Genetic diversity of Sandalwood in Imogiri, Gunung Sewu

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Abstract. Sandalwood population decreased rapidly in nature, thus strengthen the need of re-introduction to preserve its population diversity. The re-introduction materials were taken from Gunung Sewu area, which consisted of several sandalwood landraces varied in population structures and genetic diversity. One of those landraces, which is also considered the oldest based on the herbarium investigation, was Imogiri landrace in which the oldest sandalwood specimens were dated on 1853. This research applied isoenzyme analysis to estimate genetic structure of sandalwood in several sandalwood stand differed by landscapes in Imogiri. The isoenzyme samples, a juvenile leaf, were taken with 100% IS (census method), along its natural distribution in five areas: (1) Kawasan Hutan Wisata Mangunan, (2) Bukit Panguk-Kediwung, (3) Telaga Giri, (4) Bukit Mojo Gumelem and (5) Karangtengah Village. The first to third areas are formed in cliff landscapes which are lead to Oya River. The fourth and fifth areas are located in the local private yard and are arranged in an agroforestry pattern. The fifth area, which is located near the Makam Raja-Raja Mataram, is also considered as the oldest sandalwood stand in Imogiri. A 224 samples collected consisted of 136 parent trees and 88 seedlings. There were 22 trees found bearing flowers, and as well as the seedlings, mostly were found in the private yard. Only one seedling was found in the cliff landscapes. Spatially, the highest genetic diversity (He 0.512; Ho 0.672; Fis -0.313) was found on the Karangtengah Village which is considered as the oldest sandalwood stands in Imogiri. Genetic diversity on other stands are varied (He 0.436 to 0.471; Ho 0.462 to 0.603; Fis -0.382 to 0.020). Temporally, genetic diversity is higher on the older phase (parents: He 0.483; Ho 0.539; Fis -0.117) in compared to the younger generation (seedlings: He 0.407; Ho 0.419; Fis -0.030). Mating system is outcrossing which is indicated by the negative fixation index. Imogiri landrace showed the highest heterozygosity among others in Gunung Sewu and Eastern Islands of Indonesia, however it also had rare alleles that indicated the existence of genetic drift and bottleneck effect. The heterozygosity reduction at the seedling level might be attributed to the poor natural regeneration of sandalwood in Imogiri, which might occurred due to the unsynchronized flowering, low effective population size or lack of flowering trees (such as a case in Giri stand), the absence of seedbeds (a case in most of landrace grown in the verge of cliffs), and/or the absence of host (a case in Karangtengah Village). Seedlings were only found on a place where agroforestry pattern applied; implied that human interference is needed to preserve sandalwood from extinction. In order to enhance cross-mating system and prevent the increase of homozygosity, the genetic infusions with more diverse genotypes is recommended.

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1. Introduction
Sandalwood (*Santalum album* L., Genus Santalum, Family Santalaceae) was native to the southeastern part of the archipelago of Indonesia including Timor, Sumba, Flores, Alor, Solor, Wetar, Lombok, and Rote [1][2]. Sandalwood is slow growing and hemi-parasites [3]. Distilled sandalwood contains aromatic oils called sandalwood oil with strong and long-lasting fragrance. Due to its multipurpose uses, sandalwood has a high economic value. The excessive sandalwood exploitation and fragmentation led to the inclusion of this species into the category of Vulnerable A1d in 1994 according to the IUCN, which in 2009 was raised to be Endangered [4].

The re-introduction of sandalwood into its natural habitat in the Southeast part of Indonesia failed due to a low seed viability as a result of the increase of inbreeding, particularly in the highly clonalized populations [5]. The material for re-introduction purposes is taken from landraces. Landraces is a domesticated, locally adapted, traditional variety of a species that has developed over time, through adaptation to its natural environment, and due to isolation from other populations of the species [6]. According to [7], sustainable farm race will experience growth and mixed with other landraces on a genetic level. Natural and artificial selection and migration also contribute in making the land race genetically different. Most of sandalwood landraces selected for re-introduction materials of were located in Gunung Sewu Global Geopark Network. Gunung Sewu area stretches from the Parangtritis Beach (DIY) to Lake Shore Ria (Pacitan, East Java), covers an area of 1300 km2 with an altitude of 0 to 512.5 m above sea level [8].

