

Short Communication:

Genetic diversity of *Salacca edulis* from West Seram District, Maluku, Indonesia based on morphological characters and RAPD profiles

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Abstract. Elly SS, Watuguly TW, Rumahlatu D. 2018. Short Communication: Genetic diversity of *Salacca edulis* from West Seram District, Maluku, Indonesia based on morphological characters and RAPD profiles. *Biodiversitas* 19: 1777-1782. Morphological and RAPD-based genetic diversity analyses of *Salacca edulis* Reinw populations from West Seram District were performed. A survey was conducted in four locations in the village of Riring, Rumahsoal, Taniwel, Neniari, and Soya. Forty-two morphological characters and three RAPD primer were used. Data were analyzed on the NTSys program version 2.0 to perform UPGMA clustering analysis. An UPGMA dendrogram based on morphological characters resulted in two main groups with similarity value varied from 0.46-0.75 for morphology and 0.35-0.89 for RAPD. The result gives us important about cultivar of *Salacca edulis* in West Seram District Maluku which has high genetic diversity and germplasm. These results and can be used for further research for conservation as native cultivar.

Keywords: Genetic diversity, morphology, RAPD, *Salacca edulis*

INTRODUCTION

Salak (*Salacca edulis* Reinw) or snake fruit has an economic value and potentials both for domestic and export market commodity (Herawati et al. 2012). Salak is a species of palm tree that grows in clusters (Herawati et al. 2018). This plant is predominantly grown in Java and southern Sumatera. The main cultivated varieties of salak in Indonesia, are *Salacca zalacca* var. *zalacca* from Java and *Salacca amboinensis* (Becc) from Ambon and Bali. Another species related to *Salacca edulis* is *Salacca sumatrana* Becc distributed in Sumatera (Nandariyah, 2010), Sleman, Madura, and Banjarnegara (Murti, 2002).

In Maluku, salak cultivation is centered in the village of Soya, Hatalai, Wakal, Amahusu, and Hative Besar on Ambon island, and Piru, Taniwel, and Riring village on Seram island. Salak grown in West Seram is a species native to Maluku (Pattinama et al. 2007). The fruit has excellent properties, such as red fruit flesh and sweet-sour taste. The fruit is produced by crossing male and female flowers by the farmers. In this region, salak is consumed only when mature while the economic development in the form of processed products does not exist yet. Salak from Seram island and other areas of Maluku have some morphological differences. However, the diversity of the populations, their relationships and genetics have not been studied in details. □

Genetic diversity can be assessed based on variation in morphology, protein, and molecules (Govarthan et al.

2011). Morphological characters are easy to observe, straightforward, affordable, but somehow inconsistent due to subjectivity when evaluating certain characters or character states. Recent studies on genetic diversity mostly involved molecular markers, such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Length Polymorphism (AFLP), microsatellites or Simple Sequence Repeat (SSR), Inter-Simple Sequence Repeat (ISSR), Sequence Characterized Regions (SCARs), Single Nucleotide Polymorphisms (SNPs) (Semagn et al. 2006). RAPD is one of the molecular markers that can be used to study DNA polymorphism based on different sizes of DNA fragment. RAPD is widely used because it is easy, affordable, and quick in producing DNA bands polymorphism (Baig et al. 2009; Gurijala et al. 2015).

Previous studies on genetic diversity of salak from Java and Sumatera have used morphological characters and RAPD profiles (Suskendriyati et al. 2000; Murti 2002; Sudjijo 2009; Nandariyah 2010; Fatimah 2013; Herawati et al. 2012; Ariestin et al. 2015; Herawati et al. 2018). However, no study on the genetic diversity of salak from Maluku islands was reported. This present study was aimed to assess genetic diversity of *Salacca edulis* from West Seram District based on morphological characters and RAPD profiles. The results of the analysis will contribute to the improvement strategy of utilization and conservation salak as a native plant species. □

MATERIALS AND METHODS

Study site

Samples were collected from four locations in West Seram District, Maluku (Moluccas), Indonesia, i.e., the villages of Riring, Rumahsoal, Taniwel, Neniari, and a village in Ambon City named Soya as a comparison (Figure 1).

Morphological observation

Morphological characters of the species were selected and scored based on the International Plant Genetic Research Institute guidelines for coconuts genetic resources (IPGRI, 1995). Observation on plant organs included vegetative and generative organs, consisting of three root characters, nine stem characters, 12 leaf characters, ten fruit characters, four seed characters, and four thorn characters.

