

Genetic Diversity of Indonesian Snake Fruits as Food Diversification Resources

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Abstract— Indonesia is one of the megabiodiversity, which is rich with germplasm including tropical fruit. Snake fruit (*Salacca spp.*) is a native fruit of Indonesia with a scaly peel and sweet-tart taste. The genetic diversity of 17 accessions of Indonesian snake fruit was resolved using the Random Amplified Polymorphic DNA Polymerase Chain Reaction with 5 primers. The study demonstrated that the samples were grouped in six different clusters with coefficient of similarity ranged from 0.12 to 0.71. The value indicated the wide range of genetic variability among the tested plants. This variability was an important resources for the snake fruit breeding program in developing the consumer's preferred product which by the end supports the plant diversification program.

Keywords— Genetic variability; RAPD PCR; *Salacca spp.*

I. INTRODUCTION

The snake fruit also known as salak (*Salacca spp.*) was an exotic plant native to Indonesia. The salak fruit was consumed for its sweet sor, and slightly tart taste [1]. Indonesia as one of the origin of the plants has a diverse types of salak. The commercial salak in Indonesia included the 2-3 seed Salak Jawa (*S. zalacca*), 1-2 seed Salak Bali (*S. amboinensis*), and red or white fruit flesh coloured Salak Sidempuan (*S. sumatrana*). Moreover, another species of snake fruit also can be found, such as *S. wallichiana*, *S. glabrescens*, and *S. affinis* [2]. On the other hand, not every species of snake fruit is consumable. The snake fruit preferred by the consumers include those with thick fruit flesh, sweet, less or none tart taste, and spineless peel [3]. The snake fruit breeding program was one of the way to construct an ideal product. Moreover, the availability of products which are suitable with consumer's demand will increase the

The variability of the snake fruit species in Indonesia which showed a diverse characteristics was a valuable germplasm. The information about plant genetic variability can be achieved through characterization activity which included morphological, enzymatic, and genetic based methods [4]. Among these three methods, the genetic based characterization using molecular marker produced most accurate results since the data were relatively stable, were not

influenced by environment changes, and can be used to resolved either the closely or far related species [5].

One of the molecular DNA based marker which is commonly used as plant genetic marker is RAPD (Random Amplified Polymorphic DNA) [6]. The RAPD method present numerous advantages over conventional assays due to no prior knowledge of the genome are needed [7]. The RAPD technology uses random oligonucleotide primer which means that by applying a variety of primers, different banding profiles can be rapidly generated [8]. In addition, these assays are relatively cheap and do not require specialized and expensive equipments [9]. The assay also presents has the potential to detect significant genetic alterations, such as mutation, somaclonal variation, and other genetic variations [10, 11, 12]. This study was conducted to determine the genetic relationship among ITFRI's 17 snake fruit accessions as a crucial information to construct the consumers preferred product.

II. MATERIAL AND METHOD

The study was conducted starting in September 2012 at the ITFRI Laboratory. Materials used in this study were foliage from 17 accessions of snake fruit (Table 1).

The young leaves of those plants were chosen as DNA sources for the DNA extraction activity. The experiment consisted of several phases of activities, namely sampling, isolation of DNA which includes DNA extraction and

purification, test the quality and quantity of DNA, the primary selection, amplification reaction and electrophoresis.

