Genetic diversity of Mindi (Melia Spp.) and its implications on the development of seed source for community forests

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Abstract. Melia Spp. is known as fast-growing tree species and commonly cultivated in community forest and agriculture land due to its valuable wood. This species was widely distributed in some agroforestry systems, especially in Selaawi village, Garut, West Java. Based on the farmer information, two species mindi were found in this location; they were small and big seed mindi. The research aimed to get information on mindi diversity in Selaawi village and the opportunity for seed source development. For this purpose, microsatellites was used. Forty leaf samples were collected from parent tree owned by a farmer in Selaawi village. Three microsatellites markers (Ai_5, Ai_34 and Ai_48) developed for Azadirachta indica were used as transferability of PCR amplification. The result showed that genetic diversity value (He) of mindi in this location was high and ranged from 0.379 – 0.439. Big seed mindi has higher diversity than the small one. It indicates that this species is prospective to be developed as target species for the community forest. It is also potential to develop mindi in this location as seed source due to its high variability.

1. Introduction

Mindi (Melia Spp.) is a member of Meliaceae and has a wide habitat range [1]. This species has well adapted and well grown in lowlands - highlands from 0-1200m.asl with a temperature ranges from -5°C -39°C and an average rainfall 600-2000 mm/year. Naturally, mindi is distributed in India and Burma, then widely cultivated in tropical and sub-tropical regions including Indonesia. In Indonesia, mindi is classified into exotic species and distributed in Sumatra, Java, Nusa Tenggara and Papua [1].

In West Java, mindi is extensively cultivated in Bogor, Cianjur, Sumedang, Purwakarta, Subang, Kuningan, Bandung, Majalengka and Garut. Mindi is planted in agriculture lands or community forests owned by community members [2]. The species become one of targets for community forest development [3]. In Garut, especially Selaawi Village, mindi is cultivated by a farmer for the wood purpose. The seed source for cultivation is taken from parent trees in the surrounding location. Considering the increasing demand of mindi wood and the development of community forest, sustainability supply of seed iss an important factor in determining the successfulness of the program. For this purpose, it is needed seed with physically, physiologically and genetically high character from identified source. In contrary with this requirement, [4] reported that most of community forests cultivation used seed from farmer’s own trees which were usually originated from 1-5 trees. This condition caused the narrow diversity and would increase the level of inbreeding. When inbreeding continuously occurs, it will threaten the species and later will cause extinction [5].
Some researcher has conducted research on mindi genetic diversity using molecular. In West Java, according to [5], the genetic diversity value of mindi using microsatellite marker was moderate (He:0.366). Similar study using RAPD marker conducted in six mindi population in West Java also found moderate value of diversity [3]. Specific study of mindi population in Selaawi Village has not been determined yet, whereas based on interview with the farmer, there were two species of mindi in the location which were big and small seed mindi. The big mindi was characterized by its bigger seed (5 times bigger than the small one) and faster growth. Therefore, this research was conducted to get information on mindi diversity in Selaawi Village based on microsatellite and morphological markers. This information will support the seed source development of mindi in agricultural land or community forest.

2. Material and method

2.1. Materials

Forty mindi leaf samples were collected from agroforestry land in Selaawi Village, Garut Regency, West Java Province. Twenty small and twenty big mindi leaf samples were collected from parent trees for this purpose. All of the samples were then stored in plastic bags containing silica gel and kept at room temperature until DNA extraction process. DNA was processed through isolation, Polymerase Chain Reaction amplification, and data interpretation.

2.2. Method

DNA isolation was performed using CTAB (Cetyl Trimethyl Ammonium Bromide) method developed by [6]. Three microsatellites loci developed for Azadirachta indica, Ai_5, Ai_34 and Ai_48, were used to get information on mindi diversity (table 1).

| Locus    | Repeat motif | Sequences                                           | t_m (°C) | Allele size (bp) | Accession no. |
|----------|--------------|-----------------------------------------------------|----------|-----------------|---------------|
| Ai_5     | (CA)15       | F: GAAAGGAGGGTTTTCAATCA R: TCGGCCGAACACAATTTTA     | 55       | 130–182         | FM161908      |
| Ai_34    | (GA)18       | F: ATTTGTGTGTGCGTGCTAGG R: CGAGGAACTGAGACTCCTGAA  | 55       | 146–168         | FM161913      |
| Ai_48    | (CA)10       | F: TCCAGTTATCAACGTAGGC R: TCCTAACATGGATTGCTTCACA  | 55       | 105–125         | FM161914      |

PCR amplification was performed using a PCR Thermal Cycler (PTC-100). The reaction was mixture of 13.5 μL containing 2 μL DNA template, 2 μL nuclease-free water, 7.5 green Go Taq and 2 μL loci. The PCR cycling conditions were as follows: pre-denaturation at 95 °C for two min, denaturation at 95 °C for one min, annealing for two minutes with temperature following each loci procedure, extension at 72 °C and the final extension at 72 °C for five minutes. PCR products were run on 1 % polyacrylamide gel and followed by the staining process using silver staining method. Band pattern was then scored, and the scoring result was further analyzed using POPGENE ver 1.31 and NTSYS 2.02. The observed parameters for genetic diversity were the percentage of polymorphic loci (PLP), observed number of alleles (na), number of effective alleles (ne), expected heterozygosity (He), and genetic differentiation (FST).

