

Genetic variation of *Melia azedarach* in community forests of West Java assessed by RAPD

YULIANTI*, ISKANDAR ZULKARNAEN SIREGAR, NURHENI WIJAYANTO, IKG TAPA DARMA, DIDA SYAMSUWIDA

Department of Silviculture, Faculty of Forestry, Bogor Agricultural University. Fahatan Bld. Jl. Lingkar Akademik, Darmaga Campus, Bogor 16680, West Java, Indonesia. Tel. +62-251-8621947, Fax. +62-251-8621256, *E-mail: yuli_bramasto@yahoo.co.id, deptsilvik@ipb.ac.id.

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ABSTRACT

Yulianti, Siregar IZ, Wijayanto N, Tapa Darma IKG, Syamsuwida D (2011) Genetic variation of *Melia azedarach* in community forests of West Java assessed by RAPD. *Biodiversitas* 12: 64-69. *Melia azedarach* L. or mindi (local name) is one of the widely planted exotic species in Indonesia, mostly found in community forests in West Java. However, improving and increasing the productivity of mindi community plantation in West Java requires information on patterns of existing genetic diversity. The present work was aimed at estimating the genetic variation of mindi by using RAPD markers. Outcome of the activities was to propose appropriate conservation and management strategies of genetic resources in order to support the establishment of seed sources. Six populations of mindi plantation in the community forests were chosen for this research, i.e Sukaraja (Bogor-1), Megamendung (Bogor-2), Bandung, Purwakarta, Sumedang and Kuningan. Five primers (OPA-07, OPY-13, OPY-16, OPA-09 and OPO-05) producing reproducible bands were analysed for 120 selected mother trees in total, in which 20 trees per locality were sampled. Data were analysed using Popgene ver 1.31, NTSYS 2.02 and GenAlEx 6.3. Based on the analysis, the observed number of alleles per locus ranging from 1.43 to 1.60, and percentage of polymorphic loci (PPL) ranging from 43.33 to 60.00%. The levels of genetic variation were considered as moderate for all populations (H_e range from 0.1603 to 0.1956) and the the mean level of genetic diversity between population (G_{st}) was 0.3005. Cluster analysis and Principal Coordinates showed three main groups, the first group consists of 4 populations i.e Bandung, Kuningan, Purwakarta and Megamendung, the second was Sukaraja and the third was Sumedang. Based on Analysis of Molecular Variance (AMOVA), the Percentages of Molecular Variance within population (69%) is higher than that of between populations (31%). The moderate level of genetic variation in the community plantation forests, might be due to small population size, leading to reduce genetic variability. Further analysis is required to confirm this findings using other genetic marker.

Key words: *Melia azedarach*, RAPD, genetic variation, community forest.

INTRODUCTION

Mindi (*Melia azedarach* L.) is one of the family members of Meliaceae. and categorized as an exotic species because this plant was originated from the Southern Asia and spread to East Africa, Middle East, American continent and Indonesia. Mindi, in West Java can be found in Bogor, Cianjur, Bandung, Sumedang, Purwakarta, Subang, Kuningan, Majalengka and Garut in agriculture lands owned by community members or community forests (Pramono et al. 2008). Mindi grows well in soils with good drainage, deep soil, and sandy clay soils with pH of between 5.5-6.5. Mindi could grow in hills situated in low elevation, up to the high elevation (700-1400 m above sea level), with rainfall between 600-2000 mm/year and climate type A-C (Martawijaya et al. 1989; Soerianegara et al. 1995; Ahmed and Idris 1997; Wulandini et al. 2004). Information on growth site distribution and condition as well as genetic variability of mindi is necessary for developing sound strategies in the establishment of seed sources. One of the factors that influence patterns of genetic variation in nature is the pollination mechanism

(mating system) in plants (Sedgley and Griffin 1989), this mechanism depend on the structure of the flower. Mindi's flower is a kind of compound or in a series of flowers (spike), known as the panicle, flower structure is closely associated with the pollination, which could be by animals or by wind. Mindi is an individual polygamaous which is each tree consists of male flowers and also a hermaphrodite because each flower posses both male and female reproductive organs (Styles 1972).

