

GENETIC DIVERSITY OF SENGON (*Falcataria moluccana* (Miq.) Barneby & J.W.Grimes) REVEALED USING SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKERS

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GENETIC DIVERSITY OF SENGON (*Falcataria moluccana* (Miq.) Barneby & J.W. Grimes) REVEALED USING SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKERS. Knowledge on genetic diversity and relationship between population is important for genetic conservation and breeding program. This paper describes the use of Single Nucleotide Polymorphism (SNP) markers for genetic diversity and relationship of sengon (*Falcataria moluccana* (Miq.) Barneby & J.W. Grimes). Twelve single nucleotide polymorphism (SNP) markers which were analyzed from Candirotto Seed Orchard samples showed that the genetic diversity of the total population is relatively high ($H_e = 0.359 \pm 0.128$). Sengon from Wamena in Papua achieved the highest level of genetic diversity, while sengon from Java region was the least genetic diversity. The genetic relationship analysis showed three main clusters i.e. the first cluster consisted of Wamena and East Java, each close to Biak, and the Makian Island; the second cluster consisted of Central Java and West Java which are close to Mindanao Island; the third cluster consisted of Halmahera only. These genetic relationship information can be used to support breeding program and population genetic of sengon. For example in the collection of seed for the development of the next sengon seed orchard, it is important to minimize collecting genetic material from Java Island because the populations have low genetic diversity and there was close genetic relationship with sengon from Papua and Mindanao Island. Meanwhile, collecting genetic material for sengon from Biak and Wamena in Papua and Halmahera in Maluku could be directly and separately collected because of its high genetic diversity and cluster differences.

Keywords: Sengon (*Falcataria moluccana*), genetic diversity, genetic relationship, SNP markers

*KERAGAMAN GENETIK SENGON (*Falcataria moluccana* (Miq.) Barneby & J.W.Grimes) MENGGUNAKAN PENANDA SINGLE NUCLEOTIDE POLYMORPHISM (SNP). Pengetahuan tentang keragaman genetik dan hubungan kekerabatan antara populasi merupakan hal penting bagi konservasi genetik dan program pemuliaan pohon. Tulisan ini mempelajari penggunaan penanda SNP untuk mengetahui keragaman genetik dan hubungan kekerabatan sengon. Analisa dua belas penanda Single Nucleotide Polymorphism (SNP) pada sengon di kebun benih Candirotto Jawa Tengah menunjukkan bahwa total populasi mempunyai keragaman genetik yang tinggi ($H_e = 0.359 \pm 0.128$). Sengon dari Wamena mempunyai keragaman genetik tertinggi sementara sengon dari wilayah Jawa terkecil. Hasil analisa hubungan kekerabatan menunjukkan sengon terbagi dalam tiga cluster; yang pertama, sengon dari Jawa Timur dekat dengan Wamena, yang keduanya ter-cluster dengan Biak dan kemudian Pulau Makian; cluster kedua, Jawa Barat dan Jawa Tengah dengan Pulau Mindanao; dan terakhir Halmahera. Informasi hubungan kekerabatan ini dapat digunakan untuk mendukung program pemuliaan pohon dan populasi genetik sengon. Sebagai contoh untuk koleksi materi genetik sebagai bahan pembangunan kebun benih sengon selanjutnya perlu meminimalkan pengambilan materi genetik dari Jawa karena selain mempunyai keragaman genetik yang lebih rendah dari populasi lainnya, juga terindikasi mempunyai hubungan kekerabatan yang dekat dengan sengon dari Papua dan Mindanao. Sementara sengon dari Biak dan Wamena di Papua dan Halmahera di Maluku dapat secara langsung dikoleksi karena mempunyai keragaman genetik yang tinggi dan cluster yang berbeda.*

*Kata kunci: Sengon (*Falcataria moluccana*), keragaman genetik, hubungan kekerabatan, penanda SNP*

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I. INTRODUCTION

Sengon (*Falcataria moluccana* (Miq.) Barneby & J.W. Grimes), synonym names *Albizia falcataria* (L) Fosberg and *Paraserianthes falcataria* (L) Nielsen (Argent et al., 1996), is one of the valuable economic species in Indonesia. It has natural distribution in the Moluccas, New Guinea, the Bismarck Archipelago and the Solomon Islands (Soerianegara & Lemmens, 1994; Argent et al., 1996). It grows in primary and secondary rainforest, often on river flood terraces; on sandy soils up to 1600 m altitude (Argent et al., 1996) or up to 2300 m altitude (Soerianegara & Lemmens, 1994). It is a fast growing species often used for reforestation and afforestation or fire wood production (Soerianegara & Lemmens, 1994). The timber is used for light construction, house building and as furniture, sometimes to substitute pine wood; it is also planted as an ornamental and shade tree (Argent et al., 1996).

