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Genetic Variation of Ceiba pentandra (L.) Gaertn. from Three Populations in West Sumatra, Indonesia Based on RAPD Markers

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Abstract— Ceiba pentandra (L.) Gaertn. is one of the cotton-producing plants. However, there is a population decline in some areas, resulting in a decrease in genetic variation. This study aims to determine intrapopulation and interpopulation genetic variation and genetic differentiation of C. pentandra in three populations in West Sumatra. The research was conducted with the descriptive method using molecular data and sample collection with the survey method. Analysis of genetic variation using RAPD (Random Amplified Polymorphic DNA) markers on 15 individual plants collected from three populations, namely Solok, Pesisir Selatan, and Padang Pariaman. The results showed that primers OPA-01, OPA-02, and OPB-10 could detect polymorphism. The value of intrapopulation genetic variation was highest in the South Coastal population (H = 0.1857) and lowest in the Solok population (H = 0.1228). Interpopulation genetic variation (D_{ST} = 0.0326) was lower than intrapopulation genetic variation (Hs = 0.1607), with a low genetic differentiation value (G_{ST} = 0.1686) and a high gene flow value (Nm = 2.4660). Cluster analysis showed that the Pesisir Selatan and Solok populations had the farthest genetic distances (0.0580). UPGMA analysis shows that accessions do not cluster specifically based on their population.

Keywords—Ceiba pentandra, genetic diversity, RAPD

I. INTRODUCTION

Kapok *(Ceiba pentandra* (L.) Gaertn.) grows and is widely distributed in Central America, South America, Southeast Asia, and West Africa [1]. Its distribution in the Asian continent includes several countries such as India, Thailand, the Philippines, and Indonesia [2]. *C. pentandra* can grow in the lowlands up to 400 meters above sea level and in places with hot weather [3]

The main product of *C. pentandra* is fruit fiber, which produces silk cotton and is used as a textile material, pillow filler, and mattress [4, 5, 6]. Stems are used as materials for making furniture [7], pulp, paper products, agricultural equipment, matches, and firewood [8, 9, 4, 10]. Leaves as herbal medicine in treating diseases such as fever, scar removal, and diarrhea [11].

In Indonesia, *C. pentandra* is widely developed by the people, private plantations, and government plantations (BUMN). Indonesia was once the largest producer of kapok, about 80% before World War I, and about 60% of the total production at that time came from Java Island [12]. In 2014, the area of kapok plantations reached 144.30 thousand hectares, with fiber production reaching 55.30 thousand tons [13].

West Sumatra is one of the provinces where some areas still produce kapok. According to data from the West Sumatra Central Bureau of Statistics (2020) [14], kapok production in West Sumatra in 2015 was 139 tons, in 2016–2017 was 70.15 tons, in 2018 was 55 tons, and in 2019–2020 was 51 tons. The region with the highest kapok production in West Sumatra is Tanah Datar Regency, followed by Solok Regency and Padang Pariaman Regency. There is a decrease in production in several regions of West Sumatra every year. Several regions in 2015 still produced kapok, but in 2020, production became zero, such as the Pesisir Selatan District, Sijunjung District, and Agam District. Based on the preliminary survey, the population of *C. pentandra* in Solok District, Padang Pariaman District, and Pesisir Selatan District has decreased.

The decline in the population of *C. pentandra* is due to its declining economic value. *C. pentandra* is largely ignored because people are more interested in using pillows and spring bed mattresses made from synthetic fibers such as foam because they are more comfortable. In addition, the logging of *C. pentandra* without replanting, the lack of public knowledge about the other potentials of the plant, and the marketing aspect, which is considered less profitable, are also the causes of the low preservation of this plant [15]. As a result, the population of *C. pentandra* continues to decline, including in several areas in West Sumatra. This can be seen from the production of kapok, which has continuously decreased from year to year [14].