Development of community forest with mindi requires constant supply of seeds (seed procurement) of high quality, either physically, physiologically or genetically. One of the significant factors determining seed quality is the seed origin which is usually related closely with genetic quality of the seed. Generally, the use of high quality seeds in community forests is not greatly considered as shown by a study of Asmanah (2005) in Grand Forest Park Wan Abdul Rachman Lampung where only 7.8% of the farmers purchased planting stocks from outside, while most of them used any kind of planting stocks originating from stands in the surrounding areas, either in the form of seeds or in the form of uprooted seedlings. In addition. Roshetko et al.

(2004) reported that most seeds being used by forest farmers were collected from trees (seed trees) in the farmer's land or in traditional land, and the seeds from such seed sources were usually collected only from few trees (1 to 5 trees) without any knowledge concerning their origin, and it was supposed probably that their genetic variability is narrow (Dhakal et al. 2005). The same phenomena was also hypothesized to occur in mindi community plantation forest in West Java.

The status of genetic variability can be determined using several methods such as isoenzymes and other DNA based markers. One of the techniques of DNA analysis that is still used to capture roughly the levels of genetic variabilities is Random Amplified Polimorphic DNA (RAPD). According to Brown et al. (1993) and Saiki et al. (1988), this technique is usually used to show the level of DNA variability among species and also among individuals within species which are closely related, as well as being able to detect the presence of variation of nucleotide arrangement within DNA. This technique has been applied for detecting population genetic variability of species of dukuh (Song et al. 2000), citrus (Karsinah et al. 2002),

cashew (Samal et al. 2003), *Melia volkeensii* (Runo et al. 2004), sandalwood (Rimbawanto et al. 2006), ulin (Rimbawanto et al. 2006), merbau (Rimbawanto and Widyatmoko 2006), neem (Kota et al. 2006), Pulai (Hartati et al. 2007), sungkai (Imelda et al. 2007), meranti (Siregar et al. 2008), gaharu (Siburian 2009; Widyatmoko et al. 2009) and jelutung (Purba and Widjaya 2009).

There is an urgent need to initiate a program of genetic improvement, considering the increasing areas of mindi plantation in West Java. Therefore, this experiments were carried out with aims at determining the patterns of genetic diversities of mindi in West Java community plantation based on DNA marker, i.e. RAPD, to support the improvement program of seed source of mindi.

MATERIALS AND METHODS

Materials

Materials used in this research were mindi individuals growing in six locations of community plantation forests in West Java, Indonesia as described in Figure 1 and Table 1.