Knowing the genetic diversity and genetic relationship between populations is important for the genetic conservation and breeding program of sengon. Molecular genetic study using DNA markers provide an accurate method to examine genetic diversity. In Indonesia, several studies have been conducted to examine genetic diversity of sengon in natural population and plantation. Initial isoenzyme technique using one natural population in Papua and three plantations in Java (Bogor, Purworejo and Kediri) showed that the natural population of sengon in Papua has higher level of genetic diversity (0.163) than Java plantations (0.077-0.118) (Seido, Widyatmoko, & Nursinggih, 1993). Seido and Widyatmoko, 1994) also found (0.146-196) in four natural populations in Wamena, Papua. Meanwhile, a high level of genetic diversity ($H_e=0.281$) was found in a study using RAPD marker in progeny testing of sengon from Solomon in Cirangsad Experimental Forest in Jasinga Bogor (Dwiyanti, 2009).

Single nucleotide polymorphisms (SNPs) have been proposed as the new frontier for

population studies (Morin, Martien, & Taylor, 2009). SNP is a variation of a single nucleotide between individuals and these polymorphisms can therefore be used to discern small differences both within population and among different populations (Norrsgard & Schultz, 2008). SNPs offer significant advantages relative to microsatellites such as lower error rates, the ability to combine and add to data overtime and space, a simple mutation model with low homoplasy and many technologies for high genotyping efficiency (Gupta, Roy, & Prasad, 2001; Vignal, Milan, SanCristobal, & Eggen, 2002; Batley, Mogg, Edwards, O'Sullivan, & Edwards, 2003; Morin et al., 2009). SNPs have been used as markers for genetic diversity studies, genetic mapping or population structure in plants (Deulvot et al., 2010; Foster et al., 2010; Inghelandt, Melchinger, Lebreton, & Stich, 2010).

The ability of SNPs to discriminate between populations differs from that of microsatellite markers as their power comes not from the number of alleles but the large number of loci that can be assessed (Foster et al., 2010). Thus SNP markers have discriminatory power even in a low diversity species, once the rare SNPs are discovered (Foster et al., 2010). Osman et al. (2003) has also indicated that SNP markers provide more sensitive assays of genetic diversity than isozyme markers. The purpose of this paper was to examine the genetic diversity and genetic relationship of sengon population in Candiroto seed orchard, Central Java.

II. MATERIAL AND METHOD

A. Sample Collections

The Candiroto Seed Orchard in Central Java, Indonesia was established in 1994 (Susanto & Hashimoto, 1996). It consists of sengon trees collected from four regions in Indonesia: Biak and Wamena, Papua; Halmahera and Makian Island, Maluku; and West, East and Central Java, in Java; and a fourth region, Mindanao Island, Philippines (Figure 1). A total of 76 individual trees (Table 1) which are representing

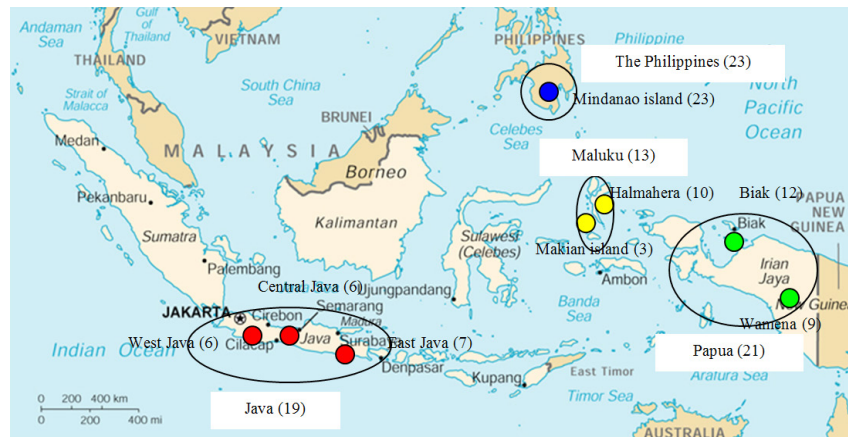


Figure 1. Sources of material of sengon (*F. moluccana*) from several regions in Indonesia and the Philippines, collected in Candirotto Seed Orchard.

