest types.

Keywords: Adaptability, *Shorea balangeran*, heath forest, Muara Kendawangan, peat forest

INTRODUCTION

Shorea balangeran (Korth.) Burk, also known as balangeran, belongs to the Dipterocarpaceae family that is distributed naturally in Indonesia, especially in the peat swamp forests. However, the existence of *S. balangeran* in their natural habitat has been threatened due to illegal logging, over-exploitation, and forest conversion.

Furthermore, *S. balangeran* has been categorized as critically endangered based on the IUCN Red List of Threatened Species (Ashton 1998). *S. balangeran* has economically and ecologically high values. For economic value, the wood is used as a good material for construction as well as furniture. Besides that, bark extracts of *S. balangeran* are potential for oligostilbenoids as anti-babesial activity against *Babesia gibsoni* (Subeki et al. 2005; Tukiran et al. 2005). For ecological value, *S. balangeran* is used as a promising species for restoring degraded peatland, a major carbon sink, and mitigating the effect of global warming (Page et al. 2011). In addition, *S. balangeran* has a wide ecological range, can grow in a variety of soil and environmental conditions, has adaptability in the open vegetation and can compete with weeds (Hidayati et al. 2009). Daryono (2006) confirmed that *S. balangeran* could grow in a dry land, very deep peat

and ultisol or latosol soils. Also, *S. balangeran* is even able to grow in the burnt areas with large grasslands (*Imperata cylindrica*) in East Kalimantan, Indonesia.

In Muara Kendawangan Nature Reserve, West Kalimantan, *S. balangeran* is found in heath forest and peat forest. Muara Kendawangan Nature Reserve is one of the biggest nature reserves in West Kalimantan (Uji 2003). However, data and information of *S. balangeran* diversity and their potentials in this area have not been well investigated. Besides, this nature reserve suffered from severe damages and disturbances due to human activities such as forest fires and illegal logging. When this condition occurred continuously, then the sustainability of the ecosystem will be increasingly threatened especially for *S. balangeran* that has good adaptability and is expected to accelerate ecosystems restoration.

To understand the ability of a species to adapt to environmental changes in their habitat, the use of genetic tools such as DNA-based markers or sequences can be applied. Despite the development of DNA-based molecular marker techniques have advanced to study genetic diversity over the last decades, information concerning the molecular adaptability of *S. balangeran* to ecological condition variation is still limited. Considering the lack of genetic data of the respective species, as a first step, we used

RAPD to conduct this study. RAPD has been used because of its fast and non-requirement of DNA sequence (Harada et al. 1994). Also, to determine the adaptability of *S. balangeran*, we need to complement genetic data with relevant ecological information. Thus, this study was aimed (i) to determine structure and species composition of two types of forest in Muara Kendawangan Nature Reserve where *S. balangeran* is found and (ii) to assess genetic diversity of *S. balangeran* at two respective forest types in Muara Kendawangan Nature Reserve.

MATERIALS AND METHODS

Study area

The study was conducted at two ecosystem types, the heath forest and peat swamp forests in the Muara Kendawangan Nature Reserve, Ketapang District, West Kalimantan Province, Indonesia (Figure 1). The nature reserve covers an area of 150,000 ha in size with a rich diversity of flora and had several types of ecosystems, i.e., lowland forest, heath forest, swamp forest, and mangrove. The climate in Ketapang is tropical humid with two peaks of rainy seasons around January and December, and a dryer period during July-August (Figure 2).