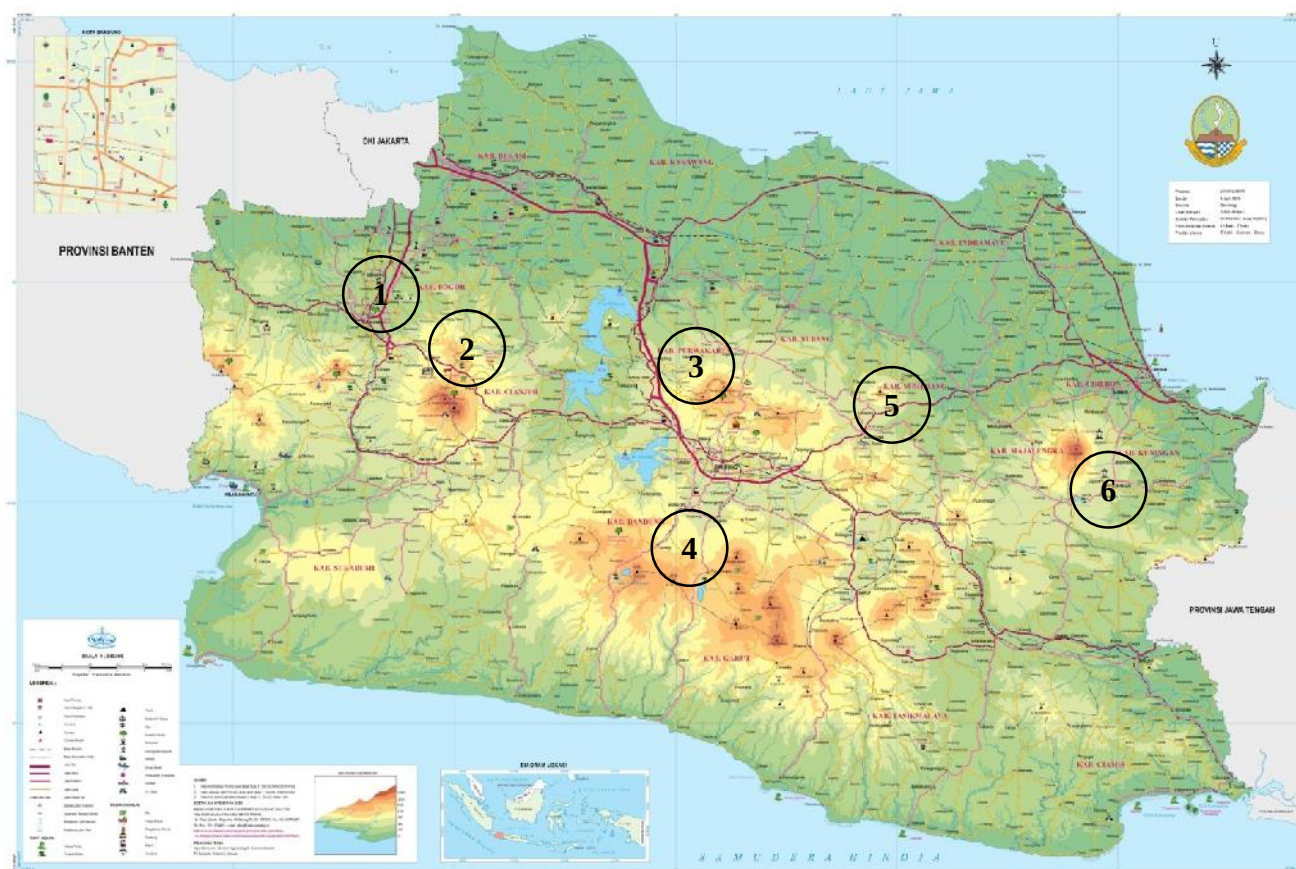


Figure 1. Approximate geographical locations of sampled populations of mindi in West Java community forests: (1) Nagrak village, Sukaraja subdistrict, Bogor, (2) Tegal Mendi, Sukakarya village, Megamendung subdistrict, Bogor, (3) Legok Huni village, Wanayasa subdistrict, Purwakarta, (4) Gambung, Mekarsari village, Pasir Jambu subdistrict, Bandung, (5) Padasari village, Cimalaka subdistrict, Sumedang, (6) Babakan Rema village, Kuningan subdistrict, Kuningan

Table 1 Details of sampled locations of mindi community plantation in West Java

Name of locations	Geographic position	Elevation (m asl)	Temp. (°C)	RH (%)
Nagrak village, Sukaraja subdistrict, Bogor	06° 40' 472" S, 106° 53' 615"E	250-350	26-27	70-75
Tegal Mendi, Sukakarya village, Megamendung subdistrict, Bogor	06° 40' 477" S, 106° 53' 635"E	711-721	24-27	70-75
Legok Huni village, Wanayasa subdistrict, Purwakarta	06° 39' 378" S, 107° 32' 479"E	617	28-29	70-75
Gambung, Mekarsari village, Pasir Jambu subdistrict, Bandung	07° 14' LS, 107° 5144' BT	700-1400	20-28	40-50
Padasari village, Cimalaka subdistrict, Sumedang	06° 47' LS, 107° 56' BT	600-700	24-28	80-85
Babakan Rema village, Kuningan subdistrict, Kuningan	06° 45' LS, 105°20' BT	417	30-36	50-60

In each location, 20 parent trees were selected. For obtaining the DNA extract using standard procedure of CTAB (Doyle and Doyle 1987). For analysis of genetic variation, five primers were used, which showing reproducible and polymorphic bands, namely OPA-07; OPY-13; OPY-16; OPA-09 and OPO-05, with DNA sequences as shown in Table 2. Those primer have been selected from twenty five random primers (Table 2)

Table 2 Five random primers from selection of twenty five primer for genetic analysis

