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Genetic Diversity of *Flacourtia rukam*, a Local Fruit Tree Using Random Amplified Polymorphic DNA Markers

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Research Article

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ABSTRACT

Flacourtia rukam, Zoll and Moritzi (Salicaceae) is a local fruit tree that is rarely found in various provinces in Indonesia including West Sumatra which is known to be rich in biodiversity. Therefore, an assessment of genetic diversity has been carried out at three locations under different conditions in West Sumatra using the RAPD technique with 18 primers. The results showed that five primers (OPA-01, OPA-03, OPA-13, OPA-16, OPB-10) exhibited DNA polymorphisms. The value of genetic diversity in the Matur population was the highest (H=0.1883, I=0.2770), while the Lubuk Alung population had the lowest genetic diversity value (H=0.0230, I=0.0341). Genetic diversity between populations was higher (Dst=0.1704) compared to the value of genetic differentiation of 0.5710. The PCoA analysis shows that the Matur genetic population was widely distributed, while in Aripan it was moderate, and in Lubuk Alung it was narrowest. This study concludes that the population of *F.rukam* in the Matur area can be used as a gene pool and source of germplasm.

INTRODUCTION

Flacourtia rukam, Zoll and Moritzi is a local fruit trees in the family Salicaceae which is found growing wild in secondary forests and farming area in Indonesia, and other countries of Southeast Asia. The fruit can be consumed and can be processed into processed food, the roots and leaves are used as herbal medicine by the local community. The population of this tree is reported to have decreased, with relative densities of 1.05% to 3.0% in several provinces in Indonesia [1-4].

The low population of *F. rukam* is caused by less economic value according to farmers compared to other edible fruit trees, and the fruiting season is only once a year [5-7]. In the last decade, these fruit trees were cut down when residents cleared the plantation land and the secondary forest was converted into plantation crops. The lower the

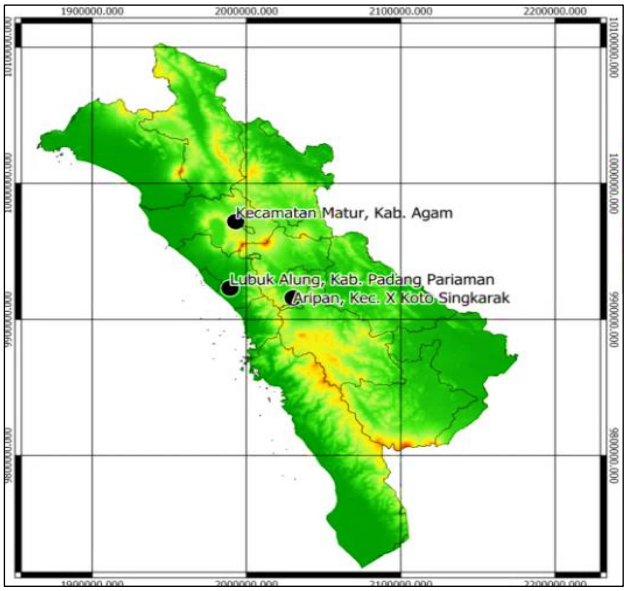
population of *F. rukam* in farming areas and secondary forests, the status will change to threatened in the future. Therefore, the ecology and genetics of *F. rukam* needs to be studied immediately in several areas in the province of West Sumatra which are known to be richer in plant diversity, as an effort to protect and develop them as local fruit germplasm.

One approach to genetic diversity uses molecular markers, namely Random Amplified Polymorphic DNA (RAPD) markers, which are universal and more economical markers using primers to amplify random DNA sequences to detect the presence of a DNA polymorphism. RAPD has been widely used as an estimate of genetic diversity, genome-specific markers and for the characterization of plant species germplasm [8]. The advantage of this marker is that it is a simple molecular marker without requiring DNA sequence information and is easy to find. Considering the simplicity of the method, the RAPD marker has been used to assess intra and inter-population genetic diversity of *F. rukam* fruit trees in different locations in West Sumatra.

MATERIALS AND METHODS

Young leaf samples were collected from 15 individuals of *F. rukam* in three populations in the province of West Sumatra area of 1) Lubuk Alung in Padang Pariaman district, a lowland residential area; 2) Matur area in Agam district, a mixed forest bordering upland secondary forest; and 3) Aripan area in Solok Regency, a medium plain area planted with local commodity trees. Each leaf sample was stored in a bag made of filter paper and put in a ziplock plastic with silica gel added (Figure 1) .

