Reproductive outputs of two sandalwood landraces with different genetic base and clonality levels in Gunung Sewu, Indonesia

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Abstract. Based on the genetic base and clonality level, sandalwood (Santalum album) formed two types of landraces along with the Gunung Sewu Global Geopark Network in the southern part of Java islands, Indonesia. These two types are represented by Bejiharjo (which has a low genetic base and high clonality) and Bleberan (which has a high genetic base and low clonality) landraces. This study aimed to observe the quality of sandalwood seeds collected from the two landraces to determine the qualified seeds. We collected and tested all produced seeds of fruiting parent trees from August 2019 to March 2020. The physical and physiological quality tests included counting the number of seeds, measuring the seed size, and testing the viability of seeds. The Bleberan landrace produced 9161 seeds with 22.06% of seed viability. Meanwhile, the Bejiharjo produced only 158 seeds, of which all failed to germinate. Lower genetic base and higher clonality increased the inbred progenies, led to inbreeding depression, and decreased seed production and viability. Therefore, the qualified seed from the Bleberan landraces is worth considering to provide future genetic materials.

1. Introduction
Sandalwood (Santalum album) is native to East Nusa Tenggara (NTT), Indonesia [1]. Its fragrant wood has high economic value, mainly for perfume, medicine, and wood carving [2]. However, the continued overexploitation of Sandalwood, forest fire, forest degradation, and forest conversion caused a significantly decreasing natural population [1, 2]. Therefore, this species is assessed as Vulnerable in the IUCN Red List [3]. Conservation actions have been carried out by establishing seed sources, plantation and conservation plots in NTT [4], and ex situ conservation plots in Yogyakarta [5]. However, good quality seeds are needed considering effective rehabilitation and conservation activities.

The sandalwood mating system influences the reproduction results. This species tends to outcross, but it can also self-breed, depending on the parents' clonality level and genetic diversity in the population. It can reproduce vegetatively by root sucker. Sandalwood clonality can occur when all individuals in the population originated from only a few parents or even a single clone through vegetative propagation [6, 7]. High clonality and a low genetic base can reduce the outbreeding rate and influence the increase in inbreeding. Inbreeding can increase if the flowering parent comes from the identic clone, which can cause the quality and quantity of seeds to decrease [6]. Some sandalwood
populations with high clonality and low genetic base, mainly originating from East Nusa Tenggara, Indonesia, experienced a decline in genetic quality and reproductive capacity [5].

Sandalwood formed landraces along with the Gunung Sewu Global Geopark Network in the southern part of Java islands. The first introduction to Gunung Sewu is not certainly known. However, rehabilitation activities using sandalwood have been recorded since 1964 in the Wanagama forest area, Playen, Gunungkidul [5]. Some landraces such as Bleberan, Mulo, Watusipat, and Banyusoco might originate from Wanagama by gene flow, while Bejiharjo was first planted in the 1970s using seed from Nglipar [8]. The different conditions of each location influenced the development of various landraces as well.

There are two types of sandalwood landraces in Gunung Sewu, based on the genetic base and clonality level. These two types are represented by Bejiharjo and Bleberan landraces. The Bejiharjo landrace has a low genetic base and a high clonality characteristic [9]. Land clearing and population decline during 2014-2017 in Bejiharjo reduced the observed heterozygosity of flowering trees (from 0.462 in 2012 to 0.249 in 2019) and dramatically increased clonality from 31.03% in 2012 to 73.77% in 2019 [10]. On the other side, the Bleberan landrace has a higher genetic base and lower clonality characteristics [11-12] as the location is relatively undisturbed. The genetic diversity of flowering individuals in the Bleberan landrace is higher (with an observed heterozygosity value of 0.559 in 2012 and 0.729 in 2019). The clonality tends to be low, i.e., 11.11% in 2012 and 12.50% in 2019, respectively [12].

Seed quality is a prerequisite for sustaining the regeneration of sandalwood, so it is necessary to determine the quality of seeds obtained from seed sources with various genetic bases and clonality levels. Therefore, this study aimed to compare sandalwood seeds’ quality collected from Bejiharjo landrace (with a narrow genetic base and high clonality) and Bleberan landrace (with a broad genetic base and low clonality). This information is essential to determine the qualified seeds as propagule in the future.