The declining population of *C. pentandra* can lead to a decrease in genetic variation caused by inbreeding, genetic drift, limited gene flow, and a small population size [16]. Low genetic variation can affect survival and reproductive fitness, limit the ability of populations to adapt to environmental changes, and increase the risk of extinction [17]. Therefore, information on the genetic variation of *C. pentandra* is needed.

As a basis for efforts to determine the genetic variation of plants, it can be done with a more accurate approach, namely the molecular approach. Molecular markers provide tools that can measure the level of genetic variation in populations [18]. One of the molecular markers used to analyze genetic variation is RAPD (Random Amplified Polymorphic DNA) [19]. Several studies on *C. pentandra* have been conducted previously. Brondani RPV et al. [20] reported high genetic diversity of *Ceiba pentandra* in the Amazon Forest using microsatellite markers, with 112 alleles detected and He values ranging from 0.814 to 0.895. Abengmeneng et al. [21] reported genetic relationships among 36 *Ceiba pentandra* genotypes using RAPD and ISSR markers, obtaining nine accessions that can be used as candidates for conservation as seed trees and in the *Ceiba pentandra* breeding program in Ghana. To assess the genetic variation of *C. pentandra* in several regions in West Sumatra where populations are declining, it is necessary to conduct research using RAPD markers, where information about this genetic variation can be used as a basis for consideration in conservation efforts.

II. Research Methods

Plant materials

The study was conducted from January 2022 to May 2022. Fifteen accessions of C. pentandra were collected from three West Sumatra populations: Pesisir Selatan, Solok, and Padang Pariaman (Figure 1). Each population consisting of five accessions. Young leaf samples were stored in plastic bags and added silica gel to dry until DNA extraction was carried out in the laboratory.