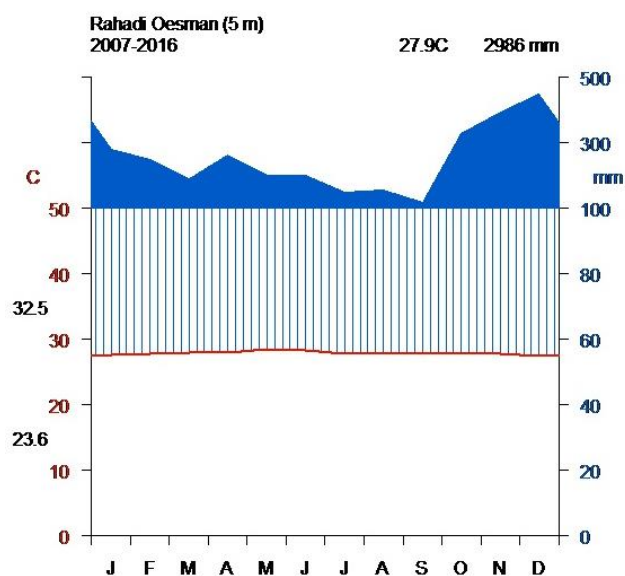


Figure 2. Average monthly temperature and rainfall at Rahadi Oesman Airport, Ketapang, West Kalimantan, Indonesia from 2007 to 2016. The relation of average monthly rainfall (solid and striped blue) to average monthly temperature (red line) illustrates Ketapang's humid climate, with mean monthly rainfall above 100 mm throughout the year

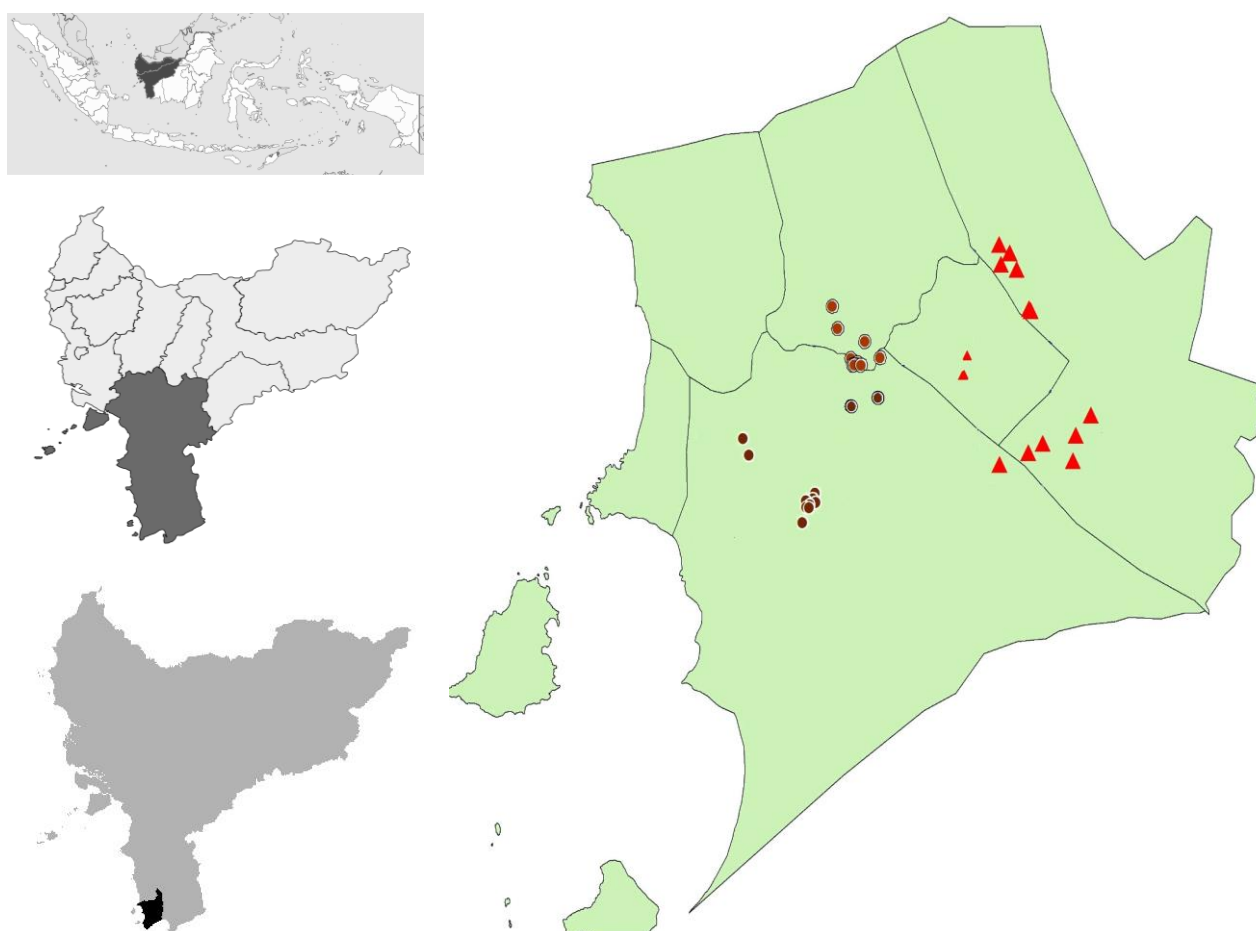


Figure 1. Location of *Shorea balangeran* populations used in this study in Muara Kendawangan Nature Reserve, Ketapang District, West Kalimantan Province, Indonesia