Gunung Sewu region consists of three GeoArea which are Gunungkidul (Yogyakarta), Wonogiri (Central Java), and Pacitan (East Java). Sandalwood presented in Yogyakarta since the 19th century, which was proven by the LIPI herbarium collection dated on 12 July 1853, where the specimen was collected from the Kawasan Makam Raja-raja Imogiri [9]. In addition to Imogiri landraces, specimens of sandalwood on the Java Island is also collected from Situbondo (1893)[9], Madura (1894)[9], Kediri (1922)[9], Wonogiri (1922)[9], Pasuruan (1922)[9], Malang (1930)[9] and Nglipar, Bantul (1960)[10]. This information strengthens the evidence that sandalwood in Java Island has been existed since more than 100 years ago.

Sandalwood landraces distributed in various locations in the region of Gunung Sewu, and possible differed in genetic and population structures [5]. Genetic structure is the distribution of the frequency of certain genetic types in the population [11]. Genetic structure of natural populations was the result from interaction between natural selection, gene flow, and genetic drift [12] and it reflects the distribution of genetic diversity at the level of population [13]. According to [14], genetic structure was influenced by three aspects i.e. genetic processes, genetic forces, and population structure. The genetic structure of a population can be detected by measuring genetic diversity in the average population, range of variation on different populations, and distance as well as variations in the genetic relationship or genetic distance between populations [15].

One of sandalwood landraces, which is also considered the oldest in Java Island based on the herbarium investigation, was Imogiri landrace in which the oldest sandalwood specimens were dated on 1853. Imogiri was located on the south-west zone of Gunung Sewu, with the geological formations were influenced by ancient volcanic activities, resulted in the recent landscapes. A 70% of area was wavy and 30% was hilly to mountainous. Several studies have been conducted in sandalwood natural distribution in Timor and Sumba [17], as well as those conducted in several landraces in Gunung Sewu [23]. Those studies revealed that sandalwood in its origin has lost the genetic diversity due to the lowered genetic base and the increase of clonality, which was resulted in the inbreeding mating system and reproductive failure [17]. Some of landraces in Gunung Sewu which have wider genetic base were

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**Keywords**: genetic structure; Gunung Sewu; Imogiri landrace; sandalwood
higher in genetic diversity, and therefore were projected to be the seed sources for rehabilitation [23].
However, information about genetic structure in Imogiri landrace was still limited. This research
aimed to estimate genetic structure of sandalwood in several sandalwood stand differed by landscapes
in Imogiri, in order to provide genetic resources for conservation efforts.

2. Material and Methods

The isoenzyme samples, a juvenile leaves, were taken with 100% IS (census method), along
sandalwood natural distribution in five areas: (1) Kawasan Hutan Wisata Mangunan, (2) Bukit
Panguk-Kediwung, (3) Telaga Giri, (4) Bukit Mojo Gumelem and (5) Karangtengah Village. The first
to third areas are formed in cliff landscapes which are lead to Oya River. The fourth and fifth areas are
located in the local private yard and are arranged in an agroforestry pattern. The fifth area, which is
located near the Makam Raja-Raja Mataram, is also considered the oldest sandalwood stand in Imogiri
(the Head of Karangtengah Village; personal communication). Inventory and sampling were
conducted in October 2018 to January 2019. Laboratory research was conducted in the Laboratory of
Tree Improvement, Faculty of Forestry, Universitas Gadjah Mada in February 2019.