RAPD analysis

RAPD analysis was conducted in the molecular laboratory of the Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University. The leaf samples were cut, packed with aluminium foil and kept in cool box. DNA isolation was carried out using a modified CTAB method (Doyle and Doyle, 1987). DNA concentration and purity was measured quantitatively using Genesys 10 spectrophotometer at the absorbance wavelength of 260/280 nm. PCR amplification of RAPD was using primers OPA-3, OPA-17, and OPA-19 (Nandariyah et al. 2004; Ediwirman and Mansya, 2011; Ayuningrum et al. 2012). The condition of PCR reaction was as follows: pre-denaturation at 94 °C for 5 minutes followed by 40 cycles consisted of denaturation at 94 °C for 30 seconds, annealing at 35 °C for 30 seconds, and

extension at 72 °C for 30 seconds, and terminated by a final extension at 72 °C for 10 minutes. The results of PCR amplification were run in an electrophoretic tank using 1.5% agarose gel and visualized in a UV transilluminator.

Data analysis

Morphological data consisted of qualitative and quantitative characters. Scoring for each character based on IPGRI (1995) and then was standardized into binary data. Data were arranged in NT-edit (Rohlf, 1998). RAPD profiles were analyzed based on the presence or absence of bands DNA generated by primers at each locus. The scored data were treated as binary data. Coefficient of similarity was determined with Simple Matching (SM) on SIMQUAL (Similarity of Quantitative Data) procedure. Cluster analysis was performed using Sequential Agglomerative Hierarchical and Nested Unweighted Pair-Group Method with Arithmetic (SAHN-UPGMA) on NTSYs program version 2.0 (reference?) □

RESULTS AND DISCUSSION □

Morphological variations of salak populations

Our observation showed that Maluku salak plant has the height of 2.5-7 m with stem circumference ranging from 20-80cm. The stem is circular and jagged with brown and dark brown color. Leaves are elongated, mostly green or dark green color. Leaves size is 18-80 x 2-7 cm, every petiole has different sizes. Each cluster produces 5-20 fruits, fruit diameter ranges from 2.5-14 cm. The rind is black and brownish black while the flesh can be yellowish white, white, or reddish white. The flesh is 0.5 -1.8 cm thick and it tastes sweet when it is ripe (Figure 2).

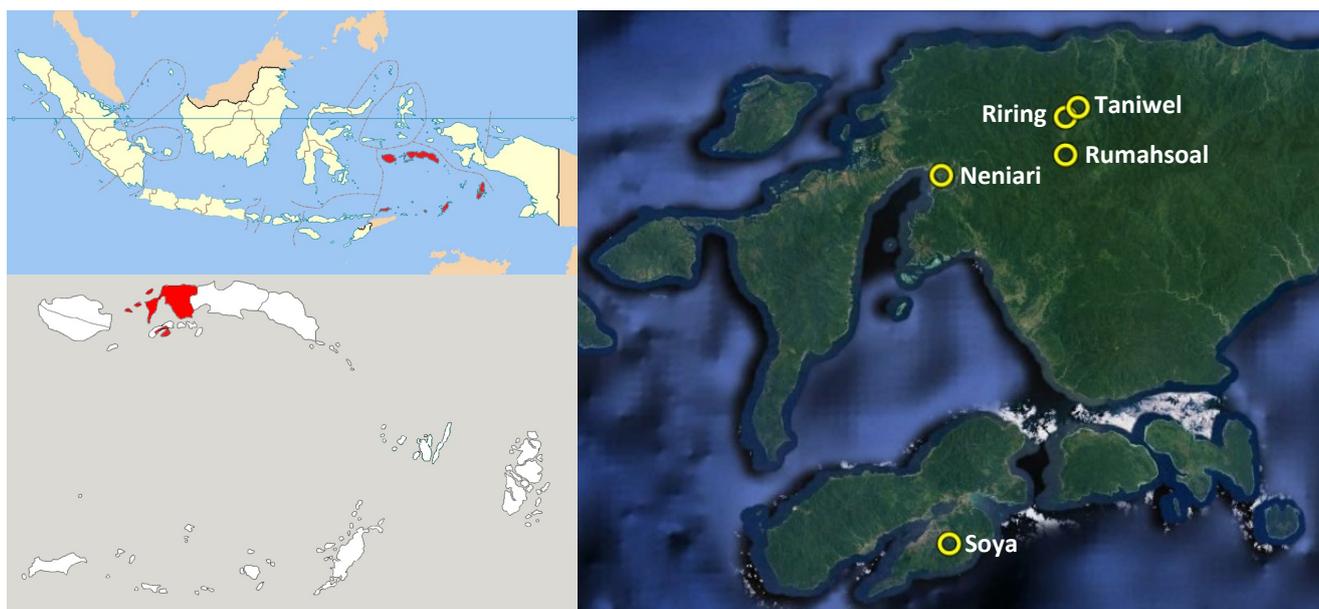


Figure 1. Study sites in West Seram District (i.e., villages of Riring, Rumahsoal, Neniari, Taniwel) and Ambon City (i.e., Village of Soya), Province of Maluku (Moluccas), Indonesia