Procedures

Plot design

Two sites were purposively selected by the existence of *S. balangeran*. The first, four plots of 0.25 ha were established. Each plot (50 m x 50 m) (Drescher et al. 2016) was gridded into 25 subplots, each 10 m x 10 m in size as workable units (Sagar et al. 2003). Plots 10 x 10 m is used for the measurement of trees and poles, while plots of 5 m x 5 m for the saplings and seedlings.

Data analysis

The dominant and co-dominant species of each site were identified by the importance value index (IVI). IVI for trees and poles was calculated by adding relative density (RD), relative dominance (RDo) and frequency relative (RF). IVI for saplings and seedlings were calculated by adding a relative density (RD) and frequency relative (RF). These following parameters were obtained:

RD = (number of individuals of species i/total number of individuals) x 100

RDo = (total basal area for species i/total basal area of all species) x 100

RF = (frequency of species i/sum frequencies of all species) x 100

The species richness, evenness, Shanon-winner, and dispersion index were calculated for each site (2 ha sample size). Different diversity indices were calculated using the following equations of Margalef (1958) (Eq 1), Shannon-Wiener (1949) (Eq 2), Whittaker (1972) (Eq 3) and Krebs (1978) (Eq 4).

$$C = \frac{S-1}{N} \quad (1)$$

$$H' = -\sum p_i \ln p_i ; p_i = n_i/N \quad (2)$$

$$E = H'/\ln S \quad (3)$$

$$\text{Mean (M)} = \sum x_i/n ; \text{Variance (V)} = \frac{\sum x_i^2 - (\sum x_i)^2/n}{n-1} \quad (4)$$

In the above equations, C is Margalef index of species richness, S = the number of species, N=the total number of individuals. H'= the Shanon-Wiener index, p_i is the proportion of individuals found in the species i, where n_i is the number of individuals in the species i and N is the total number of individuals in the community. E is Whittaker's index of evenness. Equation 4 is Morishita index; x_i is the number of species i, n is the number of plots. If $V/M=1$, the distribution species is random, $V/M>1$ means clumped and if $V/M<1$ means uniform.

DNA extraction and RAPD analysis

Genomic DNA was extracted from 0.1 g fresh leaves with CTAB (Cetyl Trimethyl Ammonium Bromide) method (Weising et al. 2005; Aritonang et al. 2007). The quality of

Table 1. RAPD primers used in this study

Locus	Primer sequences (5'-3')	T _m (°C)
OPA-02	TGCCGAGCTG	35
OPA-03	AGTCAGCCAC	35
OPA-08	GTGACGTAGG	35
OPA-09	GGGTAACGCC	35
OPB-10	CTGCTGGGAC	35
OPC-06	GAACGGACTC	35
OPC-07	GTCCCGACGA	35
OPC-08	TGGACCGGTG	35
OPN-19	GTCCGTACTG	35
OPY-05	GGCTGCGACA	35

the DNA was checked by electrophoresis on agarose gel 1% (w/v). The analysis of RAPD amplification reactions was performed using 10 primers from an Operon Technologies to produce reproducible bands (Table 1). Amplification was performed by denaturing at 94°C for 2 min followed by 40 cycles of 1 min at 95°C, 1 min at 35°C, 2 min at 72°C and a final extension at 72°C for 7 min. The PCR products were observed in agarose gel 2%, and photographed in ultraviolet light.

Data analysis

Presence or absence of loci was scored using the software pyElph (Pavel and Vasile 2012). Then the data were analyzed using POPGENE 32 ver 1.31 software (Yeh and Yang 1999), and clustering was analyzed by STRUCTURE 2.3.4 (Pritchard et al. 2000). For this analysis, runs were performed for each set with K ranging from 1 to 5, a burn-in of 1×10^5 iterations and 1×10^5 repeat after burn-in. The best-fitted number of grouping was evaluated using ΔK by STRUCTURE HARVESTER Web v0.6.94 (Earl and von Holdt 2012).