Genetic diversity was measured spatially across five sites in Imogiri, and temporally between two
generations of mature trees and seedlings. Mature trees were recorded with 100% IS (census method)
along sandalwood natural distribution in five areas. Seedlings were collected from natural
regeneration from seeds. Samples were wrapped, frozen in ice packs and taken to the laboratory for
allozyme extraction and electrophoresis. Allozyme analysis conducted at 6 putative loci following a
similar analysis previously performed for the ex situ plantations [17]. Previous study gained three
enzymes, shikimate dehydrogenase (E.C. 1.1.1.25.), esterase (E.C. 3.1.1.) and diaphorase (E.C.
2.6.4.3.) which observed to be polymorphic. Zymogram phenotypes that were interpretable were
found for only six loci, shikimate dehydrogenase Skd-1, esterase Est-1, Est-2, and Est-3, and
diaphorase Dia-1 and Dia-2. Electrophoresis procedures was conducted with vertical polyacrilamide
gel electrophoresis procedure following David-Ornstein method [16]. The leaves were homogenized in
modified extraction buffer and centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was
loaded onto polyacrylamide vertical slab (Sigma Inc., USA) gels and electrophoresed at 4 °C at 220 V
and 200 mA current for about 3 h. After electrophoresis, the gels were stained using staining solution
of each enzyme system. Three enzyme systems, shikimate dehydrogenase (E.C. 1.1.1.25.), esterase
(E.C. 3.1.1.) and diaphorase (E.C. 2.6.4.3.) were stained and the allozyme gels were genetically
interpreted. Genetic variation can be measured by the the percentage of polymorphic loci (PLP), the
number of alleles per locus (A/L), effective alleles (Ae), observed heterozygosity (Ho), expected heterozygosity is (He) and fixation index (FIS), following the formula of [16]. At each of the allozyme locus, the frequency of each allele and the genotype were counted. The A/L was obtained by counting the number of allele in each of locus; while the Ae was the number of effective allele. For each locus the number of heterozygote genotype were counted and expressed as percent observed heterozygosity (Ho). The observed heterozygosity was then pooled and averaged over all loci to determine the percent observed heterozygosity for a population. The expected heterozygosity (He) for each locus and over all loci for each population in Hardy–Weinberg equilibrium was counted. Fixation index, the deviation from expected frequencies under Hardy–Weinberg equilibrium, was measured following the formula: FIS = 1 – He/Ho. Microsoft excel was used to measure genetic variation.

3. Results and Discussion
Out of 224 samples analysed in this study, only 162 were interpretable. Fifteen out of 40 seedling samples from Mojo could not be interpreted due to overstaining, while all 47 seedlings from Karangtengah Village were failed to be sampled due to seedlings mortality as a result of long drought in Imogiri (Table 1).

Table 1. Total number of individual in each site and the effective population size

| Site       | Mature | Seedlings | Flowering plants | Effective Population Size (%) |
|------------|--------|-----------|------------------|-------------------------------|
| Mangunan   | 20     | 0         | 4                | 20                            |
| Mojo       | 44     | 40(25)a   | 7                | 16                            |
| Kediwung   | 45     | 1         | 3                | 6.7                           |
| Giri       | 13     | 0         | 0                | 0                             |
| Karangtengah | 14     | 47(0)b   | 8                | 57                            |
| **Total**  | **136** | **88**   | **22**           |                               |

aThere were only 25 interpretable samples
bThe seedlings could not be sampled for isoenzyme analysis due to seedlings mortality
Genetic variation was a result of interaction between natural selection, gene flow, and genetic drift. These factors are strongly controlled by the environment fragmentation. Fragmentation can lead to disturbed gene flow accelerating natural selection, and increase occurrence of genetic drift. This fragmentation occurred in Imogiri landrace. Imogiri was previously existed as a large landrace, but recently is separated into small groups of sandalwood (Table 1). Each group is separated by barrier in the form of karst hills and cliffs (Figure 2). Gradually, gene flow occurred only within each group of sandalwood, resulting in different genetic variation in each group. Gene flow within each group occurred through the spread of pollen with the help of pollinator, and seed dispersal with biotic or abiotic dispersers.

In this study, each group of sandalwood except Karangtengah Village (Imogiri) found on the verge of cliffs following the flow of the river. Accordingly, it was possible that gene flow not only occurred through seed dispersal by birds. Spreading the seeds through stream flow could also happened. Pollen has a low chance to spread since the distance among sites was more than 2 km, while optimum pollen distance for sandalwood was only approximately 42 m[17], therefore it was very difficult for pollen to spread between sites. The measurement of genetic diversity, both spatially (among five sites in Imogiri) and temporally (between two generations of parents and seedlings), were shown in Table 2.
Table 2. Site, plant developmental phases and genetic parameter measured

| Plant developmental phase and site | PLP (%) | A/L | Ae   | He   | Ho   | Fis   |
|-----------------------------------|---------|-----|------|------|------|-------|
| Parent tree, Mangunan             | 100     | 2.500 | 2.122 | 0.457 | 0.583 | -0.277 |
| Parent tree, Mojo                 | 100     | 2.500 | 2.081 | 0.471 | 0.462 | 0.020  |
| Parent tree, Kediwung             | 100     | 2.500 | 1.994 | 0.464 | 0.517 | -0.114 |
| Parent tree, Giri                 | 100     | 2.500 | 1.918 | 0.436 | 0.603 | -0.382 |
| Parent tree, Karangtengah         | 100     | 2.500 | 2.140 | 0.512 | 0.672 | -0.313 |
| Seedlings, Mojo                   | 100     | 2.500 | 1.763 | 0.398 | 0.414 | -0.040 |
| **Average**                       | **100** | **2.500** | **2.003** | **0.456** | **0.542** | **-0.184** |

PLP of sandalwood in each group valued 100%, indicated that all sites have polymorphic loci. This polymorphic loci indicates high allele variation [16]. Allele diversity can increase the proportion of heterozygous genotype, and therefore increase the population heterozygosity [22].

