BIODIVERSITAS
Volume 20, Number 6, June 2019
Pages: 1708-1712

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200629

Genetic diversity of mindi (*Melia azedarach*) from community forest in Deli Serdang, North Sumatra, Indonesia revealed by microsatellite marker

RIDAHATI RAMBEY♥, ARIDA SUSILOWATI, NELLY ANNA
Faculty of Forestry, Universitas Sumatera Utara. Jl. Tri Dharma Ujung No. 1, Medan 20155, North Sumatra, Indonesia. Tel./fax.: +62-61-820-1920.
♥email: ridahatirambey@yahoo.com.

Manuscript received: 4 February 2019. Revision accepted: 25 May 2019.

Abstract. *Rambey R, Susilowati A, Anna N. 2019. Genetic diversity of mindi (*Melia azedarach*) from community forest in Deli Serdang, North Sumatra, Indonesia revealed by microsatellite marker. Biodiversitas 20: 1708-1712. Melia azedarach* or locally known as mindi is fast growing and one of important tree species for community forests especially in West Java and North Sumatra, Indonesia. High demand for its wood must be accompanied by increased productivity through planting of high-quality seed. There is still lack of information about genetic diversity of mindi from different seed source in North Sumatra. Therefore, our research was conducted to determine genetic diversity of mindi from different seed source in North Sumatra through microsatellites marker. Leaf samples originated from three populations Percut Sei Tuan, Pancur Batu and Biru-Biru were used in this study. The SSR markers were from *Azadirachta indica* i.e. Ai5, Ai 11, Ai 13, Ai 14 and Ai 34. Molecular data were analyzed using Popgene and NTsys version 2.0. The result showed that number of alleles (na) ranging from 1.80 to 2.80, and percentage of polymorphic loci (PPL) ranging from 60 to 80 %. The level of genetic diversity showed that mindi in North Sumatra has moderate value (He: 0.244 to 0.269). It indicated that assessment of genetic diversity of *M. azedarach* North Sumatra is necessary for improvement and conservation of a species.

Keywords: Community forest, diversity, genetic, microsatellite

INTRODUCTION

Melia azedarach or Mindi is an important forest tree for pharmaceutical (Sharma and Paul 2013), insecticidal, pesticidal, and commercial purpose (Al-Rubae et al. 2009). This species was widely planted in India, Burma and some of tropical and subtropical area, including Indonesia (Nikoletta 2010). In Indonesia, mindi have been commonly planted in Sumatra, Java, Nusa Tenggara and Papua (Wardani 2001). It also good reforestation tree because of fast growth and adaptive for drought hardy stress condition. Mindi has reddish or pinkish brown wood, light and suitable for furniture, veneering and sports goods **(**Hasan and Alam 2001; Rahman et al. 2014). Beside it wood, the leaf, fruit and seed extract of this plant has been tested to manage malarial mosquito, dengue mosquito, lice, ticks, etc. under laboratory conditions (Maciel et al. 2006; Al-Rubae 2009).

In Deli Serdang regency, North Sumatra, Indonesia mindi were distributed in subdistrict Percut Sei Tuan, Pancur Batu, and Biru-Biru. Interview result with farmers revealed that farmers prefer to plant mindi due to the faster growth character. Not different with some location in West Java, mindi in North Sumatra also mixed with other plants like cacao through agroforestry system and also using as land boundary marker. Demand for mindi wood in this location is increasing every year. In contrary with wood requirement, the mindi material for planting was very constrained with limited seed trees (Syamsuwida et al. 2012). This condition also in accordance with Roshetko et al. (2004) that stated most of community forests cultivation using seed from farmer own trees were usually originated from 1-5 trees. This condition caused the narrow of diversity and will increase level of inbreeding.

Genetic diversity in a species allows itself to adapt, evolve and respond to environmental stress. It provides the basis for survival of a species and critically influences its evolutionary potential (Thakur 2016). The genetic diversity status of species can be determined using several molecular methods such as isoenzymes, RAPD, and microsatellite. Microsatellite was widely used for tropical tree species due to some characteristics: high levels of polymorphism, co-dominant, and is inherited following the laws of Mendel (Ujino et al. 1998; Zulfahmi 2006). Microsatellite also better used than those of morphological marker because it dominant expression, having high polymorphisms and shows random distribution throughout the genome (Susilowati et al. 2018).