TABLE I
SNAKE FRUIT ACCESSIONS USED IN RAPD CHARACTERIZATION

No.	Accessions		Morphological characteristics		
	Code	Name	Flesh fruit color	Astringent taste	presence of fruit spines
1.	PH	Salak Pondoh	White	absent	slightly sharp
2.	MWR	Salak Mawar	White	Absent	slightly sharp
3.	SDP	Salak Sidempuan	Cream	present	slightly sharp
4.	PHK	Salak Pondoh X K	Cream	present	slightly sharp
5.	PHM	Salak Pondoh X M	Cream	present	slightly sharp
6.	PHMJB	Salak Pondoh X MJ	Cream	present	slightly sharp
7.	PHMJC	Salak Pondoh X MJ	Cream	present	slightly sharp
8.	PHMW	Salak Pondoh X Mawar	Cream	present	slightly sharp
9.	MWSPA	Salak Mawar X Sidempuan	White	Absent	slightly sharp
10.	MWSPB	Salak Mawar X Sidempuan	White	Absent	slightly sharp
11.	MWSPC	Salak Mawar X Sidempuan	White	Absent	slightly sharp
12.	SPMWA	Salak Mawar X Sidempuan	Cream	present	slightly sharp
13.	SDMSJ	Salak Sidempuan merah X Sanjung	Reddish white	present	slightly sharp
14.	SPSJ	Salak Sidempuan putih X Sanjung	Cream	present	slightly sharp
15.	JKMW	Salak Jaka X Mawar	Cream	Absent	slightly sharp
16.	SBGP	Salak Gula Pasir	Cream	Absent	slightly sharp
17.	AFNS	<i>S. affinis</i>	Cream	Absent	absent

A. DNA Extraction

DNA extracted from snake fruit leaves using the CTAB method Doyle & Doyle [13] modification. A total of 100 mg the durian leaves were grinded with 1.5 ml of extraction buffer, 1% β -mercaptoethanol, and 10 mg of PVP-10 to form a paste. The leaves paste durian samples were then transferred into a 2 ml centrifuge tubes and incubated at 65°C for 60 minutes. A volume of 500 μ L of chloroform: Isoamyl-alcohol (24:1) were added to the leaf paste, homogenized, and then centrifuged at 12,000 rpm for 10 minutes. The supernatant were transferred into a new 2 ml centrifuge tube. The chloroform-isoamyl alcohol (24: 1) step was repeated three times. The supernatant was then transferred to a new tube and added with 500 μ L of cold iso-propanol, then centrifuged at 12,000 rpm for 10 minutes. The formed DNA pellets were air dried, rinsed with 70% ethanol, and dissolved in 50 μ L of TE buffer. DNA was quantified by using nano-drop equipment.

B. Reaction Amplification, and Electrophoresis

There were total of 5 primers used in this study, which were primer rapd1, rapd2, rapd4, rapd6, and D3. RAPD PCR reaction was performed with Taq PCR reaction mixture 4.25 μ L (KAPPA) with 1 μ L of primer, 1 μ L of sample DNA, and ddH₂O to a final volume of 12.5 μ L reaction. PCR was performed by 45 cycles with the following programs: pre denaturation: 95°C for 3 minutes, denaturation: 95°C for 15 seconds, annealing: 36°C for 15 seconds, extension: 72°C for 5 seconds, and final extension: 72°C for 10 minutes. DNA amplification product was separated by electrophoresis at 50 V for 20 minutes.

C. Data Analysis

Each band in RAPD profile was treated as independent loci with two alleles, presence or absence of band. The band patterns were scored using software BioDocAnalyze, and a binary matrix thus obtained.

The snake fruit coefficient of genetic similarity was determine based on SIMQUAL (Similarity for Qualitative Data) procedure on NTSYSpc ver. 2.01 [14] dan was counted based on DICE coefficient using formula:

$$S = \frac{2 n_{ab}}{(n_a + n_b)}$$

with :

S (*DICE coefficient*): individual genetic similarity.

a and b: the 2 comparative individus

n_{ab} : total similar band on the same position of individu a and b

n_a and n_b : total band on each individu a and b [15]

The data clustering analysis was conducted using the Sequential, Agglomerative, Hierarchical, and Nested (SAHN)-UPGMA (Unweighted pair-group method, arithmetic average) on software NTSYSpc ver. 2.02.

III. RESULTS AND DISCUSSION

The 5 primers of total amplified a total of 40 scoring bands, with 6 to 12 band amplified per primers, all of them were polymorphic bands (Table 2). Similarity of coefficient ranged from 0.12 (SDMSJ - PHMJB) and 0.71 (SPMWA – PH). The wide range of coefficient of similarity among the tested snake fruit indicated the wide range of genetic potential of each plants, which were an important resources in plant breeding program.