Number of alleles per locus (A/L) reflects the richness of alleles shared by every locus. In this study, each location has A/L 2.5. It means there were no missing alleles observed in this study. The result is similar to [18] which reported the value of A/L 2.5 in the sandalwood ex situ conservation area in Wanagama, Gunungkidul, which is located in the same zone of Gunung Sewu.

The effective alleles (Ae) will reach the maximum value when it has the same value to the alleles per locus (A/L). When the value is closer to the maximum value, the distribution of alleles per locus will increase. Hence, the contribution of each allele to form genetic variation will be equal [16]. In this research, the value of Ae ranged from 1.763 – 2.140. This value did not reach the maximum value since it is not equal to A/L (2.5). This may occurred due to the low frequency of alleles, with only small contribution to genetic variation.

3.1. Spatial Genetic Variation

In general, heterozygosity was high in each group of sandalwood (Table 2). The high value of heterozygosity is due to the wider genetic base in each of sites, particularly the older one (parental Ho ranged from 0.462 to 0.672). Cross- and assortative mating in each sites was indicated by the negative value of FIS. However, each site had different characters between heterozygosity and reproductive ability. In the Mangunan stand, high heterozygosity was found (Ho 0.583) and showed tendency to assortative mating (FIS - 0.277). However, despite the existence of mature parents (20 individual), it had very low reproductive potential which was represented by the absence of seedlings (Table 1). Similar case occurred in Giri stand, which had the high heterozygosity (Ho 0.603), tended to assortative mating (FIS - 0.382), had sufficient mature parents (13 individual), but lack the natural regeneration. At the time of observation, none of the parents observed bearing flowers. Hence, the low reproductive potential might related to the absence of flowers and pollinators [16]. This low ability to regenerate could be prevented by applying flowering stimulation and vegetative propagation methods in order to maintain natural cross-mating and preserve genetic variation.

Kediwung stand also showed high heterozygosity and cross-mating preference (Ho 0.517, FIS - 0.114), and also has many parent trees (45 mature individuals). However, the effective population size was low (6.7%) since only 3 individual observed bearing flowers. There was only one seedling found in this site. It might be occurred due to the low amount of flowers and pollinators, and the seedbed limitation that affects the reproductive success and survival rate. Previous study showed that less flowers and/or less pollinators reduced the pollination and fertilization rates, which in turn reduced the seed production [23]. According to [19], seeds need seedbed, seed supply, and an appropriate
environment to germinate and grow. Previous study reported that the lack of seedbed, particularly in the cliff landscapes such as Mulo Cliff of Gunung Sewu, significantly reduced the number of natural regeneration [5]. In this study, Kediwung stand was located on the verge of cliff, rugged terrain, and rocky inclined which might detained seed germination (Figure 3). In this case, the limited seedbed can be considered the main problem for seed germination. In addition, the lack of sandalwood primary host may also contribute to the poor seedling recruitments. To solve this problem, seeds supposed to be collected and germinated in other sites where seedbed is available. In the other hand, seedbeds in Kediwung site could be created by adding soils from more fertile sites, planting the pioneer herbs and shrubs, and mulching. The planting of primary hosts such as *Capsicum* sp and *Eupatorium* sp was also an option. After the environmental condition is suitable to promote natural regeneration, the seedlings can be replanted in Kediwung site.