Research on genetic diversity of mindi in North Sumatra has never been conducted yet especially using molecular marker, whereas the data was needed as basic data and reference for pursuing high-quality seed sources. Therefore, the objective of this research was to determine genetic diversity of mindi populations in Deli Serdang community forests using microsatellite marker.

MATERIALS AND METHODS

Plant material

The fresh leaf samples for genetic analysis of mindi originated from subdistricts of Percut Sei Tuan (PST),

Biru-Biru (SBB) and Pancur Batu (PB) of Deli Serdang, North Sumatra, Indonesia (Figure 1). Thirty-five leaf samples were stored in plastic bags with silica gel and kept at room temperature for DNA extraction. Five microsatellites marker from mimba (*Azadirachta indica*) developed by Boonton et al. (2008) those wereAi_5, Ai_11, Ai_13, Ai_14 and Ai_34 were used for cross amplification (Table 1). The cross amplification was utilized primer from different taxa for amplified in PCR process. The successful of cross-amplification for mindi using *A. indica* also have been reported by Abaloso et al. (2009), Lemes et al. (2011), Costa et al. (2013), Thode et al. (2013), Wang et al. (2013), Wee et al. (2013), and Irmayanti (2015).

DNA extraction

Genetic diversity of mindi was conducted through some process those were total genomic DNA isolation, DNA quality test, amplification process and result in analysis. Total genomic DNA isolation for mindi was perform using CTAB (Cetyl Trimethyl Ammonium Bromide) method developed by Murray and Thompson (1980). The result of this step will be checking on DNA quality test.

DNA quality test was perform using 1% agarose gel and horizontal electrophoresis. A TE 50 µL buffer was added to the DNA pellets then centrifuged at 10 000 rpm for 10 minutes. DNA pellets were taken as much as 3 µL and added 2 µL BJ (Blue Juice). The DNA and BJ pellet mixture was put into agarose gel, and electrophoresed for 45 minutes. Electrophoresis results were immersed in EtBr (Ethidium Bromide) solution for 15 minutes and photographed on UV transilluminator TFX-20.LM model (Aritonang et al. 2007). The sample with clear and high quality and quantities than processed for further DNA amplification process.