Remarks : Numbers in parentheses are number of trees from each region used in this study. Big open circles define populations in Java (red), Papua (green), Maluku (yellow) and the Philippines (blue).

Table 1. Number of samples used for genetic diversity study in sengon

Region	Area	Number of samples
Papua	Biak	12
	Wamena	9
Maluku	Halmahera	10
	Makian Island	3
Java	Central Java	6
	East Java	7
	West Java	6
The Philippines	Mindanao Island	23
	Total	76

all populations in the orchard was randomly selected and used as material in this study. The number of samples for each region and population varied from 13-23 samples and 3-23 samples, respectively (Figure 1 and Table 1).

B. DNA Analysis

Twelve SNP markers were multiplexed into three sets for Single Nucleotide Primer Extension (SNUPE) analyses (Yuskianti & Shiraishi, 2010a) and were used in the study. The three sets of SNUPE analyses used in this study were obtained from an extensive search for polymorphism in 288 RAPD primers (Yuskianti, 2011). The DNA analysis was performed following the procedure by

Yuskianti and Shiraishi (2010a). The genomic DNA was isolated using the modified CTAB method (Shiraishi & Watanabe, 1995) and purified using MagneSil (Promega) following the procedure described by the supplier. The DNA was analyzed using two-step amplification procedure (Yuskianti & Shiraishi, 2010a). The first amplification used balanced multiplex SCAR primers in the SCAR reaction, and the second balanced SNP extension primers. Those procedures stand into three sets of multiplex single nucleotide primer extension (SNUPE) analyses. Both PCR products were purified using Alkaline Phosphatase and Exonuclease I. The fluorescently labelled product was then diluted with Hi-Di Formamide and LIZ-120 internal

sizing standard. DNA fragment separations were determined using a 3130 Genetic Analyzer with POP7 (Applied Biosystems) and data analysis was performed using GeneMapper v3.7 (Applied Biosystems).

C. Data Analysis

Data were scored as presence and absence of major and minor alleles (homozygous) or presence of both alleles (heterozygous) for each sample. The genetic diversity parameters were mean number of observed alleles (N_a), mean effective number of alleles (N_e), the percentage of polymorphic loci (P), observed (H_o) and expected (H_e) heterozygosity based on Nei (1973), and F-statistics were calculated using POPGENE v1.31 (Yeh, Yang, & Boyle, 1999). Gene flow (N_m) was estimated using the formula $N_m = 0.25 (1 - F_{ST}) / F_{ST}$, where F_{ST} = the degree of gene differentiation among populations. The genetic distance and genetic relationship between populations were shown using an unweighted pair-group method with arithmetic means (UPGMA) dendrogram generated using the Tree program from MEGA v.5 Software (Tamura et al., 2011).

III. RESULT AND DISCUSSION

A high percentage of polymorphic loci (P) was generally obtained ($\geq 75\%$). The mean observed number of alleles (N_a) ranged from 1.833 ± 0.389 to 2.000 ± 0.000 whereas the mean effective number of alleles (N_e) ranged from 1.374 ± 0.265 to 1.622 ± 0.296 (Table 2). In all populations, the mean observed heterozygosity (H_o) was higher than its expected heterozygosity (H_e) which measures the level of genetic diversity. At the population level, H_e varied from 0.247 ± 0.147 to 0.363 ± 0.125 . The highest level of genetic diversity was Wamena, Papua ($H_e = 0.363 \pm 0.125$), followed by Biak, Papua ($H_e = 0.338 \pm 0.127$) and Makian Island, Maluku ($H_e = 0.333 \pm 0.142$). The level of genetic diversity (H_o) in the total population was 0.359 ± 0.128 (Table 2). The higher level of observed heterozygosity (H_o) than H_e has indicated anisolate-breaking

effect (the mixing of two previously isolated populations) (McDonald, 2008) as showed in Java populations. It is also showed that the population has a system of mating in which inbreeding is avoided (Gliddon & Goudet, 1994).

The level of genetic diversity obtained by SNP markers are generally lower than SSR markers. A population structure and genetic diversity based on 1,537 elite maize inbred lines using 359 SSR and 8,8244 SNP markers found the total gene diversity was 0.69 for the SSRs and half as much (0.32) for the SNPs (Inghelandt et al., 2010). A similar result was found in 2273 accessions of domesticated grapevine analyzed using 22 common SSR and 384 SNP markers, the average genetic diversity was higher for SSR loci (0.81) than for SNPs (0.34) (Emanuelli et al., 2013).