Sandalwood in Mojo and Karangtengah village existed in agroforestry pattern which indicated the human interference. In Mojo, sandalwood planted in a mix with annual plants such as chili and cassava, and other tree species such as *Dalbergia* sp; in a “tree along border” pattern. Mojo stand showed high heterozygosity and random mating preference ($H_o$ 0.462, $F_{IS}$ 0.020), and has many parent trees (44 mature individuals) with moderate level of the effective population size (16%). Many seedlings (40 seedlings) grew naturally in the aboveground of chili plantation. This may prove that the agroforestry association can provide the suitable condition for sandalwood natural regeneration. The landscape is relatively flat, slightly rocky, and the annual plants may act as the host for sandalwood. The trees may act as the secondary host for sandalwood. Altogether, it creates the suitable environment both at the belowground and aboveground levels. The reduction of heterozygosity at the seedling level ($H_o$ 0.414, $F_{IS}$ - 0.040) might occurred due to the poor natural regeneration of sandalwood, which might occurred due to the flowering asynchrony, low effective population size, lack of flowering trees and pollinators and/or the absence of host. Flowering stimulation, pollination management and genetic infusions are recommended in order to synchronize and improve flowering, and enhance outcrossing. Planting more hosts is also an option.

Sandalwood in Karangtengah Village had the fewest parents (only 14 mature individuals) but had highest heterozygosity, outcrossing rate and effective population size ($H_o$ 0.672, $F_{IS}$ - 0.313, $N_{ep}$ 57%). Sandalwood in Karangtengah Village is considered the natural regeneration of the oldest sandalwood landrace in Imogiri, since this village is located at the same place with the Makam Raja-Raja Mataram, the place where sandalwood was first discovered in 1853. Recently, sandalwood in Karangtengah planted in a mix with annual plants such as *Colocasia* sp and *Pennisetum purpureum*, and other tree species such as *Dalbergia* sp, *Swietenia* sp; *Gliricidia* sp and *Schleichera oleosa*; in a “random mixture” pattern. High heterozygosity might be occurred due to the wider genetic base, higher level of effective population size (57%), and the panmictic spread of pollen. Actually the reproductive success was higher at this site, which was shown by the abundant number of seedling recruitments (47 seedlings), indicating the suitable condition for natural regeneration. However, seedlings could not survive due to the long drought and primary host inexistence. To solve this problem, the primary host supposed to be planted to promote natural regeneration of sandalwood.
3.2. Temporal Genetic Variation

3.2.1 Genetic parameters. This study reported a slightly reduction of several genetic parameter values including $A_e$, $H_E$, and $H_O$; and the increase of fixation index to be more inbreeding, at the younger generation (Table 3). $A_e$ declination implied that allele contribution to genetic variation was decreased. It means allele frequency and distribution at each loci was also decreased. Furthermore, this showed allele dominance which increases the proportion of homozygote genotypes.

Table 3. Genetic parameters of sandalwood at parent and seedling levels

| Phase          | PLP (%) | A/L   | $A_e$  | $H_e$  | $H_o$  | $F_{is}$ |
|----------------|---------|-------|--------|--------|--------|----------|
| Mature tree    | 100     | 2.500 | 2.053  | 0.483  | 0.539  | -0.117   |
| Seedlings      | 100     | 2.500 | 1.777  | 0.404  | 0.419  | -0.037   |
| Average        | 100     | 2.500 | 1.915  | 0.444  | 0.479  | -0.077   |

Heterozygosity, which is represented by the expected heterozygosity ($H_E$) and observed heterozygosity ($H_O$), was also declined at the younger generation. The outcrossing mating system was also reduced at the younger level ($F_{is}$ parent – 0.117 and seedlings – 0.037). This reduction might be attributed to the poor natural regeneration of sandalwood in Imogiri. In this study, poor natural regeneration particularly by sexual reproduction, may occurred due to the flowering asynchrony, low effective population size, or lack of flowering trees and pollinators (such as a case in Giri stand), the absence of seedbeds (a case in most of landrace grown in the verge of cliffs), and/or the absence of host (a case in Karangtengah Village).
According to [20], a negative and approaching zero $F_{IS}$ values indicate that the mating system is assortative (outcrossing). Other study [21] stated that in assortative mating, individual tend to mate with individuals with genotype similarities. This may resulted in the increase of offsprings with homozygous genotypes. They further explained that the increase of homozygous genotype will reduce genetic variation since it tends to produce recessive and deleterious alleles, particularly when the natural selection and genetic drift were large. In order to enhance the cross-mating system and prevent the increase of homozygosity, the genetic infusions with more diverse genotypes supposed to be applied. The natural regeneration by sexual reproduction should be supported by flowering stimulation, pollination management, providing seedbeds, and planting more primary and secondary hosts.