2. Materials and Methods
The Bejiharjo landrace is located in Karangmojo Sub-District, Gunungkidul District, while the Bleberan landrace is located in Playen Sub-District, Gunungkidul District. Both landraces are parts of the Yogyakarta Special Region, Indonesia. The sandalwood populations were naturally regenerated. Observations were carried out at the peak of the fruiting session in both landraces, namely the third and fourth week of August 2019 in Bejiharjo and the third and fourth week of September 2019 in Bleberan. The reproductive potential of each landrace is calculated based on the percentage of individuals producing flowers, fruit, and seeds to the total adult individuals [13].

We collected, extracted, and tested all produced ripe seeds of fruiting parent trees in both landraces. The physical and physiological quality of the seed tests included counting the number of seeds, measuring the size of the sample seeds, and testing the viability of seeds [14].

3. Results and Discussion
3.1. Reproductive potential
A total of 15 out of 61 mature individuals (25%) were fruiting in Bejiharjo, while more individuals (53 out of 64 mature individuals or 83%) were fruiting in Bleberan (Table 1-2). Thus, although both landraces have a similar number of mature individuals, the reproductive potential in Bejiharjo is much lower. This is because the reproductive potential in the Bejiharjo landrace is disrupted by massive logging for ecotourism development in the area. In contrast, sandalwood in the Bleberan area has a higher reproductive potential because its habitat is relatively undisturbed by human activities.

Habitat loss and over-exploitation cause the sandalwood population to become fragmented or isolated [5]. This condition interferes with pollination and fertilization activities between populations and can cause inbreeding. Inbreeding occurs when flowering individuals are separated from other pollen sources. The risk of inbreeding in isolated populations is higher than that of individuals in mixed populations [15]. Inbreeding can cause physiological stress [16], reduce embryo viability,
seedling survival, vigor, and seed production [13], increase homozygosity and rare alleles in the next generation [17]. In addition, sandalwood in the Bejiharjo landrace shows vegetative propagation through root suckers [10]. Clones resulting from vegetative propagation have identical genotypes to their parents so that gradually it will narrow the genetic diversity in the population. Based on isozyme markers, sandalwood parent trees in Bleberan tend to outcross [12] compared to parent trees in Bejiharjo [10].

Based on the distance that allows for gene flow, the sandalwood population in the Bejiharjo landrace can be categorized as a small population and isolated from other groups. This population is more than 30 km apart from other groups. Species with isolated and smaller populations will experience extinction due to their ecological, demographic, and genetic conditions. Small and isolated populations will experience decreased genetic variation due to genetic shifts towards homozygotes and increasing rare alleles. In addition, it resulted in inbreeding and lowered reproductive capacity [5].

Table 1. The number of seeds, seed dimension, and seed viability collected from each mature fruiting individual in Bejiharjo.

| No. | Number of seed | Length (mm) | Width (mm) | Viability (%) |
|-----|----------------|-------------|------------|---------------|
| 1   | 62             | 7.72        | 7.00       | 0.00          |
| 2   | 49             | 7.12        | 6.52       | 0.00          |
| 3   | 5              | 7.92        | 7.46       | 0.00          |
| 4   | 15             | 7.03        | 6.42       | 0.00          |
| 5   | 1              | 7.21        | 6.40       | 0.00          |
| 6   | 4              | 7.84        | 7.18       | 0.00          |
| 7   | 12             | 7.63        | 6.94       | 0.00          |
| 8   | 1              | 8.32        | 8.00       | 0.00          |
| 9   | 1              | 7.25        | 6.55       | 0.00          |
| 10  | 2              | 7.18        | 6.56       | 0.00          |
| 11  | 1              | 7.39        | 6.15       | 0.00          |
| 12  | 2              | 7.13        | 6.00       | 0.00          |
| 13  | 1              | 7.98        | 7.11       | 0.00          |
| 14  | 1              | 7.00        | 6.23       | 0.00          |
| 15  | 1              | 7.89        | 7.34       | 0.00          |
|     | Average        | 10.53       | 7.51       | 6.79          | 0.00          |

Table 2. The number of seeds, seed dimension, and seed viability collected from each mature fruiting individual in Bleberan.