RESULTS AND DISCUSSION

Ecological aspect

Shorea balangeran is one of the forest tree species that has potentials to be a species of choice for peat swamp forest restoration. Although *S. balangeran* naturally grow in peat swamp forest, it also can grow well in heath forest (dry land) in which this condition is similar to degraded lands characterized by low nutrient and organic matters contents. Based on Moran et al. (2000) and Metali et al. (2015) heath soil have a low concentration of nitrogen in soil solution and topsoil compared to mixed dipterocarp forest soil. Consequently, the restoration project needs a tree species of choice which can grow well in an extremely sub-optimal environment both of dry or flooded land and low or high-temperature conditions (Graham et al. 2013). However, recently the existence of *S. balangeran* is rarely found in natural forests due to illegal logging, forest fire, and forest conversion. This condition occurred obviously in Muara Kendawangan Nature Reserve.

The distribution of individuals per hectare for all species in Muara Kendawangan is presented in Figure 3. In general,

the frequency distribution showed that the regeneration population (seedling and sapling) is more than that of tree stage. However, the condition differed for number of individuals per hectare for *S. balangeran* (Figure 4). It indicated that the regeneration of *S. balangeran* species is not sufficient enough to secure the natural forest development. It also confirmed the current status of *S. balangeran* that is being critically endangered.

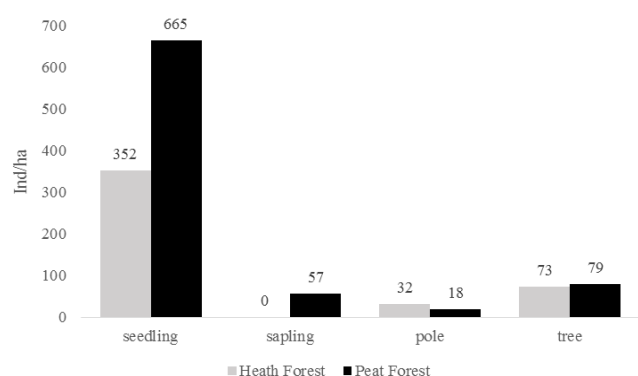


Figure 3. Total number of individuals for all species in Muara Kendawangan Nature Reserve, West Kalimantan, Indonesia

On the basis of the importance value index, the two sites differed regarding the combination of dominant species (Table 2). *S. balangeran* was dominant at heath forest and peat swamp forest at the tree and pole stages. However, at the seedling stages, *Cratoxylon arborescens* was found to be dominant, and at sapling stage, *Melaleuca cajuputi* was also dominant. In the future, should the condition has continued regarding the dominance of *C. arborescens* and *Melaleuca cajuputi*, *S. balangeran* would have reduced significantly.

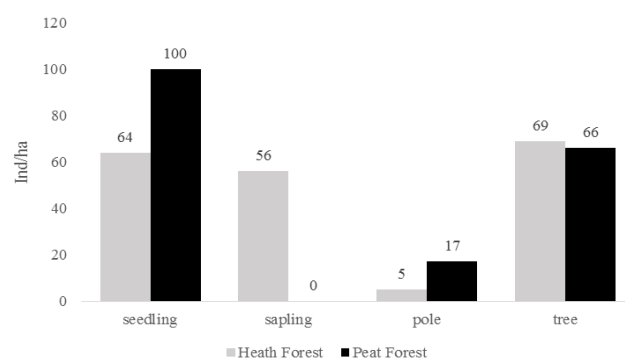


Figure 4. The number of *Shorea balangeran* in Muara Kendawangan Nature Reserve, West Kalimantan, Indonesia

Table 2. Important value index of two types forest in Muara Kendawangan Nature Reserve, West Kalimantan, Indonesia