3.2.2 **Allele frequency and distribution between generations.** Each locus dominated by different alleles (Appendix 1). Locus *Est-1*, *Dia-1*, and *Dia-2* are dominated by 'b' allele, while locus *Est-2* and *Est-3* are dominated by 'a' allele. Locus *Shd-1* is dominated by the 'c' allele. This pattern of allele domination occurred both on parents and seedlings. The interesting findings were the rare alleles which were found on locus *Est-3*, *Dia-1*, *Dia-2*, and *Shd-1* at the parent level; and were found only on locus *Shd-1* at the seedling level. It indicated that most of rare alleles were inherited and derived to the next generation. These rare alleles could be used as an indicator of genetic drift and inbreeding. On the fragmented sites, chance of genetic drift was larger than inbreeding. Inbreeding could be detected by the positive value of $F_{IS}$ [22]. Most of observation sites performed negative $F_{IS}$ despite the occurrence of barriers, indicated that inbreeding can still be ignored. To prevent the loss of more rare alleles, natural cross-mating system should be improved by conducting genetic infusion and enrichment planting with the selected genotypes, particularly those which have higher flowering rate and cross-ability. Flowering stimulation and synchronization were also an option. According to [5,23], flowering of sandalwood in Gunung Sewu occurred twice a year, in the beginning of dry season in May-June, and in the middle of rainy season in December-January. The exchange of genetic materials with high allele frequency could be applied among sites to improve the frequency of rare alleles.

3.3. **Comparison to Sandalwood Genetic Variation in Gunung Sewu and Eastern Islands of Indonesia**

According to the herbarium investigation, the specimen dated in year 1853 has proven that Imogiri is by far considered the oldest landrace in Gunung Sewu, and possibly also in Java Island. Therefore, it is assumed that the landrace has the most complete alleles which can be used as a source of genetic materials in any of conservation and improvement programs. Isoenzyme was used to determine the genetic variation of landraces in Gunung Sewu [16] [23] (Table 4). By far, the highest parameters of genetic variation was found in Imogiri landrace.
It was hypothesized that the center of origin of a species is most likely the location with the highest genetic variation (center of biodiversity) [24]. This hypothesis may strengthen the assumption that Imogiri landrace is the oldest landrace in Java Island. However, it specifically explained that the center of origin should have the abundance of natural distribution and had endemic character of species [24]; two of requirements which are still unknown in Imogiri. There are only 136 mature individuals found in Imogiri, and among these parents, only 22 observed bearing flowers. At the molecular level, endemic character could be appeared in the form of private allele. Private alleles could not be detected by isoenzyme analysis, since it works only on living tissues, therefore can only detect fewer forms of polymorphic character. More detail research could be conducted in order to obtain more information on genetic variation. DNA markers could bring more polymorphic characters and therefore could be used to find the origin of species and determine more accurate inheritance.

4. Conclusions
Spatially, the highest genetic diversity (He 0.512; Ho 0.672; Fis -0.313) was found on the Karangtengah Village. Genetic diversity on other stands is varied (He 0.436 to 0.471; Ho 0.462 to 0.603; Fis -0.382 to 0.020). Temporally, genetic diversity is higher on the older phase (parents: He 0.407; Ho 0.419; Fis -0.030) in compared to the younger generation (seedlings: He 0.483; Ho 0.539; Fis -0.117). Generally, sandalwood in Imogiri performed outcrossing mating system which is indicated by the negative fixation index. Imogiri showed the highest heterozygosity compared to other sandalwood landraces in Gunung Sewu and Eastern Islands of Indonesia. Seedlings were only found on a place where agroforestry pattern applied. It implied that human interference is needed to preserve sandalwood from extinction. In order to prevent the increase of homozygosity, the genetic infusions with more diverse genotypes is recommended.

References
[1] Heyne K 1987 Tumbuhan Bergunan Indonesia II (Jakarta: Yayasan Sarana Wana Jaya)
[2] Orwa C Mutua R Kindt R Jamnadass A Simons 2009 Agroforestry Database: a tree reference and selection guide version 4.0. [Online] Available at: 