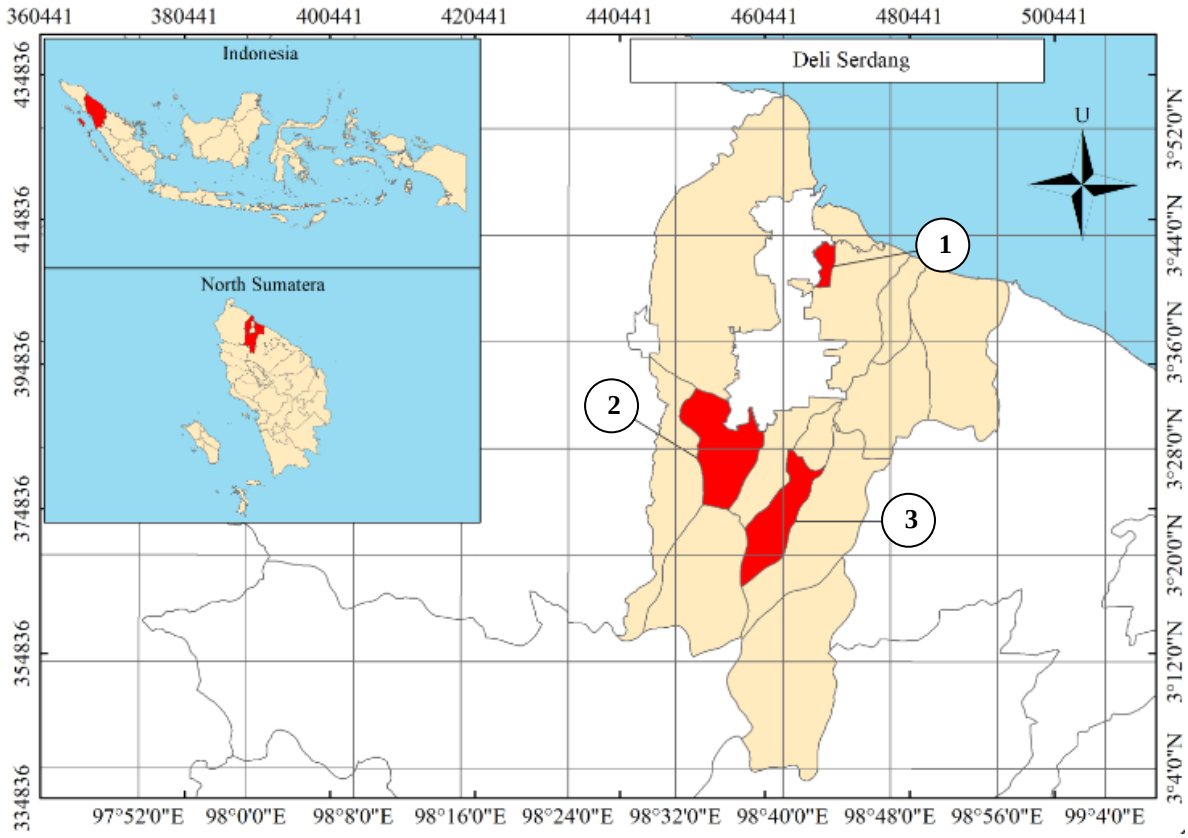

Figure 1. The research location for mindi on Deli Serdang, North Sumatra, Indonesia. 1. Percut Sei Tuan, 2. Biru-Biru, and 3. Pancur Batu

Table 1. Primer was used in PCR-Microsatellite genetic analysis (Boonton et al. 2008)

Locus	Primer sequences	Ta (°C)	Allele size (bp)	Gene bank assesion
Ai5	F: GAAAGGAGGGTTTTCAAATCA; R: TCGGCCGAACACAATTTTA	55	130-182	FM161908
Ai11	F: GCATCAGTCAGCCATAGTGC; R: TTGAAAAATCCTGGCGAGTG	55	175-219	FM 161910
Ai13	F: CCACAAACAAATGGGAAACC; R: CCCTTATTACAAAAGAGAGGGA	55	158-188	FM 161911
Ai14	F: GTCCACGCAAACAGAGACAC; R: TTGGCTTGGCTTTCTCTTTC	55	224-232	FM 161912
Ai34	F: ATTTGTGTGTGCGTGCTAGG; R: CGAGGAACTGAGACTCCTGAA	55	146-168	FM161913

SSR marker analysis

PCR amplification process was performed using a PCR Thermal Cycler (PTC-100). The process of PCR consisted of four-step, initial denaturation, denaturation, annealing, and final extension. Initial denaturation was conducted at temperature 95°C for 2 min. Denaturation step was performed at temperature 95°C for 1 min, while annealing step was at temperature 52°C followed by polymerization at 72°C for 2 min. The last step was final extension conducted at 72°C for 7 min. The process was used reaction 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 follow: pre-denaturation at 95°C for 2 min, denaturation at 95°C for 1 min, annealing for 2 minutes with temperature following each loci procedure, extension at 72°C and final extension at 72°C for 5 minutes. PCR products were run into 1% polyacrylamide gel and stained by silver staining method (Bonbouza et al. 2002). Band patterns then were scored and analyzed using POPGENE ver 1.31 (Yeh and Yang 1999), NTSYS 2.02 and GenAlexVer 6.5 (Rohlf 2008).

Data collection analysis

Genetic parameters were measured for genetic diversity within population and between population. The parameters were Percentage of Polymorphic Locus (PPL), number of observed alleles (na), effective number of alleles (ne) and expected heterozygosity (He). On the other hand, in relation to parameters being observed for genetic diversity between population, we used cluster analysis to estimate genetic distance. Genetic distance data from POPGENE were used for cluster analysis in UPGMA (unweighted pair group with arithmetic average) method using NTsys version 2.0 software. Principal Component Analysis (PCA) was also calculated to detect clustering pattern using GenAlexVer 6.5 (Blyton and Nicola 2006).