Figure 1. Map of *F. rukam* sample collection locations in West Sumatra (***Note:** 1-Lubuk Alung area in Padang Pariaman District, at coordinates S 0°40'41" and E 100°15'51", altitude 265 masl, 2-Matur area in Agam District, at coordinates S 0°14'49.2" and E 100°17'45.6", altitude 1172 masl, 3-Aripan Area in Solok District, at coordinates S 0° 44'21.4" and E 100 ° 37'19.2", altitude 459 masl).



A total of 0.1 grams of young leaves were isolated using the modified Bioline's Isolate II Plant kit protocol. Testing the quality of DNA isolated by electrophoresis in 2% agarose gel in a buffer solution of 10 x TBE, added 6.5 µl of Ethidium Bromide to the gel, and then visualized using a set of gel documentation tools [9].

DNA samples were amplified using 18 RAPD primers. The composition of the PCR mix reaction consisted of 12.5 µl My Taq TM Red Mix Bioline, 2 µl primer, 6.5 µl Nuclease Free Water, and 4 µl DNA isolate. DNA amplification includes: 1) pre-denaturation step at 94 ° C for 2 minutes; 2) denaturation step 39 cycles for 1 minute at 94 ° C; 3) annealing stage for 1 minute at 34 ° C, and 2 minutes 30 seconds at 72 ° C; and 4) the final extension stage is 72 ° C for 10 minutes, followed by cooling to 4 ° C (Sambrook and Russell, 2001) (Table 1).

Table 1. List of primers to be tested on the DNA of three populations of *F. rukam*.

No	Primer	Nucleotide Sequences (5' ☐ 3')	Reference
1	OPA-01	CAGGCCCTTC	Przyborowski and Sulima
2	OPA-02	TGCCGAGCTG	Przyborowski and Sulima
3	OPA-03	AGTCAGCCAC	Hariyati et al.
4	OPA-04	AATGGGGCTG	Przyborowski and Sulima,
5	OPA-05	AGGGGTCTTG	Przyborowski and Sulima
6	OPA-06	AGGGGTCTTG	Hariyati et al.
7	OPA-07	GAAACGGGTG	Przyborowski and Sulima
8	OPA-08	GTGACGTAGG	Przyborowski and Sulima
9	OPA-09	GGGTAACGCC	Przyborowski and Sulima
10	OPA-10	GTGATGGCAG	Hariyati et al.
11	OPA-11	CAATCGCCGT	Przyborowski and Sulima
12	OPA-12	TCGGCGATAG	Przyborowski and Sulima
13	OPA-13	CAGCACCCAC	Przyborowski and Sulima
14	OPA-16	AGCCAGCGAA	Hariyati et al.
15	OPAC-12	GGCGAGTGTG	Akza et al.
16	OPAC-15	TGCCGTGAGA	Sen et al.
17	OPB-08	GTCCACACGG	Przyborowski and Sulima
18	OPB-10	CTGCTGGGAC	Przyborowski and Sulima

Scoring of RAPD data is based on the presence or absence of a band, which is given a score of 1 if there is and 0 if there is no band. All DNA bands with the same migration rate were assumed to be homologous loci. The results of the binary data matrix were analyzed using the POPGENE software program version 1.32[10]. Parameters observed included the percentage of Polymorphic Loci (PLP), Nei's genetic diversity (H), Shannon's diversity index (I), heterozygosity in the population (Hs), total heterozygosity (Ht), coefficient of genetic differentiation (Gst), and gene flow (Nm) (Finkeldey, 2005). Individual distribution patterns of each population were analyzed by Principal Coordinate Analysis (PCoA) using MVSP 3.2 software [11].

RESULTS

The results of the selection of 18 RAPD primers have obtained 5 primers that can produce polymorphic bands (Figure 2). The profile of the bands in each primer has a size that varies from 138-1600 bp. Based on the number of bands produced from five primers, four of them produced 1 monomorphic band (OPA-01=500 bp; OPA-03=413 bp; OPA-16=430 bp and OPB-10=758 bp). The percentage of polymorphic bands from 5 primers obtained in *F. rukam* was high at 93.42% (Table 2).

Figure 2. Profile of amplified DNA bands in the population of *F. rukam* (***Note:** 1- OPA-01, 2) OPA-03, 3) OPA-13, 4) OPA-16, 5) OPB-10, (*) = monomorphic band).