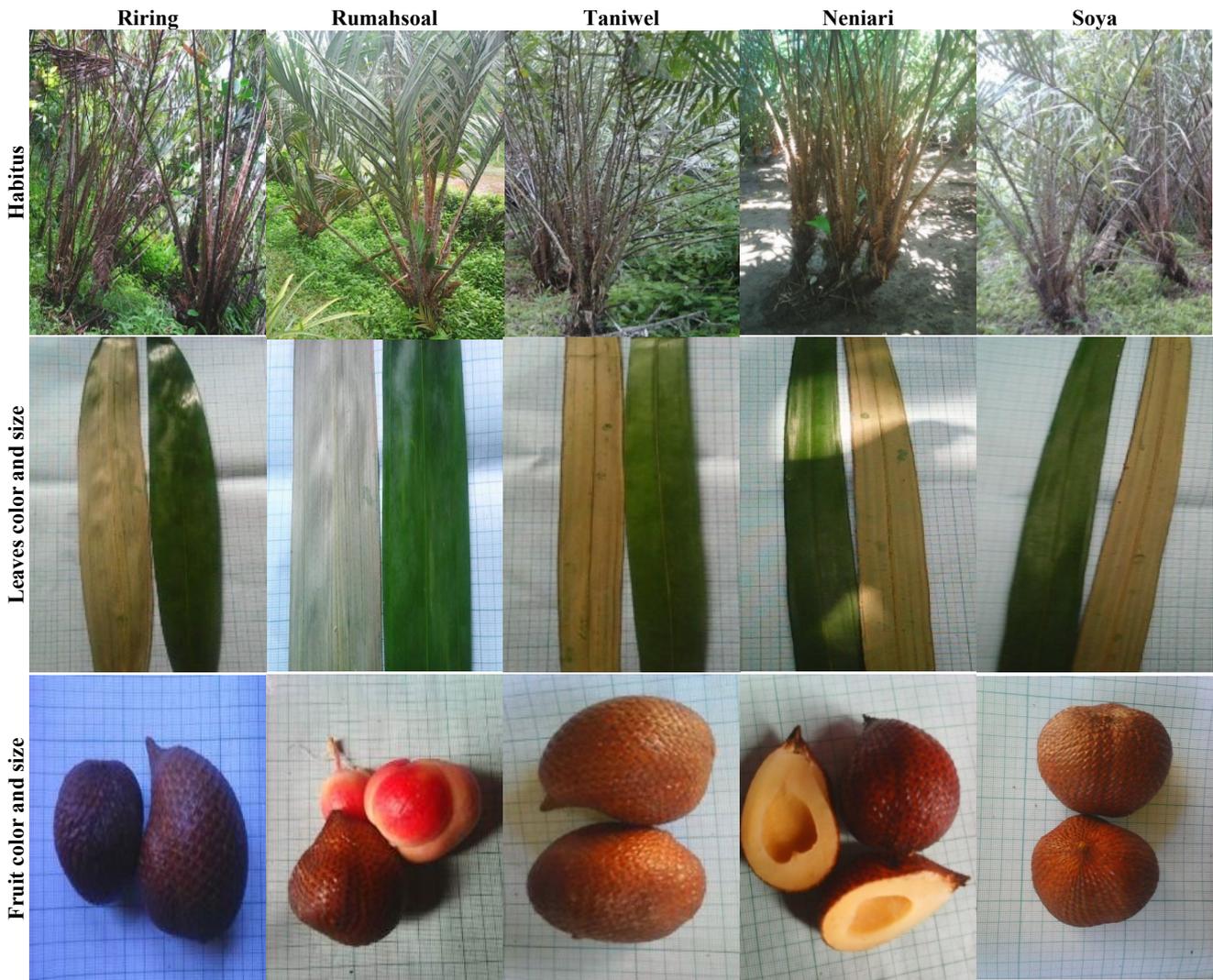


Figure 2. Morphological variation of *Salacca edulis* from West Seram District, Maluku, Indonesia

The results of the present research are in line with Suskendriyati et al. (2000) who state that the varieties of salak can be differentiated based on the texture of the flesh, the color of the skin, the size of the fruit, the taste, and the habitus.