3. Results and discussion

3.1. Diversity within population

The diversity value (He) for big mindi is 0.439 and for small mindi is 0.373 (table 2). It indicates that both of mindiin the location have a high value of diversity. This result is in accordance with [7] in Pakistan and Turkey, but it is in contrary with the research conducted by [8] on small mindi using RAPD.
marker showing lower diversity (He:0.16-0.19). The high value of genetic diversity indicates higher possibility which allows the tree to adapt under gradual environmental changes.

**Table 2.** Genetic diversity value of big mindi and small mindi in Selaawi village.

| No | Population     | N  | PPL | Na   | Ne   | He   |
|----|----------------|----|-----|------|------|------|
| 1  | Big seed mindi | 20 | 100 | 2,333| 1,997| 0,439|
| 2  | Small seed mindi| 20 | 100 | 2,333| 1,736| 0,373|

Noted: n = Amount of sample, PPL = Percentage of Polymorphic Loci, na = Observed number of alleles, ne = Effective number of alleles, He = Gene diversity

Genetic diversity indicator of forest management sustainability [9]. High value of diversity in this location indicates that mindi has high adaptability to environmental changes.

3.2. Diversity between population

The parameters to characterize variation between population was genetic distance. Genetic distance is measured by the differences of genetic structure between two populations at a particular gene locus [10]. The pairwaise genetic distance is presented in table 3.

**Table 3.** Genetic diversity between mindi populations.

| Noted | Population | N | Ht   | Hs   | Gst  |
|-------|------------|---|------|------|------|
| Average| 2          | 40| 0.110| 0.406| 0.555|

Note: Ht = Genetic diversity of total population, Hs = Genetic diversity of sub-population, Gst = Genetic differentiation between populations

Based on table 3, genetic diversity between populations (Gst) based on Nei (1972) is 0.555. This value is higher than the value of genetic diversity within populations (Hs: 0.1459). The genetic composition of individuals in one population tends to be more uniform than individuals outside the population. Variations between populations are based on genetic distance calculations. Genetic distance is one of the parameters indicating the relationship between populations. UPGMA dendogram based on genetic distance is presented in figure 1.

**Figure 1.** Dendogram mindi based on genetic distance.

Figure 1 shows that mindi population in Selaawi Village is clustered into two group, those were big seed mindi and small seed mindi. It can be concluded that genetically big and small mindi are different although one of individual small mindi is clustered together with the big ones. The value of genetic diversity of a species in a population is influenced by several factors including the size of effective population area, flower production, pollen flow between stands and the mating system [11]. Mindi flower was hermaphrodites with female and male reproductive organ reside in the same flower [3]. This condition will increase the selfing rate of species which will decrease genetic variability of a population.
Combining group using morphological observation and genetic character was also measured in this research. Morphological characters (fruit diameter, fruit length, leaf fin number, and leaf area) was measured and observed to differentiate big and small mindi. Whereas genetic character was quantified using alleles on Ai5 and Ai34 primers. The result showed that big and small seed mindi were clustered into different group (figure 2).

![Dendrogram](image)

**Figure 2.** Dendrogram combining morphological and genetic traits, number 1 s.d 10 (large mindi), number 11 s.d 20 (small mindi).

Information on genetic distance and genetic relatedness between individual trees are important for the breeding program [12]. Largest genetic distance between two individuals indicates that two individuals have quite wide genetic differences. The genetic resources conservation of a species must begin with clearly identifying what the conservation goals are. The second stage is genetic resources selection based on available knowledge of the spatial pattern of genetic variation. Furthermore, it is followed by choosing a conservation method to conduct physical preservation. The final stage of the conservation program is a regeneration of genetic resources [13].

Based on previous research conducted by [3], big seed mindi were only found in Selaawi Village. At least 65,000 mindi seed have been cultivated in this area for a household need. These activities can support in situ conservation effort of mindi. According to farmer information, some farmers in surrounding Selaawi Village and Cianjur are also interested in mindi, and hopefully they will support mindi community forest development through agroforestry scheme and support the national timber needs.

### 3.3. Implications of genetic diversity of mindi on community forest development

Big seed mindi is potential to be developed as community forest species. The trees can be harvested at five years after planting, and the remain bole after cutting will sprout and be harvested again after four years later. Compared to Jabon, sengon, afrika wood and other fast-growing species, the price of mindi wood is quite high in the local market. The wood of mindi has durability from termite attacks, attractive features and it is commonly utilized for part of houses.

According to [3], the limited species cultivated in community forest might be caused by the preference of local species which are naturally distributed in Indonesia. Lack of information and regulation that supports the development of exotic species such as mindi. Our research concludes that big seed mindi with higher diversity value is potential to be developed. The initial activities can be conducted through selected the number of parent trees in farmer agricultural land or forest based on best morphological appearance and genetic trait.
4. Conclusion
Based on DNA microsatellite analysis, genetic diversity value (He) of mindi in Selaawi Village was high. The value for big mindi was 0.439 and small mindi was 0.373. Dendrogram pattern shows that big and small mindi have a different cluster and different genetic structure. This information is essential for mindi breeding program, especially for seed source development. Agroforestry using mindi species is also considered due to the fact that it is easy to cultivate, fast-growing characters and high value of wood.

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