Sengon in the total population of the Candiroto Seed Orchard was considered to have a high level of genetic diversity ($H_e = 0.359$). This level is comparable with other study using 372 SNPs in 300 representative rice inbred lines from 22 rice growing countries worldwide; the average level of genetic diversity was 0.358 (Chen et al., 2011). Several specific markers such as SCAR markers (Yuskianti & Shiraishi, 2010b), SNP markers (Yuskianti & Shiraishi, 2010a) and eight microsatellite markers (Saito, Lian, Ishio, & Ide, 2014) have been developed, however, only isoenzym and RAPD markers are the most commonly used markers for sengon studies (e.g. Suharyanto, Rimbawanto, & Isoda, 2002; Dwiyantri, 2009). The result from SNP markers that rely only on one base differences in DNA sequences provided high discrimination power; almost 100% (1.000 of Discrimination Power/DP) (Yuskianti & Shiraishi, 2010a). The low number of samples from Makian Island has limited its application for the area, however other information is important to support breeding program and population genetic studies in sengon.

The level of genetic diversity of sengon in this study was high ($H_e = 0.359$) (Table 2), higher than previous sengon studies. Previous studies

Table 2. Summary of genetic diversity in sengon (*Falcataria moluccana*) populations[#]

Region	Population	N_a	N_e	P	H_o	H_e
Papua	Biak	2.000 (0.000)	1.563 (0.304)	100	0.724 (0.104)	0.338 (0.127)
	Wamena	2.000 (0.000)	1.622 (0.296)	100	0.719 (0.166)	0.363 (0.125)
Maluku	Halmahera	1.917 (0.289)	1.523 (0.320)	91.67	0.783 (0.189)	0.314 (0.156)
	Makian Island	1.917 (0.289)	1.559 (0.310)	91.67	0.722 (0.193)	0.333 (0.142)
Java	Central Java	1.833 (0.389)	1.374 (0.265)	83.33	0.681 (0.219)	0.247 (0.147)
	East Java	1.917 (0.289)	1.525 (0.379)	91.67	0.762 (0.141)	0.306 (0.169)
	West Java	1.750 (0.452)	1.529 (0.395)	75	0.736 (0.241)	0.299 (0.199)
Mindanao Island	Mindanao Island	2.000 (0.000)	1.561 (0.347)	100	0.670 (0.153)	0.329 (0.152)
Total		2.000 (0.000)	1.619 (0.321)	100	0.715 (0.109)	0.359 (0.128)

Remarks: $^{\#}N_a$ =Mean observed number of alleles, N_e =Mean effective number of alleles, P=The percentage of polymorphic loci, H_o =Mean observed heterozygosity, H_e =Mean expected heterozygosity. Values in parentheses are standard deviations.

using isoenzym showed that H_e of five natural stands in Papua varied between 0.146 and 0.196 (Seido et al., 1993; Seido & Widyatmoko, 1994), and 0.077-0.118 for three plantations of sengon in Java (Seido et al., 1993). Furthermore, RAPD marker studies obtained H_e =0.226 for an introduced population at Kediri, East Java (Siregar, Basyuni, Sudarmonowati, & Iriantono, 1998), 0.2183 for progeny trial of sengon from Solomon in Cirangsad trial, West Java (Dwiyantri, 2009) and 0.2349 from community forest in Java (Siregar & Olivia, 2013).

Though the varying genetic diversities obtained may be caused by differences of DNA markers used, number of samples and also origin of collected materials; however, this study support previous genetic studies in sengon. Sengon in the SSO collected from Wamena, Papua had the highest level of genetic diversity (H_e =0.363) has corroborated the finding that the Wamena population is the highest levels

of genetic diversity (Seido et al., 1993). While the level of genetic diversity of sengon from Java in this study (H_e =0.247 to 0.306) (Table 2) was comparable with previous RAPD studies (Dwiyantri, 2009; Siregar et al., 1998; Siregar & Olivia, 2013).

The genetic relationship among all population shows in genetic distance data (Tabel 3) and UPGMA dendogram (Figure 2). The genetic distance analysis indicated a close genetic relationship among population of sengon in Java region and between Java and Papua. East Java and Wamena has the lowest genetic distance (0.017), followed by 0.028 for West Java and Central Java, 0.038 for East Java and Central Java, and Central Java and Wamena (Table 3). Sengon from Maluku especially Halmahera consistently showed its distinctive genetic composition with other populations (Table 3).