RESULTS AND DISCUSSION

Genetic diversity within populations

The number of polymorphic loci in genetic diversity analysis determines level of diversity in a population. Variable is used to characterize genetic variation within population were Percentage of Polymorphic Loci (PPL), average number of alleles per locus (A/L), and genetic variation (He) (Finkeldey 2005). In this study, the mean value of observed allele (Na) was 1.9333, PLP was 73%, and genetic diversity in population (He) was 0.252 (Tabel 2). According to Weising et al. (2005) the value of genetic diversity range from 0 to 1. Based on this classification, the value of mindi genetic diversity classified into moderate. Rambey (2011) explained that genetic diversity of big mindi was 0.439 and that of small mindi was 0.773 and categorized as high diversity. Furthermore, Irmayanti et al. (2015) reported genetic diversity of mindi in West Java Community Forest was 0.366 categorized as moderate.

Table 2. The value of number of observed alleles (Na), expected alleles (Ne), percentage of polymorphic loci (PLP) and heterozygosity (He)

Population	N	N_a	N_e	PLP (%)	H_e
PST	12	2.0000	1.4436	80	0.2444
SBB	10	1.8000	1.4380	80	0.2370
PB	13	2.0000	1.5464	60	0.2692
Mean		1.9333	1.4760	73	0.2502

The highest genetic variation of mindi in this study was obtained in PB population (0.2692), whereas the lowest genetic variation of mindi in SBB population (0.2370). Based on Table 2, the value of genetic diversity (He) on mindi population was lower compared to previous research. It might be caused by lower number of mindi population from North Sumatra. The value of variation in population could be affected by pattern of plant distribution (Hamid et al. 2008). According to Yulianti (2011), Genetic diversity of *Melia azedarach* in community forests of West Java assessed by RAPD marker (He: 0.1603 -0.1956) categorized as moderate. Thakur (2016) found genetic diversity and population structure of *Melia azedarach* in North-Western Plains of India assessed by microsatellite marker (He = 0.712) categorized as high diversity

Genetic quality needs to be considered because it is related to with the capacity to adapt to the growth environment. The higher the genetic diversity showed that the plant has greater chance to adapt to the environment change. The lower value of genetic variation of mindi in SBB population (0.2370) from other population might be caused by inbreeding process. Continuous inbreeding will result in extinction of species in the long term period (Hamilton 2009). The serious effort needed to prevent mindi in North Sumatra from extinction.

Mindi genetic resources could be maintained by in situ and ex situ conservation. In situ conservation is a method and tool to protect species, genetic variation and habitat in its natural ecosystem. On the other hand, ex situ conservation comprises method and tool to protect species, microorganism, and genetic variation outside their natural habitat or ecosystem. To increasing genetic variation of mindi several activities could be conducted, one of them is genetic infusion. Genetic infusion is activity to introduce low variability plant population with high variability of species for improving its genetic variability. This activity expected will improve variability of the population in the next generation (Nurtjahjaningsih 2007).

Genetic variation between population

In general, the genetic difference between two or more population was analyzed by using genetic distance matrix in pairwise combination (Finkeldey 2005). A pairwise genetic distance from this study was presented in Table 3. Genetic distance measures the difference in genetic structure between populations at a particular gene locus. Based on Table 3, the widest genetic distance was obtained from SBB and PB population (0.3291) whereas the small distance obtained from SBB and PST (0.2480). The large

genetic distance indicates that the genetic relatedness between both of population was far and not closely related. Conversely, the population with small genetic distance indicated that both of the population were closely related.

Besides the genetic distance, other variables which can be used to characterize genetic variation between populations is cluster analysis or genetic distance dendrogram between populations (Figure 1). Mindi population in this research grouped into two clades. The first clade consisted of PST and SBB population, whereas PB population separated in the second clade. It indicated that genetically PST and SBB population was closely related.