Primer	Sequences	Primer	Sequences
OPU-08	5'-GGCGAAGGTT-3'	OPY-20	5'-AGCCGTGGAA-3'
OPU-09	5'-CCACATCGGT-3'	OPA-14	5'-TCTGTGCTGG-3'
OPO-11	5'-GACAGGAGGT-3'	OPY-16	5'-GGGCCAATGT-3'
OPB-13	5'-TTCCCCCGCT-3'	OPA-16	5'-AGCCAGCGAA-3'
OPO-05	5'-CCCAGTCACT-3'	OPY-18	5'-GTGGAGTCAG-3'
OPA-09	5'-GGGTAACGGG-3'	OPU-14	5'-TGGGTCCCTC-3'
OPB-09	5'-TGGGGGACTC-3'	OPO-13	5'-GTCAGAGTCC-3'
OPO-10	5'-TCAGAGCGCC-3'	OPA-05	5'-AGGGGTCTTG-3'
OPO-16	5'-TCGGCGGTTC-3'	OPY-12	5'-AAGCCTGCGA-3'
OPB-08	5'-GTCCACACGG-3'	OPA-07	5'-GAAACGGGTG-3'
OPB-20	5'-GGACCCTTAC-3'	OPY-13	5'-GGGTCTCGGT-3'
OPA-03	5'-CATCCCCCTG-3'	OPA-12	5'-TCGGCGATAG-3'
OPU-04	5'-ACCTTCGGAC-3'		

Procedures

Isolation of DNA from each individual tree was extracted from leaves following a standard CTAB procedure (Doyle and Doyle 1987). PCR reaction (total volume about 13.5 µL) was performed using 2 µL DNA with Go taq green master mix (Promega) as much as 7.5 µL, and 2.5 µL nuclease-free water (H₂O) and 1.5 µL primer (0.5-2 M). This mixture was subsequently put into PCR-PTC100 MJ Research with regulated temperature and reaction steps with total cycle of 37 times, namely 1 time pre-denaturation (95°C for 2 minutes), followed by denaturation at 95°C for 1 minute, and afterwards annealing at 35°C for 2 minutes and extension at temperature of 72°C for 2 minutes. These steps occurred 35 times and the last was 1 time final extension (72°C for 5 minutes). The replication must be done if the band of DNA still not clear.

Results of PCR were subjected to electrophoresis by using agarose 2% at voltage of 100 v for ± 25 minutes and then followed by soaking in EtBr (1%, v/v) for 15 minutes. DNA electropherogram was visualised under observation in UV transluminator and photographed. The photograph results were analyzed using binary scoring of the banding

patterns, i.e. presence or absence of band. Estimation of genetic variability within and between populations was computed using softwares of POPGENE ver. 1.31 (Nei 1972), NTSYS 2.02 (Rohlf 1998) and GenAlEx 6.3 (Peakall and Smouse 2006).

Genetic variability was estimated using following parameters: percentage of polymorphic loci (PLP); number of observed alleles (na); number of effective alleles (ne); expected heterozygosity (He), genetic differentiation (Gst). In addition, Principal Coordinates Analysis (PCA) and Analysis of Molecular Variance (AMOVA) were also performed to determined the partition of genetic variation.

RESULTS AND DISCUSSION

Amplification and DNA polymorphism

Examples of DNA polymorphic patterns were shown in Figure 2A, B and C.

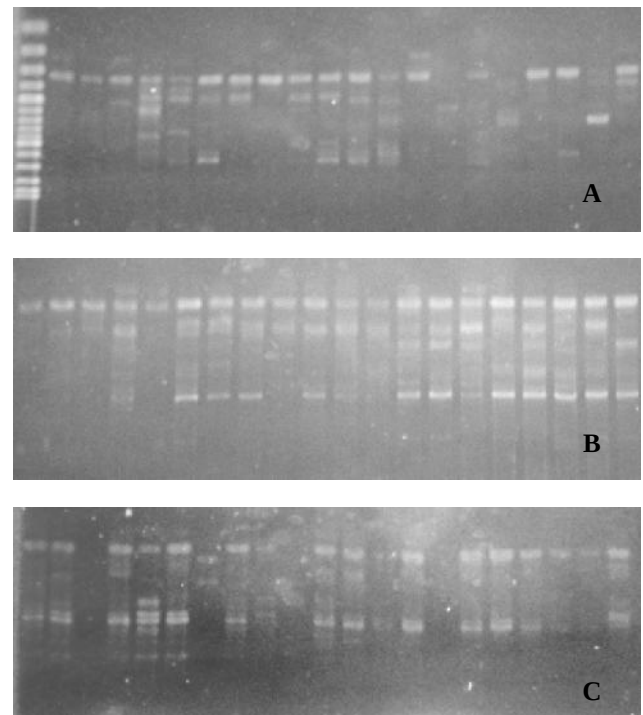


Figure 2. Polymorphic pattern at primer OPA-07 of mindi population from (A) Megamendung (Bogor-2), (B) Sukaraja (Bogor-1) and (C) Sumedang

Genetic variation within population

Values of genetic variability were shown in Table 4.