### Table 4. Genetic parameters of sandalwood landraces in Gunung Sewu, Timor and Sumba

| Landraces in Gunung Sewu and Timor | Genetic Parameters | Source |
|-----------------------------------|-------------------|--------|
|                                   | H₀ Parent | H₀ Seedling | H₀ Parent | H₀ Seedling | Fis Parent | Fis Seedling |        |
| **Gunung Sewu:**                  |          |             |          |             |            |              |        |
| GSN1-Nglanggeran                  | 0.269    | 0.231       | 0.261    | 0.284       | 0.029      | -0.231       | [23]    |
| GSN2-Sriten                       | 0.308    | 0.296       | 0.317    | 0.348       | -0.031     | -0.175       | [23]    |
| GSN3-Bejiharjo                    | 0.315    | 0.210       | 0.312    | 0.102       | 0.008      | 0.514        | [23]    |
| GSM1-Bleberan                     | 0.282    | 0.308       | 0.294    | 0.333       | -0.042     | -0.079       | [23]    |
| GSM2-Mulo                         | 0.259    | NA          | 0.243    | NA          | 0.062      | NA           | [23]    |
| GSM3-Wanagama                    | 0.347    | 0.375       | 0.406    | 0.439       | -0.172     | -0.171       | [23]    |
| GSS1-Petir                       | 0.210    | 0.219       | 0.153    | 0.117       | 0.271      | 0.464        | [23]    |
| GSS2-Botodayakan                  | 0.367    | 0.306       | 0.324    | 0.313       | 0.117      | -0.025       | [23]    |
| **Timor:**                        |          |             |          |             |            |              |        |
| Tm-Soe                            | 0.044    | NA          | 0.016    | NA          | 0.638      | NA           | [17]    |
| Tm-Netpala                        | 0.240    | NA          | 0.193    | NA          | 0.197      | NA           | [17]    |
| **Sumba:**                        |          |             |          |             |            |              |        |
| Sb-Sumba                          | NA       | 0.209       | NA       | 0.163       | NA         | 0.223        | [17]    |
| **Imogiri**                       | 0.483    | 0.404       | 0.539    | 0.419       | -0.117     | -0.037       | This study |
http://www.worldagroforestry.org/af/treedb[Accessed 16 Oktober 2018].

[3] Britanica 2018 *Sandalwood*. The Encyclopedia of Britannica [Online] Available at http://www.britannica.com/plant/sandalwood[Accessed 16 Oktober 2018]

[4] IUCN 2001 *IUCN Red List Categories and Criteria: Version 3.1*. IUCN Species Survival Commission International Union for Conservation of Nature and Natural Resources (Cambridge: Author)

[5] Ratnaningrum Y W N, Indrioko S, Faridah E, and Syahbudin A 2018 *Gene Flow And Selection Evidence of Sandalwood (Santalum album) under Various Population Structures in Gunung Sewu (Java, Indonesia), and Its Effects on Genetic Differentiation (Biodiversitas vol 18)* pp 1493-1505 (Solo: Universitas Sebelas Maret)

[6] Kell S P, Maxted N, Allender C, Astley D, and Ford-Lloyd B V I 2009 *Vegetable Inventory of England and Wales* (Birmingham: University of Birmingham)

[7] Casanas F, Simo J, Casals J and Prohens J 2008 *Safety Assessment of Sandalwood Oil (Santalum album Linn)* (Amsterdam: Elsevier) vol 46 pp 421-432

[8] Haryono E and Suratman 2010 *Significant feature of Gunung Sewu Karst as Geopark Site Procc. 4th Int. UNESCO Conf. April 2010*

[9] Lembaga Ilmu Pengetahuan Indonesia (LIPI) 1853 s.d. 1930 Spesimen herbarium *Santalum album* Bidang Botani dan Mikrobiologi. Pusat Penelitian Biologi (Cibinong: Lembaga Ilmu Pengetahuan Indonesia)

[10] Museum Biologi UGM 1960 Herbarium specimen of *Santalum album* (Yogyakarta: Faculty of Biologi)

[11] Finkeldey R and Hattemer H 2007 *Tropical Forest Genetics* (Gottingen:Springer)

[12] Hamrick J L and Schnabel A 1984 *Understanding The Genetic Structure of Plant Population: Some Old Problem and A New Approach* In ed Gregorius Hans-Rolf Population Genetics in Forestry Procc. of the Meeting of the IUFRO Working Party “Ecological and Population Genetics

[13] Hamrick J L 1989 *Chapter 4 Isozymes and the Analysis of Genetic Structure in Plant Population* In Eds. D. E. Soltis, & P. S. Soltis (Eds.), *Isozymes in Plant Biology* (Oregon: Dioscorides Press)