One of the significant factors determining seed quality is the seed origin which is usually closed with the genetics of the seed (Yulianti et al. 2011). As known that seed quality is determined by a number of genetic and physiological characteristics. The maintenance of seed source genetic diversity was important to the sustainability seed production. Our research showed that the mindi population from 3 location especially from Percut Sei Tuan (PST) and Biru-Biru were closely related (Figures 2-3). Breeding between close relatives generally entails a fitness cost, known as inbreeding depression and mainly results from the increased homozygosity of recessive deleterious alleles (Charlesworth and Willis 2009). Considering this condition, there was need some effort for increasing the genetic diversity of mindi in this location, one of which through genetic infusion. Closely related population should be planted in different location to prevent the inbreeding process. Infusion for increasing the diversity of closely related population (PST and SBB) should be conducted using PB population or other location such as West Java population.

The individual clustering of all samples from the three population showed that there are several individuals from population PST which clustered together with the SBB population. In contrary, all of individual of PB population is separated and grouped into one clade. Based on this clustering, urgent task for increase genetic diversity of mindi in Deli Serdang community forests was needed.

Table 3. A pairwise genetic distance matrix between mindi (*Melia azedarach*) populations

Population	PST	SBB	PB
PST	0.0000	-	-
SBB	0.2480	0.0000	-
PB	0.3250	0.3291	0.0000

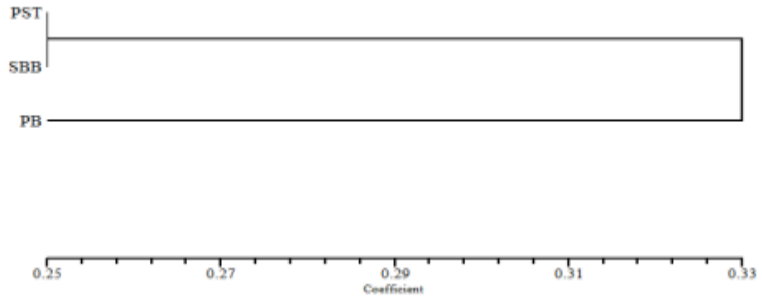

Figure 2. Dendrogram of grouping between mindi (*Melia azedarach*) populations based on genetic distance