| No. | Number of seed | Length (mm) | Width (mm) | Viability (%) |
|-----|----------------|-------------|------------|---------------|
| 1   | 6              | 6.66        | 6.35       | 0.00          |
| 2   | 3              | 7.07        | 6.89       | 66.67         |
| 3   | 191            | 7.39        | 7.01       | 9.42          |
| 4   | 22             | 7.45        | 7.20       | 4.55          |
| 5   | 4              | 6.62        | 6.38       | 0.00          |
| 6   | 397            | 6.95        | 6.59       | 4.03          |
| 7   | 5              | 6.04        | 5.60       | 0.00          |
| 8   | 7              | 7.41        | 7.15       | 71.43         |
| 9   | 2              | 7.37        | 6.69       | 0.00          |
| 10  | 1              | 6.24        | 5.92       | 0.00          |
| 11  | 2              | 7.03        | 6.87       | 50.00         |
| 12  | 5              | 7.14        | 6.89       | 40.00         |
| 13  | 140            | 7.00        | 6.73       | 67.14         |
3.2. Physical and physiological seed quality
The Bejiharjo landrace produced only 158 seeds from 15 fruiting parent trees, far less than the Bleberan landrace, namely 9161 seeds from 53 fruiting parent trees (Table 1 and 2). This study shows that the high level of inbreeding in the Bejiharjo landrace [10] causes decreased fruit production. Previous research on Santalum lanceolatum, which has experienced clonality in the remaining population, showed sexual reproduction failure due to sterile pollen and pistil dysfunction [18]. Hence, the outcrossing pattern in Bleberan [12] resulted in higher reproductive output of seeds.

The mean length and width of the seeds collected from the Bejiharjo landrace (7.51 x 6.79 mm) were higher than the Bleberan landrace (6.94 x 6.61 mm) (Table 1 and 2), but only the seed length was significantly different. However, all seeds from the Bejiharjo landrace failed to germinate. A bigger seed size does not always correlate with higher seed viability [19]. Therefore, the germination failure...
was assumed to be due to a higher inbred rate in the Bejiharjo landrace. The mean viability of the seeds of the Bejiharjo landrace was 22%, with a wide range of 0-60% (Table 2). The seeds germinate first on the 14th day after sowing. The last seeds germinate on the 74th day. The number of seeds germinating varies, from one to 258 seedlings per tree.

Differences in the mating patterns of parent trees lead to variations in the viability of the seeds produced. Previous sandalwood research in Bleberan showed a decrease in the genetic diversity of sandalwood offspring compared to their parent due to inbreeding [11] which can cause inbreeding depression through an expression of deleterious alleles [20] in the form of a decrease in offspring quality. In this study, several harvested fruits were empty. An empty fruit is caused by the absence of an embryo and cotyledon due to self-fertilization [21]. In addition to the disturbance in seeds, there was also disease interference with the seedling. The dead seedlings were excluded in the calculation of the total seed viability. The number of seedlings calculated for seed viability testing referred to the number of normal seedlings [15]. 2% of sandalwood seedlings died with dry conditions in the cotyledons and root collar, with symptoms of black cotyledons (Figure 1a.) or root collar (Figure 1b.) like burnt. Fungi also caused seedling root rot as soil-borne pathogens [22].

![Figure 1. Seedlings affected by a fungus in the: (a) cotyledon and (b) root collar area.](image)

3.3. Future perspectives
Sandalwood genetic material taken from the Bleberan landrace with a broader genetic base than Bejiharjo is essential to conserve and develop sandalwood genetic resources in the future. Genetic infusion originating from sexual reproduction by a random mating or outcrossing can add to the genetic base of the Bejiharjo landrace. The addition of new alleles can increase genetic diversity and restore populations experiencing inbreeding depression [20]. Planting heterozygous seedlings with varying alleles from other locations is essential in the rehabilitation program. When these individuals reach the reproductive phase, they will interbreed with local individuals and produce offspring with higher genetic variation.

The sandalwood population in the Bleberan landrace is very potential as a population for genetic resources conservation. Landraces with a broad genetic base should be developed to maintain parents capable of producing abundant seed production. Furthermore, adding individual sandalwood from other genetic sources is essential to increase population genetic variation. Therefore, mapping the genetic structure, population structure and density, reproductive potential, and suitable habitat in each landrace along the Gunung Sewu Global Geopark Network in the southern part of Java islands is indispensable to arrange an ex-situ conservation strategy for sandalwood in Java.

4. Conclusion
The qualities of sandalwood seeds produced in the Bleberan landrace were much better than those from the Bejiharjo landrace. However, the lower genetic base and higher clonality increased the inbred
progenies and led to inbreeding depression. The expression of inbreeding depression also decreased seed production and viability in the Bejiharho landrace. Therefore, the qualified seed from the Bleberan landraces is worth considering to provide future genetic materials.

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