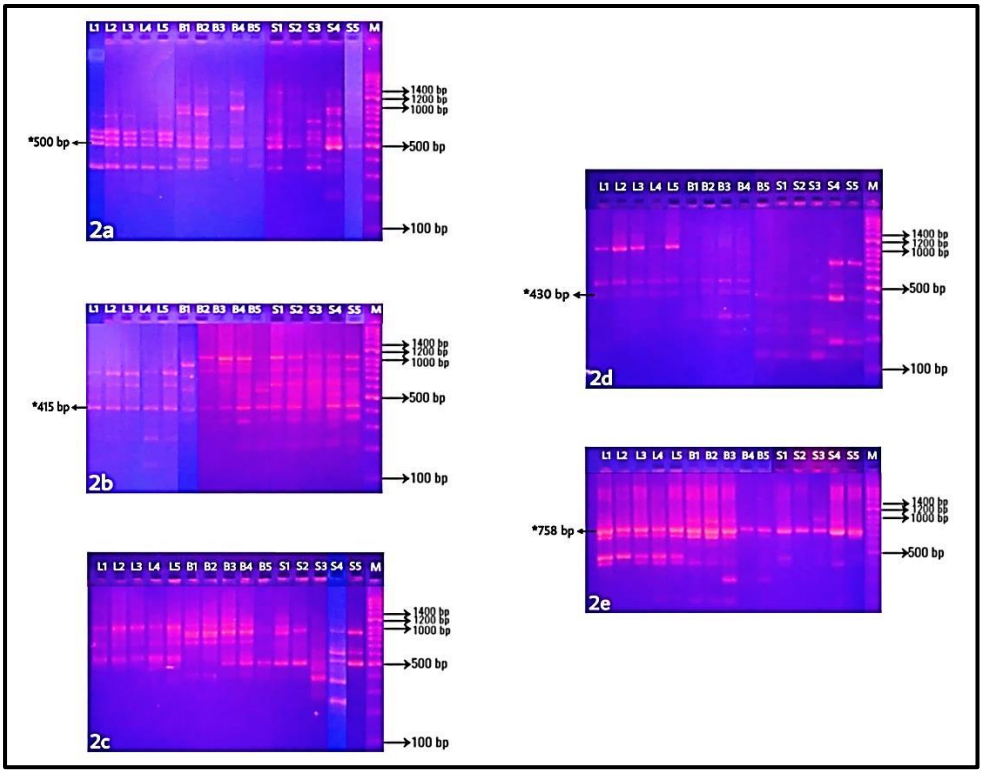


Table 2. The number of bands produced on DNA amplification from the three populations of *F. rukam*.

Primer	Total number of bands	Number of polymorphic bands
OPA-01	14	13
OPA-03	15	14
OPA-13	15	15
OPA-16	10	9
OPB-10	11	10
Total	65	51
Percentage		93,42%

In the three populations, specific bands were found using five primers. OPA-13 primers represented one specific band in each population, measuring 557 bp in Lubuk Alung, 828 bp in Matur and 600 bp in Aripan. The other four primers did not give rise to specific bands in each population. Therefore, the specific band with OPA-13 can be used to identify the origin of *F. rukam* population (Table 3).

Table 3. Specific bands of *F. rukam* DNA with several primers at each location (**Note:** (-) no specific band).

Population	Specific band size (bp) using primer:					Number
	OPA-01	OPA-03	OPA-13	OPA-16	OPB-10	
Lubuk Alung	807	245, 750	577	-	-	4
Matur	-	550, 900	828	-	300	4
Aripan	-	-	600	200, 525	-	3

The highest genetic diversity was found in the Matur population with a heterozygosity value (H) of 0.2770 and Shanon index (I) of 0.2770, while the lowest was in the Lubuk Alung population, which was 0.0341 and Shanon index (I) of 0.0341. These results are consistent with the growing habitat conditions, where the Matur population bordering the secondary forest still has a number of individuals that are easy to find, while the Lubuk Alung population has a limited number of individuals because it is a densely populated village and is dominated by rice fields (Table 4).

Table 4. Genetic diversity of intrapopulation *F. rukam* (**Note:** observed allele (Na), effective allele (Ne), number of polymorphic loci (N), heterozygosity value (H), Shanon index (I), percentage of Polymorphic Loci (PLP).

Population	Number of samples	Na	Ne	N	H	I	PLP
Lubuk Alung	5	10,615	10,402	4	0,0230	0,0341	6,15%
Matur	5	14,923	13,339	32	0,1883	0,2770	49,23%
Aripan	5	15,538	12,785	36	0,1727	0,2671	55,38%

The results of the analysis of interpopulation genetic diversity show that the value of intrapopulation heterozygosity (Hs=0.128) is smaller than the value of interpopulation heterozygosity (Dst=0.1704). The data show that gene flow (Nm) between populations is low (0.3757), while genetic differentiation (Gst) is higher (0.5710). The low intrapopulation genetic diversity is related to the limited number of individuals in the population so that the chance of self-pollination is greater than that of cross-pollination. This value also indicates that there is no gene flow between the three populations, which results in genetic differentiation in each population (Table 5).