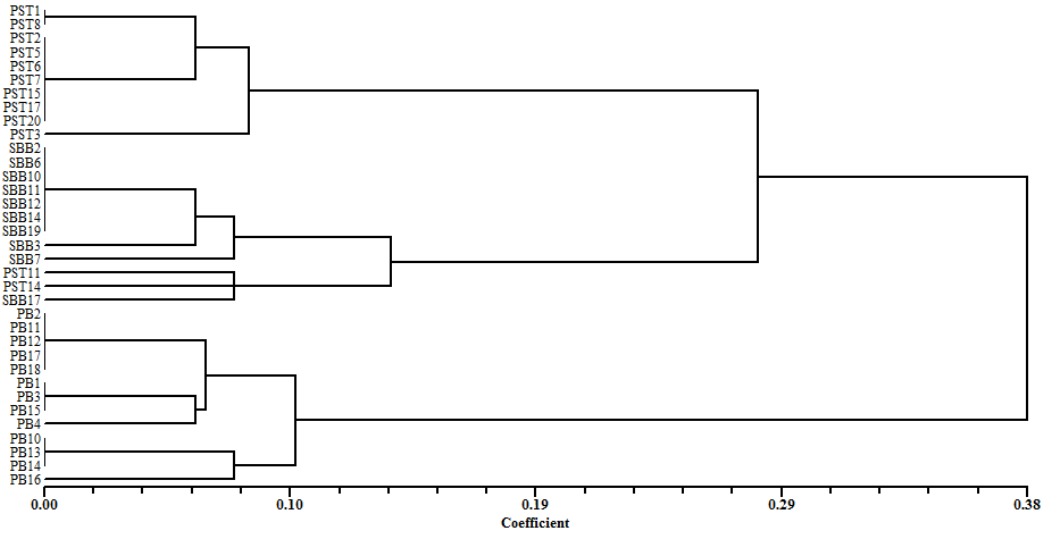

Figure 3. Dendrogram of grouping between mindi (*Melia azedarach*) individuals

Implications genetic diversity of *Melia azedarach*

Development of mindi seed source for community forest in Deli Serdang should be supported by sustainability high quality of seed. High quality of seed indicated by both physically or genetically (Yulianti et al. 2011; Pamoengkas 2012). In our research, microsatellites marker was used for determined genetic diversity of mindi in Deli Serdang-North Sumatra. The result showed that value of genetic diversity of mindi was moderate. Conservation of genetic resources should be done to prevent the existing population and collecting genetic resources from the center of genetically diverse population. The highest genetic diversity population (PB) could be chosen as priority population for the protection of original habitat. The variability of individual within population, showed that individual tree selection will be effective way for genetic improvement program. The infusion of parent trees also needed for improving the genetic diversity of mindi in Deli Serdang.

ACKNOWLEDGEMENTS

This research was supported by TALENTA grant of Universitas Sumatera Utara contract No: 6051/UN5.1/R/PPM/2016. Our sincerely thank also goes to PT Socfindo Sumatera Utara, for laboratory assistance.

REFERENCES

Abasolo MA, Fernando ES, Borromeo TH, Hautea DM. 2009. Cross-species amplification of shorea microsatellite DNA markers in *Parashorea malaanonan* (Dipterocarpaceae). Philippine J Sci. 138 (1): 23-28.

Al-Rubae AY. 2009. The potential uses of *Melia azedarach L.* as pesticidal and medicinal plant, Review. Am-Eur J. Sustainable Agric 3 (2): 185-194.

Aritonang KV, Siregar IZ, Yunanto T. 2007. Manual of Plant Forest Genetic Analysis at the Silviculture Laboratory of the Faculty of Forestry, Bogor Agricultural Institute. Faculty of Forestry IPB, Bogor.

Boonton C, Pandey M, Chaangtragon S. 2008. Isolation and characterization of microsatellite markers in Indian neem (*Azadirachta indica* var. *indica* A. Juss) and cross-amplification in Thai neem (*A. indica* var. *siamensis* Valenton). J Conserv Genet. DOI: 10.1007/s10592-008-9610-5.

Charlesworth D, Willis, JH. 2009. The genetics of inbreeding depression. Nat Rev Genet 10: 783-796.

Costa BF, Rodrigues LA, Ruas LA, Vieiera BG, Conson ARO, Ruas PM. 2013. Characterization of nine microsatellite loci for the tree species *Parapiptadenia rigida* (Fabaceae-Mimosoideae) and their transferability. Genet Mol Res 11 (3): 2338-2342.

Finkelday R. 2005. An Introduction to Tropical Forest Genetics. Institute of Forest Genetics and Forest Tree Breeding, Georg-August-University, Gottingen, Germany.

Hamid AH, Ab-Shukor NA, Senin AL. 2008. Morphometric and genetic variation of six seed sources of *Azadirachta excelsa* (Jack). Jacobs. J Biol Sci 8: 702-712.

Hamilton MB. 2009. Population Genetics. Wiley-Blackwell, New York, United States.

Irmayanti L, Siregar IZ, Pamoengkas P. 2015. Genetic diversity of mindi (*M. azedarach*L.) in community forests West Java assessed by microsatellite markers. J Silvikultur Tropika 6: 1-8.

Lemes MR, Esashika T, Gaoue OG. 2011. Microsatellite for mahoganies: Twelve new loci for *Swietenia macrophylla* and its high transferability to *Khaya senegalensis*. Amer J Bot 5: 207-209.

Maciel MV, Morais SM, Bevilaqua CML, Camurça-Vasconcelos ALF, Costa CTC, Castro CMS. 2006. Ovicidal and larvicidal activity of *Melia azedarach* extracts on *Haemonchus contortus*. Vet Parasitol 140: 98-104.

Nikoletta GN. 2010. Cytotoxic tirucallane triterpenoids from *Melia azedarach* fruits. Molecules 15: 5866-5877.

Nurtjahjaningsih ILG. 2007. Potential genetic infusion population to expand the genetic variation of *Pinus merkusii* seedling seedlings in Jember. J Pemuliaan Tanaman Hutan 3 (2): 73-81.