Stage	Species	Peat swamp forest	Heath forest
Seedling	Gerunggang (<i>Cratoxylon arborescens</i>)	49.46	31.68
	Ladi (<i>Ptenandra rostrata</i>)	21.46	-
	Ubah Putih (<i>Eugenia</i> sp)	20.89	-
	Balangeran (<i>Shorea balangeran</i>)	17.48	-
	Pentapaian (<i>Haemocharis ovalis</i>)	14.37	14.19
	Pacat-pacat (<i>Harpulia arborea</i>)	-	21.69
	Puspa (<i>Schima wallichii</i>)	-	21.32
	Gelam (<i>Melaleuca cajuputi</i>)	-	20.60
Sapling	Gelam (<i>Melaleuca cajuputi</i>)	-	57.89
	Balangeran (<i>Shorea balangeran</i>)	-	45.61
	Gerunggang (<i>Cratoxylon arborescens</i>)	-	33.33
	Puspa (<i>Schima wallichii</i>)	-	31.58
	Jambu hutan (<i>Bellucia axinanthera</i>)	-	8.77
Pole	Balangeran (<i>Shorea balangeran</i>)	167.08	101.13
	NN (<i>Eugenia</i> sp)	63.60	-
	Puspa (<i>Schima wallichii</i>)	39.13	27.19
	Gerunggang (<i>Cratoxylon arborescens</i>)	19.74	54.91
	Bintangur (<i>Callophyllum spp</i>)	10.45	-
	Pacat-pacat (<i>Harpulia arborea</i>)	-	29.66
	Prapat (<i>Combretocarpus rotundatus</i>)	-	26.09
Tree	Balangeran (<i>Shorea balangeran</i>)	269.33	259.92
	Puspa (<i>Schima wallichii</i>)	10.62	10.80
	Ubah Merah (<i>Eugenia</i> sp)	5.31	-
	NN (<i>Eugenia</i> sp)	4.75	4.30
	Prapat (<i>Combretocarpus rotundatus</i>)	9.99	16.58
	Pentapaian (<i>Haemocharis ovalis</i>)	-	4.23

Note: NN= No local name

Table 3 shows the growth rate of seedling, sapling, and poles in the peat forest and heath forest has a low dominance index because of a C value close to 0, while the trees have dominance index close to 1. Table 2 shows the growth rate of seedling, sapling, and pole in the peat forest and heath forest has a low dominance index because of C value close to 0, while the trees have dominance index close to 1. This indicated that there is a concentration of *S. balangeran* species on the tree stage. The diversity index of plants in the seedling stage either in the peat swamp forest and heath forest were classified as moderate ($2 < H' < 3$). Meanwhile, the diversity indices in pole and tree stage either in the peat swamp forest and heath forest were classified as low ($H' < 2$). The results show the evenness index in the peat swamp forest at all stages of the tree growth was moderate. In the heath forest, evenness index relatively low at the stage of the tree, moderate in seedlings and saplings and high on the stage of the pole.

The distribution of individual species in various forest types found in heath and peat forests was identified where *S. balangeran* and *Eugenia* sp. had the same distribution pattern in the two types of forest (Table 4). On the other hand, puspa (*Schima wallichii*) and prapat (*Combretocarpus rotundatus*) have different distribution patterns.

For analysis of species dispersion, almost all the species in this study showed no effect of different habitat on dispersal behavior. Only two species (i.e., Puspa and Prapat) showed different dispersal behavior from uniform to clumping or reverse. The uniform dispersion patterns of species largely enable the maintenance of high levels of diversity (Connell 1971). The change dispersion may reflect due to change in habitat conditions. The clumping dispersion according to Odum (1971) is common in nature, and a random distribution pattern happens when the environment is very uniform. The clumping distribution pattern of *S. balangeran* is expected due to seed dispersal.

Genetic diversity

Besides the ecological aspect, an important factor for the restoration is to consider adaptive genetic variations as expressed in the phenotype with habitat origin of *S. balangeran*. In addition, genetic diversity is the basis for forest adaptation to environmental changes. Based on the genetic results, Nei gene diversity (H_e) values for both peat forest and heath forest was considered moderate for *S. balangeran* (Table 5) compared to the other *Shorea* species studied using AFLPs (Cao et al. 2009). The breeding system in trees is one of the factors that affect genetic variation within and among the population. In general, endemic tree species and species with a narrow geographic distribution harbor less genetic variation than widespread species (Hamrick et al. 1992). But not all types of rare species have low genetic variation. Cao et al. (2009) indicated that the rare species *Shorea blumutensis* exhibited genetic diversity higher than widespread species. However, our result confirmed the statement of Hamrick et al. (1992).