Table 5. Value of interpopulation genetic diversity of *F. rukam* (**Note:** Heterozygosity in population (Hs), the value of heterozygosity between populations (Dst), Total heterozygosity (Ht), gene flow (Nm), genetic differentiation between populations (Gst).

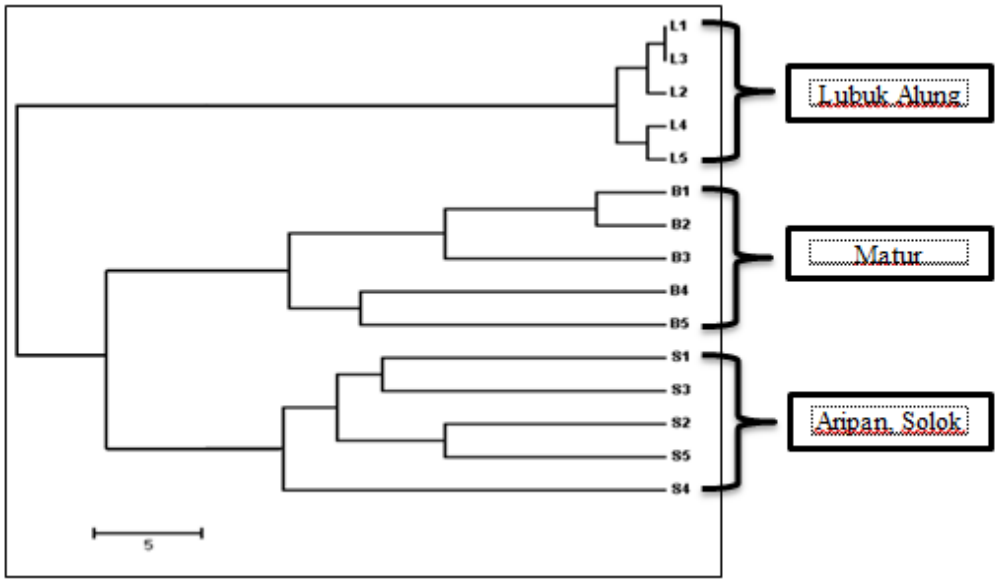
Jumlah individual	Hs	Dst	Ht	Nm	Gst
15	0,1280	0,1704	0,2984	0,3757	0,5710

Aripan and Lubuk Alung populations have the highest genetic distance (0.4398), while Aripan and Matur populations have the lowest genetic distance is 0.2365 (Table 6). Genetic distance is the degree of gene difference (genome differences) between these populations. Based on the dendrogram, it has been shown that the accessions in Matur and Aripan are in one group and separate from the Lubuk Alung group. All accessions were not scattered among other groups and subgroups. These data indicate that individuals in each population are indigenous accessions or no introduction accessions (Figure 3).

Table 6. Matrix of genetic distance in three populations of *F. rukam*.

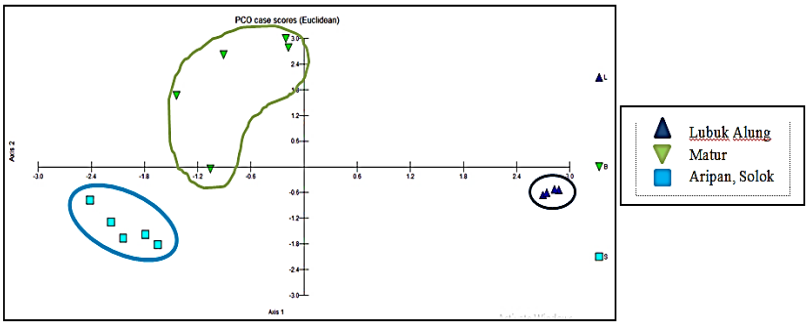
Population	Lubuk Alung	Matur	Aripan, Solok
Lubuk Alung	-		
Matur	0,3055	-	
Aripan, Solok	0,4398	0,2365	-

Figure 3. Dendrogram of accession in three populations of *F. rukam*: Lubuk Alung (L), Matur (B), Aripan Solok (S).