Pamoengkas P, Siregar IZ, Wijayanto N, Yulianti, Syamsuwida D. 2012. Seed sorce development of mindi (*Melia azedarach*) for community forest in West Java. J Ilmu Pertanian Indonesia 17: 1 36-40.

Perez RS, Ruiz D, Dicenta F, Egea J, Gomez PM. 2005.Application of Simple Sequence Repeat (SSR) markers in apricot breeding: Molecular characterization, protection, and genetic relationships. J Scientia Horticulturae 103: 305-315.

Rahman MK, Asaduzzaman M, Rahman MM, Das AK, Biswas SK. 2014. Physical and mechanical properties of ghoraneem (*Melia azedarach*) plywood. Bangladesh J Sci Industr Res 49 (1**)**. 47-52.

Rambey R. 2011. Local Knowledge of Mindi Agroforestry Systems (*M. azedarach* L.) (Case Study in Selaawi Village, Talegong District, Garut Regency, West Java Province) [Tesis]. Institut Pertanian Bogor, Bogor.

Rohlf FJ. 2008. Numerical Taxonomy and Analysis System (NTSYSpc) Version 2.0. University of New York, New York.

Roshetko JM, Mulawarman, Iriantono. 2004. Seed for Community. Cooperation inDirectoratee of Forest Plant Seedlings IFSP, World Agroforestry Center ICRAF, and Bina Swadaya, Suplemen Gedeha Edition XIV Year 2004.

Sharma D, Paul Y. 2013. Preliminary and pharmacological profile of *Melia azedarach* L.: An Overview. J Appl Pharmaceut Sci 3 (12): 133-138

Susilowati A, Rachmat HH, Siregar IZ, Supriyanto. 2018. Genetic diversity of resin yielder *Pinus merkusii* from West Java-Indonesia revealed by microsatellites marker. IOP Conf. Series: Earth and Environmental Science 122 (2018): 012060. DOI :10.1088/1755-1315/122/1/012060.

Syamsuwida D, Palupi ER, Siregar IZ, Indrawan A. 2012. Flowerinitiationn,morphologyy, and developmental stages of flowering-fruiting of mindi. Jurnal Manajemen Hutan Tropika 18 (1): 2-17.

Thakur S, Choudhary S, Bhardwaj P. 2016. Genetic diversity and population structure of *Melia azedarach* in North-Western Plains of India. J Trees 30 (5): 1483-1494.

Thakur S. 2013. Variation l Studies in *M. azedarach* Linn. Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni Solan, India.

Thode VA, Backes A, Mader G, Kriedt R, Bonnato SL, Freitas LB. 2013. Development of microsatellites for *Verbenoxylum reitzii* (Verbenaceae), a tree endemic to the Brazilian Atlantic Forest. Appl Plant Sci 1 (8): 1-3.

Varshney RJ, Chabane K, Hendre PS, Aggarwal RK, Graner A. 2007. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elit ebarleys. J Plant Sci 173: 638-649.

Wang HW, Duan JM, Zhang P, Cheng YG, Wu JW, Wang GZ. 2013. Microsatellite markers in *Paulownia kawakamii* (Scrophulariaceae) andcross amplificationn in other paulownia species. Genet Mol Res 12 (3): 3750-3754.

Wee AKS, Takayamaka K, Kajita T, Webb EL. 2013. Microsatellite loci for *Avicennia alba* (Acanthaceae), *Sonneratia alba* (Lythraceae) and *Rhizophora mucronata* (Rhizophoraceae). J Trop For Sci. 25 (1): 131-136.

Weising K, Nybon H, Wolff K, Kahl G. 2005. DNA Fingerprinting in Plants: Principles, Methods, andApplicationss. CRC Press Taylor & Francis Group, USA.

Yeh FC, Yang R. 1999. POPGENE Version1.31: User Guide Centre for International Forestry Research. University of Alberta, Edmonton, Alberta.

Yulianti, Siregar IZ, Wijayanto N, Darma IGKT, Syamsuwida D. 2011. Genetic variation of *M. azedarach* in community forests of West Java assessed by RAPD. Biodiversitas 12 (2): 64-69.