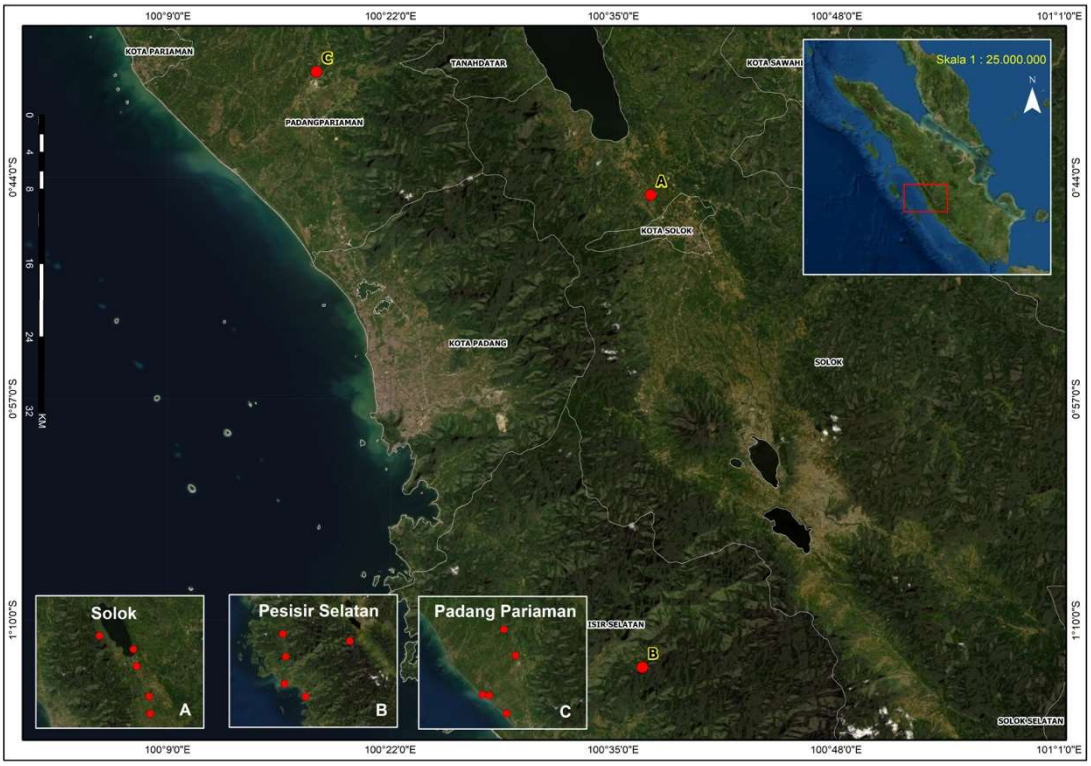


Figure 1. Sampling locations in theree populations in West Sumatra: Solok, Pesisir Selatan, and Padang Pariaman

DNA extraction

DNA was extracted from young leaves using the CTAB (Cetyl Trimethyl Ammonium Bromide) method, according to Doyle and Doyle (1987) [22]. The DNA extraction was carried out with the following steps: 15 mg of young leaf tissue was frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle. The pulverized materials were transferred to a microtube, and 750 mL of extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM Na2EDTA, 1.4 M NaCl, 2% (w/v) CTAB, and 0.2% (v/v) mercaptoethanol) solution was added. The tubes were vortexed for a few seconds and incubated at 65 °C for 45 minutes (every 10 minutes, the tube is vortexed). Samples were centrifuged for 10 minutes at 27 °C and 12.000 rpm. The supernatant was transferred to a new sterile tube and chloroform: isoamyl alcohol (24:1) was added equal to the volume of the supernatant and vortexed. The tube was centrifuged at 27 °C and 12.000 rpm for 10 minutes. The supernatant was transferred to a sterile microtube, and isopropanol equal to the volume of the supernatant was added. The tube was centrifuged at 4 °C and 12.000 rpm for 10 minutes. The supernatant was removed, added 200 mL of cold ethanol 70% was. The tube was centrifuged at 4°C and 12.000 rpm for 5 minutes. The supernatant was removed and rinsed again with 200 mL cold ethanol 70%. The tube was centrifuged at 4°C and 12.000 rpm for 2 minutes. The DNA pellet was dried over the tissue for 1-2 hours by inverting the tube. Then, added 50 mL TE buffer to dissolve the DNA pellet. Furthermore, it was stored as a stock at -20 °C.

RAPD primer selection and DNA amplification

A total of 10 RAPD primers were used for selection (Table 1). The selected primers that produced polymorphic bands were used for PCR amplification. The PCR was carried out at a total volume of 25 µL containing a mixture of 12.5 µL My Taq TM Red Mix Bioline as PCR reagent (10 mM dNTPs, 50 mM MgCl2, 1 unit of Taq DNA Polymerase), 2 µL primer, 6.5 µL nuclease-free water, and 4 µL DNA isolate. Amplification was carried out in a SensoQuest thermocycler with a cycle programmed for 45 cycles of each of the following conditions: initial denaturation at a temperature of 94°C for 2 minutes, followed by denaturation at 94°C for 1 minute, annealing at 34°C for 1 minute, elongation at 72°C for 2 minutes 20 seconds, and the final condition was elongation at 72°C for 10 minutes.

Table 1. RAPD primer name and sequence were used for selection in this study

Primer name	Sequences (5'-3')	Reference
OPA-01	CAGGCCCTTC	[23]
OPA-02	TGCCGAGCTG	[24]
OPA-03	AGTCAGCCAC	[25]
OPA-07	GAAACGGGTG	[25]
OPA-08	GTGACGTAGG	[26]
OPA-10	GTGATCGCAG	[25]
OPA-13	CAGCACCCAC	[25]
OPA-16	AGCCAGCGAA	[27]
OPB-08	GTCCACACGG	[28]
OPB-10	CTGCTGGGAC	[29]

Electrophoresis

The PCR results were electrophoresed on a 2% agarose gel with 60 Volt, 150 mA, and 20 Watts for 2 hours. To determine the size of the DNA band, a 100-bp DNA ladder was inserted into the gel well in as much as 5 µL. Staining was done by soaking the gel in SYBRTM Safe DNA Gel Stain for 30 minutes. The resulting amplified bands were observed using a GelDoc UV Transilluminator.