The overall degree of population differentiation was low ($G_{ST} = 0.030$), this low value also has been reported for

S. leprosula in Peninsular Malaysia (Lee 2000). The low variation among populations depends on the efficiency of gene flow, a factor that usually supported by effective seed dispersal and cross-pollination. However, this is not the case in *Shorea* as it characterized by poor seed dispersal and poor pollen (Cao et al. 2009). *Shorea* species are pollinated mainly by a small insect which can migrate only over a limited distance (Appanah and Chan 1981). The seed of *Shorea* species is dispersed by wind or gravity (Takeuchi et al. 2004).

The genetic diversity revealed by RAPD has been attributed to the difference in the distribution of markers in individuals. Based on STRUCTURE analysis, the ΔK values showed a clear peak at $K=2$ (195.19). The result of this analysis showed unclear separation between *S. balangeran* populations in both peat forest and heath forests (Figure 5).

Table 3. Diversity indices of two types forest in Muara Kendawangan Nature Reserve, West Kalimantan, Indonesia

Forest	Stages	Dominance index	Diversity index	Evenness index
Peat forest	Seedling	0.145	2.361	0.403
	Sapling	-	-	-
	Pole	0.365	1.224	0.353
	Tree	0.820	0.438	0.391
Heath forest	Seedling	0.093	2.619	0.403
	Sapling	0.239	1.639	0.405
	Pole	0.167	1.981	0.685
	Tree	0.767	0.559	0.128

Table 4. Dispersion pattern of tree species

Species	Peat forest	Heath forest
Balangeran (<i>Shorea balangeran</i>)	C	C
Puspa (<i>Schima wallichii</i>)	U	C
Ubah Merah (<i>Eugenia</i> sp)	R	-
NN (<i>Eugenia</i> sp)	R	R
Prapat (<i>Combretocarpus rotundatus</i>)	U	C
Pentapaian (<i>Haemocharis ovalis</i>)	-	R
Gerunggang (<i>Cratoxylon arborescens</i>)	-	R

Note: C: clumping, U: uniform, R: random. NN= No local name

Table 5. Genetic diversity within the population of two types of forest in Muara Kendawangan Nature Reserve, West Kalimantan, Indonesia

Population	n	PPL (%)	Na	Ne	He	G _{ST}
Heath forest	16	76.79	1.77	1.15	0.12	
Peat forest	11	72.02	1.72	1.18	0.13	
Average		74.41	1.74	1.17	0.125	0.030

Note: n: number of individuals, PPL: Percentage of Polymorphic loci, Na: number of alleles observed, Ne: average number of effective alleles, He: average gene diversity index, G_{ST}: coefficient of genetic differentiation

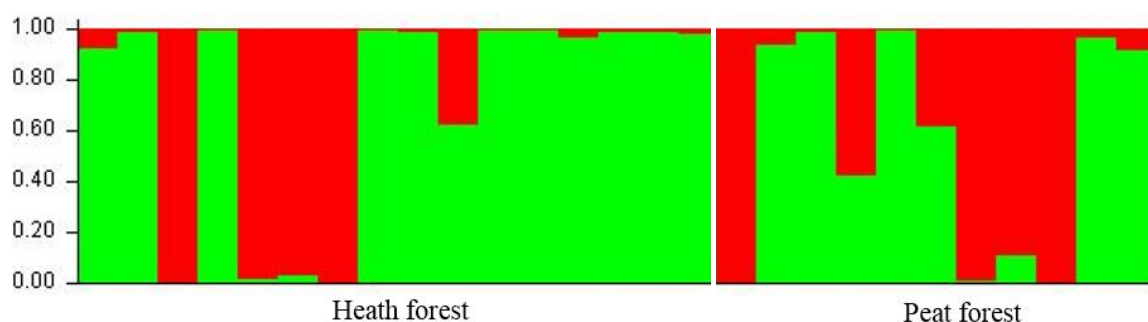


Figure 5. The result of STRUCTURE analysis from *Shorea balangeran* in peat and heath forests

Considering the habitat origin and current genetic diversity status, *S. balangeran* as previously recommended is a potential species for restoration project that may be carried out technically by enrichment plantings of degraded peatlands or through other appropriate paludiculture systems. The main problem as shown from the research findings is that the current existence of *S. balangeran* already becomes rare in natural ecosystems with moderate genetic diversity levels and therefore we suggest the management policies should consider these findings in order to maintain the appropriate population size and to protect its natural habitat. The success of this effort will have important implications for in situ conservation and sustainable utilization of *S. balangeran* genetic resources.

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