Table 4. Parameter values of genetic variability of mindi population in community forests of West Java

Population	N	PPL	na	ne	He
Bandung	20	53.33	1.533	1.2748	0.1613
Kuningan	20	53.33	1.533	1.2665	0.1603
Megamendung	20	53.33	1.533	1.3007	0.1790
Purwakarta	20	51.67	1.516	1.2933	0.1712
Sukaraja	20	43.33	1.433	1.2922	0.1612
Sumedang	20	60.00	1.600	1.3198	0.1956

Notes: PPL= Percentage of polymorphic loci; na = Observed number of alleles; ne = Effective number of alleles; He = Gene diversity

Genetic variation, within population of mindi, i.e. He, was categorized as moderate ranging between 16-19%. This level of variation can also be observed from other variables such percentage of polymorphic loci (PPL) ranging from 43.3-60.0%, with average value of 52.50. Population which harbored the lowest variability was Kuningan (0.1603), followed in terms of rank by populations of Sukaraja and Bandung (0.1612 and 0.1613), whereas population of Purwakarta and Megamendung exhibited variability of 0.1712 and 0.1790, respectively. Among the six populations being tested, Sumedang population possessed the highest genetic diversity, namely He = 0.1956. There are several drivers that affect the level of genetic diversity of a species within a population, such as effective size of population, mutation, genetic drift, migration, mating system, selection and production of flower and pollen (Siregar 2000; Lemes et al 2003; Finkeldey 2005; Hamid et al. 2008). The moderate level of genetic diversity of mindi at community forest or farmland could be as a consequence of a small population size, this situation have been proved by *Milicia excelsa* at traditional agroforestry system in Benin, Western Africa (Ouinsavi and Sokpon 2008). This could be due to scattered distribution of trees and plantation boundaries or sparsely grown which could lead to the reduction in population size. The small population size also can increase the possibility of genetic drift, which will reduce the genetic variability as a result of bottlenecking and inbreeding (Hamid et al. 2008)

Other genetic parameter being measured was value of genetic differentiation (Gst) and the value of Gst was 0.3005. This implies that average genetic variability between populations of mindi plants in community forests was around 30%. On the basis of AMOVA, as shown in Table 5, genetic variability which was contained within population was as large as 69%, whereas genetic variability between population was 31%. This is in accordance with opinion of Hamrick and Godt (1996) that most genetic variability was stored within population, whereas differences between populations were small. Craft and Ashley (2007) reported that *Quercus macrocarpa* populations harboured genetic variability within population

was 97.31%, whereas that between population was only 2.69%.

Table 5. Results of Analysis of Molecular Variance (AMOVA)

Source of variability	Degree of freedom	Sum of Square	Mean sum of square	Est. var.	%
Between pop.	5	265.092	53.018	2.380	31
Within pop.	114	617.150	5.414	5.414	69
Total	119	882.242		7.794	100

Genetic variation between populations

Genetic distance was calculated on the basis of Nei (1972) to measure genetic variation between population (Table 6).

Table 6. Genetic distances (Nei 1972) between mindi populations in community forests in West Java

	Bandung	Kuningan	Megamendung	Wanayasa	Sukaraja	Sumedang
Bandung	0.0000					
Kuningan	0.0352	0.0000				
Megamendung	0.0626	0.0770	0.0000			
Purwakarta	0.0740	0.0718	0.0976	0.0000		
Sukaraja	0.0919	0.0881	0.1357	0.1127	0.0000	
Sumedang	0.1459	0.1560	0.1681	0.1201	0.1920	0.0000

Table 6 shows that populations of Bandung, Kuningan and Megamendung possess genetic distances which are considerably close. The highest genetic distance is seen between population of Sumedang and Sukaraja (0.1920). Genetic relationship and grouping patterns of populations on the basis of genetic distances can be seen in Figure 3 and 4.