Data analysis

Each known size DNA band was scored. The DNA band was given a score of one (1) if present and a score of zero (0) if absent. The results of the binary data matrix were analyzed using the software POPGENE32 [30]. The obtained matrix data were then subjected to cluster analysis using the unweighted pair group method with the arithmetic mean (UPGMA) method based on the genetic distance matrix introduced by Nei [31].

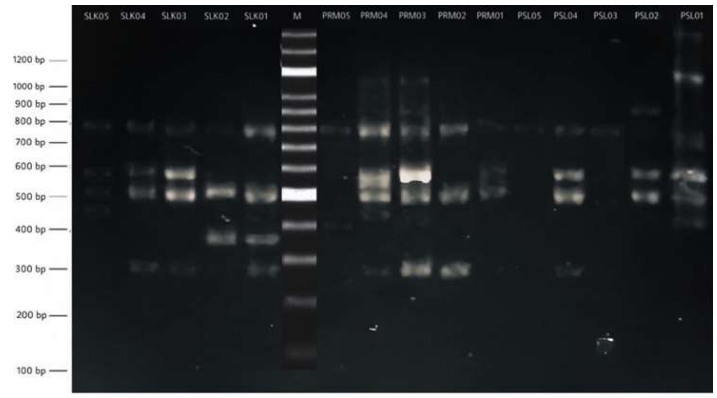
III. Results and Discussion

RAPD profile

Three useful primers (OPA-01, OPA-02, and OPB-10) were produced from ten primers that were screened. Amplification of the total DNA genome using these three RAPD primers in 15 *C. pentandra* samples produced clear and reproducible PCR products, as shown in Figure 2.

The result revealed 45 DNA fragments ranging from 154 bp to 1150 bp with 91,67–100% polymorphic DNA. Three primers produced 44 bands, averaging 14.67 bands per primer. Primer OPA-01 produced the highest bands (n = 19), whereas primer OPB-10 produced the lowest bands (n = 12) (Table 2). Based on research of Said [23] primer OPA-01 on three plants of the Malvaceae family produced DNA band lengths ranging from 200 bp to 1300 bp. Alfy et al. [29] reported that the OPB-10 primer on *Chorisia insignis* produced DNA band lengths ranging from 250 bp to 1300 bp. Hariyati et al. [24] reported that the OPA-02 primer on *Durio zibethinus* produced DNA band lengths ranging from 200 bp to 1100 bp.

There was one monomorphic band with a band size of 530 bp on primer OPB-10. Polymorphic bands produced by some sample individuals indicate the presence of genetic variation, while monomorphic bands, owned by all sample individuals, indicate the absence of genetic variation. Thus, the three primers are suitable for markers to detect genetic variation in *C. pentandra*.



(a)

(b)

(c)

Figure 2. RAPD amplification product of PCR using three primers, (a) OPA-01, (b) OPA-02, (c) OPB-10

Table 2. Polymorphism analysis results of three RAPD primers amplification of *C. pentandra*

No.	Primer name	Sequence (5'-3')	Number of bands	Number of polymorphic bands	Number of monomorphic bands	Percentage polymorphic bands (%)	Band size range (bp)
1	OPA-01	CAGGCCCTTC	19	19	0	100	264-1147
2	OPA-02	TGCCGAGCTG	14	14	0	100	212-1150
3	OPB-10	CTGCTGGGAC	12	11	1	91,67	154-1112
Total			45	44	1	291,67	
Average			15	14,67	0,33	97,23	

The research results on three populations of *C. pentandra* showed the presence of specific bands (specific alleles) that are only found in one population (Table 3). In the Solok population, one specific band is only found in that population and not in other populations. The specific band in the Solok population is found in the OPA-02 primer with a band size (allele) of about 435 bp, while the Pesisir Selatan and Padang Pariaman populations do not show specific bands. The specific bands found in *C. pentandra* in the Solok population indicate that the OPA-02 primer can be used as a marker primer in this population. Suparningtyas et al. [32] reported that the presence of specific bands illustrates the presence of unique genotypes that can be used to identify several clones of *Hevea brasiliensis*.