Using genetic distance data, it was possible to construct a phylogenetics tree showing genetic relationships for 17 accession of *Salacca* spp. The resulting dendrogram showed a small differentiation into six main clusters (Fig. 1). Cluster 1 contains accessions of PH, MWR, PHM, and PHMJ; cluster 2 contains MWSPC, MWSPB, SPMWA, PHMW; cluster 3 contains PHK, PHMJB; cluster 4 contains MWSPA; cluster 5 contains SPSJ, AFNS; whereas cluster 6 contains SDP, SDMSJ, SBGP, and JKMW.

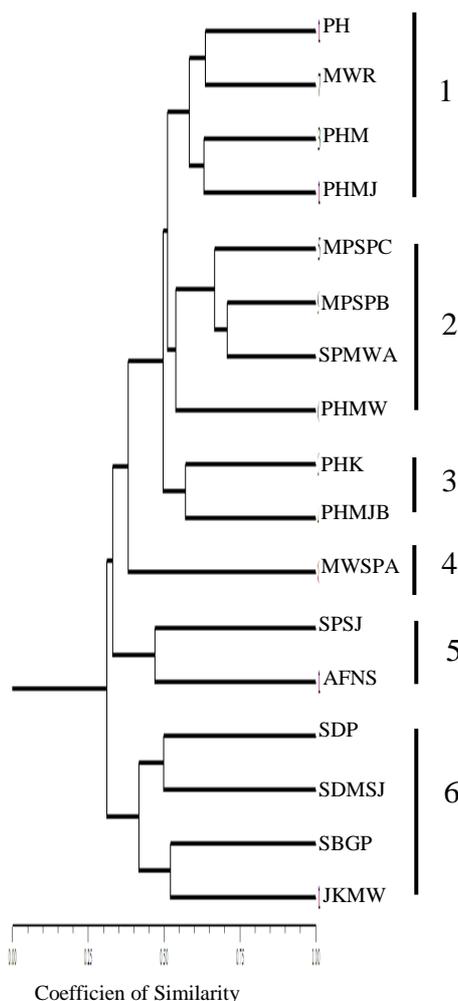


Fig. 1 The phylogenetic tree of 17 accessions of snake fruit (*Salacca* spp.) as resolved using RAPD PCR analysis with 5 primers.

TABLE II
SELECTED PRIMERS TOTAL AMPLIFIED BANDS, POLYMORPHIC BAND,
AND POLYMORPHIC PERCENTAGE

Primers	Sequence (5'-3')	Total of amplified band	Polymorphic band (%)
Rapd1	GCGGGTGGAA	7	7 (100)
Rapd2	GTT TCGCTCC	8	8 (100)
Rapd4	AAGAGCCCGT	7	7 (100)
Rapd6	CCCGTCAGCA	6	6 (100)
D3	GTCGCCGTC	12	12 (100)

Generally, the identification of snake fruit was on the basis of several phenotypic characters, namely leaf shape, bark type and fruits traits, but this has often being tricky. Therefore, a genetic identification was introduced. RAPD method had provide sufficient data as a genetic marker on barley [16], rice [17], *Eucalyptus* [18], ryes [19], tongkat ali (*Eurycoma longifolia*) [20], and medicinal plants [21, 22]. Although RAPD markers should in theory cover all types of DNA, but the some factors may influence their distribution in the genome, such as the scoring of un-ambiguously RAPD profile, preferential amplification due to copy number of target DNA as well genotypic backgrounds. Therefore, for more detailed taxonomic studies it is important to combine

different methods in order to accurately define snake fruit populations.

The snake fruit germplasms could be combine through breeding activities to construct new variety which had desirable traits that meet the consumer's demand. Such an ideal fruit was an alternative food which were diverse the people's choice of vitamin, minerals, and fiber's sources. This diversity of food supported the Indonesian goverment of food diversification.

IV. CONCLUSIONS

The ITFRI's snake fruit had a wide range of genetic similarity thus became a valuable assets in developing the fruit to be an alternative in food diversification program.

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