The genetic distance data is also supported

Table 3. Genetic distance of eight populations of sengon (*F. moluccana*)

Pop ID	Biak	Wamena	Halmahera	Makian Island	Central Java	East Java	West Java	Mindanao Island
Biak	0							
Wamena	0.018	0						
Halmahera	0.056	0.088	0					
Makian Island	0.054	0.051	0.109	0				
Central Java	0.068	0.038	0.229	0.082	0			
East Java	0,033	0.017	0.131	0.085	0.038	0		
West Java	0.086	0.047	0.204	0.093	0.028	0.057	0	
Mindanao Island	0.055	0.036	0.171	0.059	0.028	0.042	0.032	0

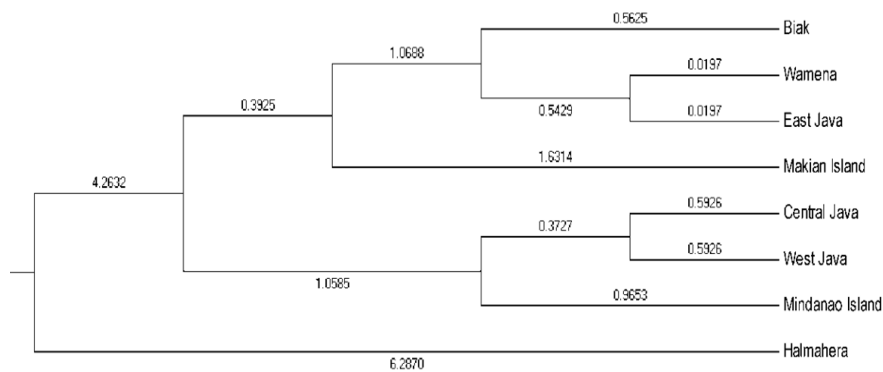


Figure 2. UPGMA dendrogram showing the genetic relationships among sengon populations

by the UPGMA dendrogram that showed different clusters between Maluku and other areas. The UPGMA showed three main clusters. The first cluster consisted of Wamena and East Java, each close to Biak, then Makian Island; the second cluster consisted of Central Java and West Java which were close to Mindanao Island; and the third cluster consisted of Halmahera only (Figure 2).

The result obtained from this study supports the previous study using isoenzyme (Seido et al., 1993). The low genetic diversity (Table 2) and low genetic distance between population in Java (Table 3) indicated the similarity of genetic composition of sengon from East, West and Central Java population. Seido et al. (1993) also found that genetic composition of Java population (Bogor, Purworejo and Kediri) were

closely related to each other and genetically very similar to one another. Both studies have clarified that sengon in Java is the result of man-made forests (plantation or community forest) and it is not part of a natural distribution of sengon in Indonesia. Sengon was firstly introduced from an area i.e. Banda Island to Bogor Botanical Garden in 1871 and spread to other parts of Java Island (Heyne, 1987).

This study also confirmed the previous study using RAPD markers that sengon in Java was closely related to the sengon from Biak and Wamena, Papua (Suharyanto et al., 2002). Our study indicates that only sengon from East Java has close genetic relationship with sengon from Wamena and Biak (Figure 2) while others have different relationships. The indication of close relationship between sengon from West

Table 4. Summary of F-statistics and Gene flow (N_m) for 12 SNP loci

Locus	Sample size	F_{IS}	F_{IT}	F_{ST}	N_m
A1	152	0.158	0.331	0.205	0.967
A2	152	-0.197	-0.095	0.085	2.692
A3	150	0.242	0.317	0.099	2.279
A4	152	-0.172	-0.120	0.044	5.481
B1	142	0.513	0.543	0.062	3.780
B2	150	-0.009	0.118	0.127	1.722
B3	150	0.205	0.367	0.204	0.975
B4	152	0.577	0.663	0.203	0.983
C1	150	-0.057	0.011	0.064	3.679
C2	152	0.181	0.226	0.055	4.3077
C3	152	0.044	0.115	0.074	3.135
C4	152	-0.160	-0.136	0.020	12.015
Mean	150	0.124	0.226	0.117	1.895

and Central Java with sengon from Mindanao, Phillipines, as this is the first time to observe, it is important to further examine. The number of samples from Java and Mindanao is limited in this study, so more studies using more number of samples to clarify this relationship is needed.