Table 3. Specific DNA band of *C. pentandra*

Population	Primer			Total
	OPA-01	OPA-02	OPB-10	
Pesisir Selatan	-	-	-	-
Padang Pariaman	-	-	-	-
Solok	-	435	-	1

Genetic variation within the population

Genetic variation of C. pentandra varied for each population (Table 4). The population of *C. pentandra* in Pesisir Selatan has the highest value for all parameters of genetic variation [33], they were H (0.1857), Ne (1.2908), PLP (66.67%), Na (1.667), and I (0.2948).

Tabel 4. Parameter value of genetic variation of *C. pentandra* populations

No.	Population	Total samples	Na	Ne	H	I	N	PLP
1	Pesisir Selatan	5	1.6667	1.2908	0.1857	0.2948	30	66.67%
2	Padang Pariaman	5	1.6000	1.2637	0.1735	0.2744	27	60.00%
3	Solok	5	1.4000	1.1950	0.1228	0.1909	18	40.00%

Note: Na = The average number of alleles observed, Ne = The average number of effective alleles, H = Heterozygosity, I = Index Shannon, N = Number of polymorphic loci, PLP = Percentage of polymorphic loci

Based on the heterozygosity (H) values in the populations of Pesisir Selatan, Padang Pariaman, and Solok, they are 0.1857, 0.1735, and 0.1228, respectively (Table 5). Based on these values, the three populations can be categorized as having low genetic variation. Of the three populations that have low genetic variation, Pesisir Selatan has higher genetic variation compared to the Padang Pariaman population and the Solok population. This is based on the determination of the value of heterozygosity by

Nei [31], which states that the value of heterozygosity ranges from 0 (zero) to 1 (one). If the H value ranges from 0.1–0.4, it means low genetic diversity; a value of 0.5–0.7 means moderate genetic diversity; and a value of 0.8–1.0 means high genetic diversity.

Based on the values of Na, Ne, H, I, and PLP, it shows that the Pesisir Selatan population has higher genetic variation than the Padang Pariaman population and the Solok population. This is probably related to differences in habitat conditions in each population. In the Pesisir Selatan population, the *C. pentandra* habitat remains undisturbed. Meanwhile, in the Solok population, *C. pentandra* habitat has generally been disturbed due to community activities. Jump and Penuelas [34] reported that habitat fragmentation in Fagus sylvatica caused a reduction in population size and increased inbreeding, resulting in a reduction in genetic variation. Fuchs et al. [35] reported that in fragmented forests, there were significantly fewer pollinator visits than in natural forests due to reduced resources.

Genetic variation among the population

The total of genetic variation in all populations (H_T) is 0.1932 with the value of genetic variation in the population (H_S) is 0.1607 and the value of genetic variation among population (D_{ST}) is 0.0326. D_{ST} values that are lower than H_S indicate that genetic variation within populations is higher than genetic variation among populations. This is probably due to the outcrossing mating system that occurs between populations of *C. pentandra.* Hamrick and Godt [36] reported that outcrossing species have low interpopulation genetic variation values due to genetic mixing between populations.