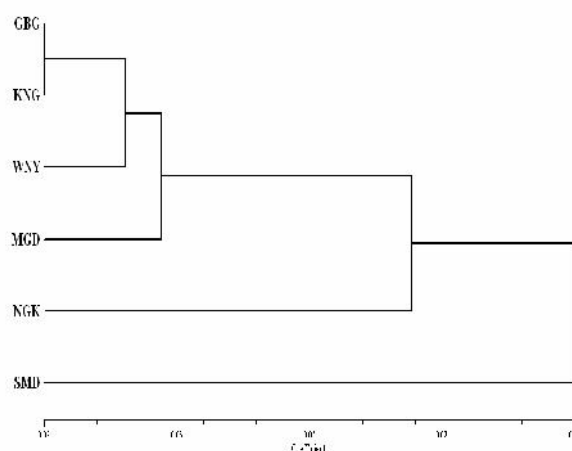


Figure 3. UPGMA-dendrogram of mindi populations of plantation community forests in West Java. Notes: GBG: Bandung; KNG: Kuningan; WNY: Purwakarta; MGD: Megamendung; NGK: Sukaraja and SMD: Sumedang

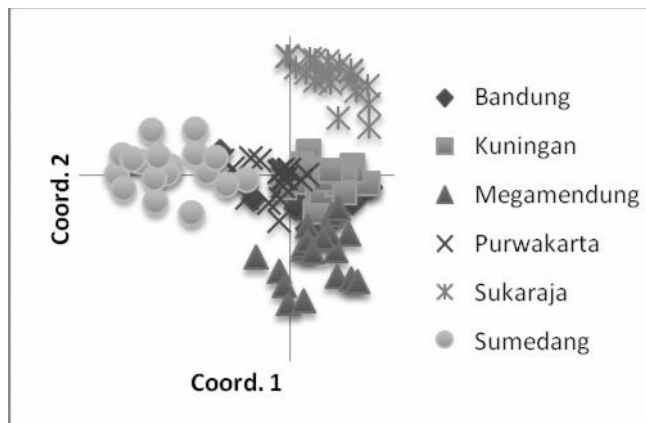


Figure 4. Groupings of mindi populations in community forests of West Java based on Principal Coordinates Analysis (PCA)

Based on dendrogram of genetic distances (Figure 3) and groupings by PCA (Figure 4), it could be seen in general that there are three groupings of mindi populations. First group consists of Bandung, Kuningan, Megamendung and Purwakarta populations. The second cluster comprises only population of Sukaraja, and the third cluster comprises also one single population of Sumedang. These groupings imply that populations of Bandung, Kuningan, Megamendung and Purwakarta have close genetic distances. However, in relation with geographic the four populations have considerably far distances. From the historical point of view, it is known that mindi populations were introduced for the first time in tea plantation area. Of the four populations, Bandung, Purwakarta and Megamendung were actually part of and close to the tea plantations. It is most likely that mindi population occurring in community lands were originated from these plantation areas. The situation was different for populations of Sukaraja and Sumedang which are not plantations. This may be a cause of existing genetic structures showing considerably differences from those of other populations.

The existing patterns of genetic variation may indicate the current status of genetic resources of mindi in West Java. Moderate genetic variation, i.e. below 20%, observed in all investigated populations indicated that genetic resources of mindi plants in the community forests are still maintained by evolutionary factors. The sustainable utilization of existing resources in particular can be recommended in form of seed sources. This condition is also explained by the fact that mindi is an exotic species. It is very likely that during the initial introduction to Indonesia, seeds used for the initial planting were not originated from many sources. According to Roshetko et al. (2004) and Dhakal et al. (2005), most seeds which were used by forest farmers were collected from trees (seed trees) in farmer's land or traditional land, and seeds from these seed sources were often collected from only a few trees (1 to 5 trees) without any knowledge concerning the origins of seed. Based on the results of this study, mindi population in Sumedang have the highest diversity, so mindi plantation of this location could become a candidate of seed sources. It is expected that the seeds produced from

this population could be used to increase the genetic diversity in other populations, especially at the same site of this seed source.

CONCLUSION

Genetic variation of mindi from six populations in community forests in West Java, showed moderate levels ranging from 16-19%. To increase genetic diversity of mindi in community forests in West Java, a population of mindi that has a highest genetic variation can be used as a source of genetic material to other populations to increase genetic diversity. On the basis of genetic distances, there were identified three clusters namely (i) Bandung, Kuningan, Megamendung and Purwakarta, (ii) Sukaraja and (iii) Sumedang. The information of genetic distance among population can be used in the through exchange of genetic material between populations.

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