Population genetic studies provide insights into evolutionary processes, and F_{ST} , which plays a central role in ecological and evolutionary genetic studies (Willing, Dreyer, & van Oosterhout, 2012), is among the most widely used measure of genetic differentiation. Our study revealed genetic differentiation among the eight sengon population examined was moderate ($F_{ST}=0.117$) with a moderate level of gene flow for all population ($N_m=1.895$) (Table 4).

Estimation of F_{ST} and N_m can be biased if limited by the loci used. SNPs are biallelic markers (Vignal et al., 2002), so the number and type required for the greatest statistical power has been vigorously debated; ~30 SNPs is suggested to be sufficient to detect moderate ($F_{ST}=0.01$) levels of differentiation (Morin et al., 2009). Inghelant et al. (2010) proposed the use of 7 and 11 times more SNPs than SSRs for respectively analyzing population structure and genetic diversity. However population sample

size (n) can be significantly reduced ($n=4$ to 6) when using an appropriate estimator and a large number (>1,000) of bi-allelic genetic markers (Willing et al., 2012). Analysis of genetic diversity of sengon in this study is estimated to be sufficient to obtain reliable data because it is used 12 SNP markers that are proven to have almost a 100% discrimination power (Yuskianti & Shiraishi, 2010a).

This high genetic diversity of sengon in Candiroto Seed Orchard showed the succesful genetic material collection in the orchard. The orchard, however could not be further utilized because of illegal logging and pest and disease attack that limit the number of existing trees in the orchard. Though the seed orchard could not be further utilized as the information of the genetic relationship is still possible to be used in supporting tree improvement of sengon in the future. The study shows that sengon from Mindanao and Central and West Java are in one cluster, Wamena, East Java and Biak then Makian Island in different cluster while Halmahera stands alone in a separate cluster (Figure 2), brings an impact for management of seed orchards. For example in sample

collection, sengon from Halmahera (this study has strengthened the distinctive genetic relationship of sengon from Halmahera as found in RAPD study (Suharyanto et al., 2002)) and sengon from Papua (Wamena and Biak) can be separately collected. Careful collection is needed when doing collection from Java Island because sengon from different areas in Java indicated close relationship with sengon from Mindanao and Papua (Figure 2). This caution need to be considered to avoid inbreeding depression.

Other results could also be used to support population genetic studies of sengon. This study indicates that Papua and Maluku might be the center of gene (gene pool) of sengon in Indonesia. This allegation is based on the genetic diversity (Table 2) and genetic relationship (Table 3 and Figure 2). This indication is possible because the natural distribution of sengon is in the Moluccas, New Guinea, the Bismarck Archipelago and the Solomon Islands (Soerianegara & Lemmens, 1994; Argent et al., 1996).

Furthermore, there is also an indication of the presence of effective border for sengon deployment in Eastern of Indonesia. Papua and Maluku though located in close area, however both areas are surrounded by sea and rich with small islands. The presence of sea is effective to avoid spreading of sengon to other areas. This condition found in our SNP study (Figure 2) and RAPD study (Suharyanto et al., 2002) where sengon from Halmahera in Maluku has different cluster than other areas in Indonesia. The remoteness and difficulty to access the island has caused limited deployment of sengon from Halmahera to other areas. This is different with sengon from Papua that has large sample size as it is located on a big island (Figure 1) and relatively easy to access. This accessible location has caused sengon from Papua to easily spread to other areas such as to Java. Human activities seems to bring significant impact to seed transfer from Papua to other areas in Indonesia. More studies using more samples from many natural distributions therefore are

needed to understand gene flow, seed transfer and evolution of sengon in Indonesia.

IV. CONCLUSION

A high genetic diversity was found from the total population of sengon in Candiroto Seed Orchard indicating a successful collection of genetic material for seed orchard. The seed orchard could not be further utilized because of limited number of existing trees, however the genetic relationship information can be used to support tree improvement and population genetics of sengon. The genetic relationship between sengon from several areas bring an impact on, for example in sample collection. Sengon from Halmahera, Maluku, and Wamena and Biak in Papua could be separately collected while genetic material collected from Java needs a caution because of its low genetic diversity and close relationship with sengon from Papua and Mindanao. The indication of a possible gene pool and the presence of effective border for sengon deployment in eastern Indonesia was also discovered in this study. More studies using more number of samples are required to understand the evolution of sengon in Indonesia.

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