Tabel 5. The mean value of genetic variation based on analysis of Nei (1978) using RAPD marker

Total samples	H_T	H_S	D_{ST}	G_{ST}	Nm
15	0.1932	0.1607	0.0326	0.1686	2.4660

Note: H_T = The total population heterozygosity, H_S = Heterozygosity within population, D_{ST} = Heterozygosity among population, G_{ST} = Genetic differentiation, Nm = Gene flow value

The value of genetic differentiation among populations (G_{ST} = 0.1686) in this study is relatively low. Low genetic differentiation among populations is probably due to gene flow among populations, which causes a low level of diversity between populations. Kinho et al. [37] reported that the G_{ST} value varies from 0 to 1, and if the value is close to 0, then the genetic difference among populations is getting smaller. Loveless and Hamrick [38] reported that outcrossing plants have a G_{ST} value of less than 10%.

The results showed that the value of gene flow (Nm = 2.4660) was high. High gene flow is probably influenced by the contribution of pollinator agents and outcrossing reproductive systems. High outcrossing causes high gene flow, so plants will tend to have uniform genetic variation. According to Wright [39], Nm values are divided into three categories: low ($0.0 < Nm < 0.249$), medium ($0.250 < Nm < 0.99$), and high ($Nm \geq 1$). Slatkin and Maddison [40] reported that if the gene flow value of $Nm > 1$, it will result in little genetic differentiation between populations.

Outcrossing in *C. pentandra* can be assisted by bats as pollinators. Singaravelan and Marimuthu [41] reported that there are three species of bats (Pteropodidae) that frequent *C. pentandra* trees, namely *Cynopterus sphinx* and *Pteropus giganteus* that visit trees throughout the night and *Rousettus leschenaulti* that visit trees in the afternoon. The movement of pollinators affects the genetic diversity among populations because pollinators have the capacity to move pollen over relatively long distances and encourage outcrossing.

Genetic distance and cluster analysis

Based on genetic distance, the population of Pesisir Selatan and Solok has the highest genetic distance (0.0580). In contrast, the population of Pesisir Selatan and Padang Pariaman has the closest genetic distance (0.0186) (Table 6). This indicates that the Pesisie Selatan population is genetically more similar to the Padang Pariaman population than the Solok population. Arsih et al. [42] reported that a high genetic distance indicates the relationship between the two populations is distant, and a small genetic distance value indicates the relationship between the two populations is close. The smaller the genetic distance (value close to zero), the more similarities between populations [43].

Cluster analysis with UPGMA on the three populations (Figure 3), shows that the Pesisir Selatan population and Padang Pariaman population are in one cluster and separated from the Solok population. This is probably because Pesisir Selatan and Padang Pariaman are on the west coast of Sumatra (lowland), while Solok is on the east coast of Sumatra (highland). This also indicates that the population on the west coast of Sumatra has begun to differ genetically from the population on the east coast of Sumatra due to the barrier in the form of Bukit Barisan. Maideliza and Mansyurdin [44] reported that in *Dioscorea bulbifera* (L.), there had been genetic differentiation between populations in the west and east of Bukit Barisan so that the PYK (Payakumpuh) population is separated from the PDG (Padang) and BKL (Bengkulu) populations.

Table 6. Genetic distance matrix of three populations *C. pentandra* based on Nei 1978

Population	Pesisir Selatan	Padang Pariaman	Solok
Pesisir Selatan	-	-	-
Padang Pariaman	0.0186	-	-
Solok	0.0580	0.0371	-

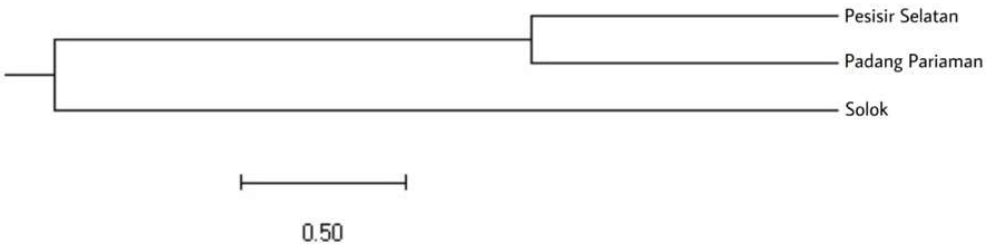


Figure 3. Dendogram of three populations *C. pentandra* based on Nei 1978 genetic distance using the UPGMA method