[14] Young A D, Boshier D, Boyle T 2000 *Forest Conservation Genetics: Principles and Practices*. (Collinwood Australia: CSIRO Publishing)

[15] Brown A H D 1978 *Isoenzyme, Plant Population Genetic Structure and Genetic Conservation* (Theor.Appl.Genet. vol 52) pp 145-147 (Amsterdam: Elsevier)

[16] Seido K 1993 *Manual Isozyme Analysis* (Jakarta: JICA and Directorate General of Reforestation and Land Rehabilitation, Ministry of Forestry in Indonesia)

[17] Indrioko S and Ratnaningrum YWN 2015 *Habitat loss caused clonality, genetic diversity reduction and reproductive failure in Santalum album, an endangered endemic species of Indonesia* (Procedia Environ Sci vol 5) pp 613-620

[18] Irmawati M A 2007 *Keragaman Genetik Cendana (Santalum album Linn) dari 2 Provenan dan 2 Ras Lahana di Wanagama I dengan Penanda Isoenzim* (Yogyakarta: Fakultas Kehutanan UGM) unpublished

[19] Leadem C L, Gillies S L, Yearsley H K, Sit V, Spittlehouse D L and Burton P J 1997 *Field Studies of Seed Biology* (Victoria: Research Branch B. C. Ministry of Forestry)

[20] Hamilton M 2009 *Population Genetics* (Oxford: John Wiley and Sons)

[21] Hartl D R 2001 *Assortative Mating* In eds S. Brener dan J. H. Miller *Encyclopedia of Genetics*. (Cambridge: Academic Press Inc)

[22] White T L, Adams W T and Neale 2007 *Forest Genetics* (Oxfordshire: CABI Publisher)

[23] Ratnaningrum Y W N, Indrioko S, Faridah E, and Syahbudin A 2015 *The effects of population size on genetic parameters and mating system of sandalwood in Gunung Sewu, Indonesia (Indon J Biotech vol 20)* pp 182-201

[24] Hummer K E and Hancock J F 2015 *Vavilovian Centers of Plant Diversity: Implications and
Impacts (HortScience vol 50) pp 780-783 (Berlin: ResearchGate)
Appendix 1 Allele frequency and distribution

| Sites and Developmental Phases | Alel | Lokus |
|-------------------------------|------|-------|
|                               | EST1 | EST2  | EST3 | DIA1 | DIA2 | SHD1 |
| Manganun Cliff; Mature phase  |      |       |      |      |      |      |
| a                             | 0,175| 0,75  | 0,625| 0,100| 0,325| 0,575 |
| b                             | 0,825| 0,25  | 0,375| 0,575| 0,6  | 0,050 |
| c                             |      |       | 0,325| 0,075| 0,375|      |
| Mojo; Mature phase             |      |       |      |      |      |      |
| a                             | 0,239| 0,875 | 0,761| 0,140| 0,233| 0,429 |
| b                             | 0,761| 0,125 | 0,239| 0,407| 0,465| 0,190 |
| c                             |      | 0,453 | 0,302| 0,381|      |      |
| Kediwung Cliff; Mature phase  |      |       |      |      |      |      |
| a                             | 0,233| 0,878 | 0,667| 0,178| 0,089| 0,553 |
| b                             | 0,767| 0,122 | 0,333| 0,356| 0,556| 0,158 |
| c                             |      | 0,467 | 0,356| 0,289|      |      |
| Giri Cliff; Mature phase      |      |       |      |      |      |      |
| a                             | 0,346| 0,577 | 0,923| 0,077| 0,077| 0,308 |
| b                             | 0,654| 0,423 | 0,077| 0,500| 0,808| 0,231 |
| c                             |      | 0,423 | 0,115| 0,462|      |      |
| Karangtengah Village; Mature phase |      |       |      |      |      |      |
| a                             | 0,286| 0,786 | 0,500| 0,227| 0,036| 0,389 |
| b                             | 0,714| 0,136 | 0,500| 0,318| 0,500| 0,167 |
| c                             |      | 0,455 | 0,464| 0,444|      |      |
| Mojo; Seedling phase          |      |       |      |      |      |      |
| a                             | 0,104| 0,813 | 0,667| 0,194| 0,104| 0,688 |
| b                             | 0,896| 0,188 | 0,333| 0,472| 0,771| 0,031 |
| c                             |      | 0,409 | 0,125| 0,281|      |      |

Rare allele