The PCO analysis show that the genetic population in Matur was widely distributed, while in Aripan it was moderate, and in Lubuk Alung it was narrower. Thus, the genetic population in Matur still has high heterozygous, while the Lubuk Alung genetic population tends to be homozygous (Figure 4).

Figure 4. Diagram of PCoA accession in three genetic populations of *F. rukam*. ***Note.** ▲ Lubuk Alung; ▼ Matur; ■ Aripan, Solok



DISCUSSION

The discovery of 5 RAPD primers capable of producing polymorphic bands provides initial information for further molecular studies on *F. rukam*. The percentage of polymorphic bands from 5 primers obtained in *F. rukam* was high at 93.42%. No RAPD primers have been reported in the genus Flacourtia, but reported that OPA-01 and OPA-13 and OPB-10 produced 6 polymorphic bands in Salix viminalis which belongs to the family Salicaceae. The percentage of polymorphic bands with the three primers reached 94% [12,13].

OPA-13 primers represented one specific band in each population, measuring 557 bp in Lubuk Alung, 828 bp in Matur and 600 bp in Aripan. The other four primers did not give rise to specific bands in each population. Therefore, the specific band with OPA-13 can be used to identify the origin of *F. rukam* population. The hybrid-specific bands generated in the RAPD analysis with OPA-18 primers could be used for the identification of F1 cowpea (Vigna unguiculata) hybrids.

The Matur population has the highest genetic diversity compared to the Aripan and Lubuk Alung populations, with a Heterozygosity value (H) of 0.2770 and a Shanon Index (I) of 0.2770. These results are consistent with the growing habitat conditions, where the Matur population bordering the secondary forest still has a number of individuals that are easy to find, while the Aripan population is moderately located in fruit tree plantation areas and is not densely populated, and the Lubuk Alung population has a limited number of individuals because it is a densely populated village and is dominated by rice fields. Additional information that both Lubung Alung and Aripan populations are separated from secondary forest. Populations in natural habitats with large population sizes tend to be able to maintain genetic variability, so that genetic drift and inbreeding are less likely to occur [14]. The opposite event was reported on the Salix phylicipolia species that there is a decrease in genetic diversity as a result of genetic drift and inbreeding. Low population size is a major factor in the loss of genetic diversity [15,16].

The value of intrapopulation heterozygosity (Hs=0.128) is smaller than the value of interpopulation heterozygosity (Dst=0.1704). The data show that gene flow (Nm) between populations is low (0.3757), while genetic differentiation (Gst) is higher (0.5710). The low intrapopulation genetic diversity is related to the limited number of individuals in the population so that the chance of self-pollination is greater than that of cross-pollination. The high intrapopulation homozygosity indicates that pollen and seed transfer are limited within the same population [17]. This value also indicates that there is no gene flow between the three populations, which results in genetic

differentiation in each population. 2008 reported that increasing spatial distance as a result of fragmentation will decrease genetic connectivity, and the population is not in gene flow/drift equilibrium [18].

The highest genetic distance was found between Aripan and Lubuk Alung with a value of 0.4398, while the lowest was between Aripan and Matur populations with a value of 0.2365. The Lubuk Alung population is geographically separated from the Aripan population by the Barisan hills that cross the island of Sumatra, while the Matur and Aripan populations are located within the hill range. According to genetic distance between populations is important information for studying the transfer of DNA from one population to another so that it can influence the microevolution of a population or species (gene flow). Based on the dendrogram, it has been shown that the accessions in Matur and Aripan are in one group and separate from the Lubuk Alung group. All accessions were not scattered among other groups and subgroups. These data indicate that individuals in each population are indigenous accessions or no introduction accessions [19-22].

The Mature genetic population showed a wider distribution than the other two populations. Thus the genetic population in Matur still has high heterozygous, while the Lubuk Alung genetic population tends to be homozygous. Therefore, the genetic population in Matur needs to be maintained and conserved as a gene pool and a source of *F. rukam* germplasm in West Sumatra. According to, the gene pool consists of all the genes and their alleles present in all such individuals, which hybridize or can hybridize with each other. The gene pool is important in selecting germplasm to use in hybridizations for plant improvements [22-25].

CONCLUSION

The population of *F. rukam* in the Matur of Agam district in West Sumatra has a higher genetic diversity value than the other two populations. Intrapopulation heterozygosity is smaller than the value of interpopulation heterozygosity as a result of the low gene flow between populations is low, so that genetic differentiation occurs higher. Therefore, in an effort to preserve and develop *F. rukam* germplasm in West Sumatra, the population in Matur area in Agam District needs to be maintained.

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