The cluster analysis of 15 accessions of *C. pentandra* in three populations is shown in Figure 4. Based on the dendrogram, accessions tend to cluster based on their population origin. However, accessions are joining other populations but are still in the same subgroup. All accessions clustered into two large groups, namely group A, consisting of two subgroups (A1 and A2) originating from three populations (Padang Pariaman, Pesisir Selatan, and Solok), and group B, originating from one population (Pesisir Selatan). Some accessions are mixed into other populations. They may have come from the same parent. Another possibility is due to seed dispersal assisted by bats. Syamsuardi et al. [45] reported that in *Morus macroura*, the accession B7 (Tanjung Raya) joining population A11 (Batipuh) was probably to be due to the accession originating from the Batipuh or X Koto populations or due to seed dispersal patterns assisted by birds and human factors.

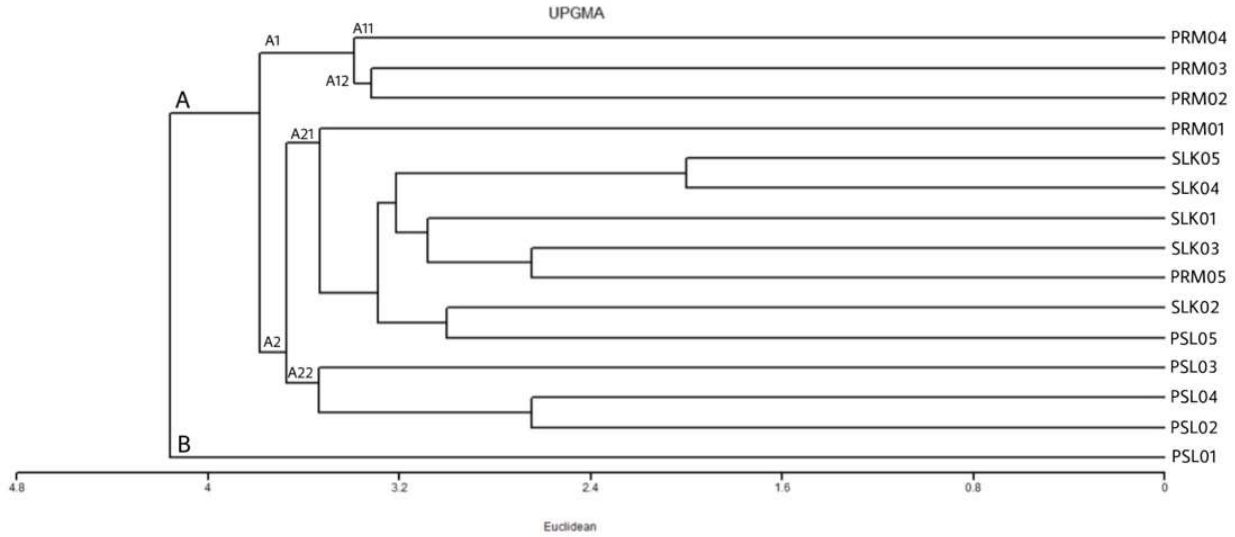


Figure 4. Dendogram UPGMA of 15 *C. pentandra* accessions in three population. (PSL01-05 = Pesisir Selatan, SLK01-05 = Solok, PRM01-05 = Padang Pariaman)

IV. CONCLUSION

Based on the results of the research that has been done, it can be concluded *C. pentandra* in the Pesisir Selatan population has higher genetic variation than the Padang Pariaman and Solok populations. Genetic variation within populations is higher than genetic variation among populations with low genetic differentiation values.

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