Traditionally, diversity is estimated by measuring variation in phenotypic or qualitative and quantitative traits. However, this approach is often limited and expression of quantitative traits is subject to strong environmental influence (Kameswara, 2004). Genetic diversity is a prerequisite for the genetic improvement of a plant. But rational use of the genetic diversity present in germplasm collections requires a good knowledge about their characteristics. □

The coefficient similarity was ranged from 0.4609-0.7500 (Table 1), with the highest similarity (0.7500) was observed in sample A3 and A4; while the lowest similarity (0.4609) was found in A1-A5 and A6-A9 sample. Based on cluster analysis, the dendrogram showed two main groups (Figure 5). The first group consisted of Riring (A1, A2, A3) and Rumahsoal (A4, A5) and the second group was

composed of Taniwel (A6), Neniari (A7, A8) and Soya (A9).

High similarity value suggests high similarity of characters among the OTU. Generally, each location shared the same qualitative characters such as habitus, shape of canopy, appearance of leaf upper and lower surface, shape flowers, and fruit texture. Individual A1 to A8 are samples from the same locations, which is from an island. A9 sample was located on a separate island, but clustered with A6 that was from different island (Figure 5). This proves that island differences cause variations in environmental factors that can cluster in different clusters.

Genetic diversity in salak populations based on RAPD markers

PCR amplification of Salak cultivar from 9 locations using three RAPD primers yielded 87 bands to which 43.68% are polymorphic bands. The length of polymorphic band ranging from 50-1000 bp with number of polymorphic ranging from 1 to 6 for each location and 12-13 band per primer. The highest polymorphism was

observed in primer OPA-3 (50-600) and OPA-19 (50-1000) (Table 2). The DNA polymorphic bands could be found in different sizes and positions of loci for each sample. The number of polymorphic DNA fragments is an essential factor in determining the genetic diversity level of a population. The difference in the number and polymorphism of DNA bands generated by each primer describes the complexity of plant genomes (Nandariyah et al. 2004). High polymorphism level suggests high genetic diversity in plant samples. Polymorphism generated in this study (43.68%) was lowered than that was reported by Nandariyah (2010), who studied 12 cultivars of Salam from with 68.4% polymorphism.

In general, DNA bands produced from the RAPD amplification had different numbers, sizes, and intensity even though they were amplified using the same primers (Figure 6). These variations may often be resulted in polymorphism at individual level. Harkinto in Herawati et al. (2018) explains that DNA polymorphism can be caused by the differences in an individual's genome sequence, this was implied by the presence and absence of bands in each sample. The intensity of the DNA bands appearance might

be influenced by the charging weigh of migrated distinct molecules and number of DNA band copies which had been amplified. Lee (1998) reported that large molecules can lead to improperly separated bands so that the DNA bands become much thicker. The fact that some primers could not generate bands indicated that the primers were not complementary with the DNA genome template.

Cluster analysis resulted in similarity values ranging from 0.34-0.88 (Table 3). The highest similarity coefficient was observed between A1 and A2 (0.8888) while the lowest similarity coefficient was found between A6-A9 (0.3472). UPGMA dendrogram two main groups. The first group was composed of a group of samples from A1 and A2, and another individual group of A5, A3, and A4. The second group consisted of A6 and A7, A8, and A9 (Figure 7). Sample A1 and A2 populations were in one group because they were from the same location of Riring. They were grouped in a cluster with A3, A4, and A5.

The results of the current research indicated that salak populations found in West Seram District had moderate genetic diversity based on RAPD profiles but low morphological variations.

Table 1. Similarity coefficients based on morphological characters

Sample locations	A1	A2	A3	A4	A5	A6	A7	A8	A9
A1	1								
A2	0.6875	1							
A3	0.5521	0.5521	1						
A4	0.5521	0.5521	0.75	1					
A5	0.5521	0.5521	0.7188	0.7188	1				
A6	0.4609	0.4609	0.4609	0.4609	0.4609	1			
A7	0.4609	0.4609	0.4609	0.4609	0.4609	0.5469	1		
A8	0.4609	0.4609	0.4609	0.4609	0.4609	0.5469	0.5938	1	
A9	0.4609	0.4609	0.4609	0.4609	0.4609	0.625	0.5469	0.5469	1

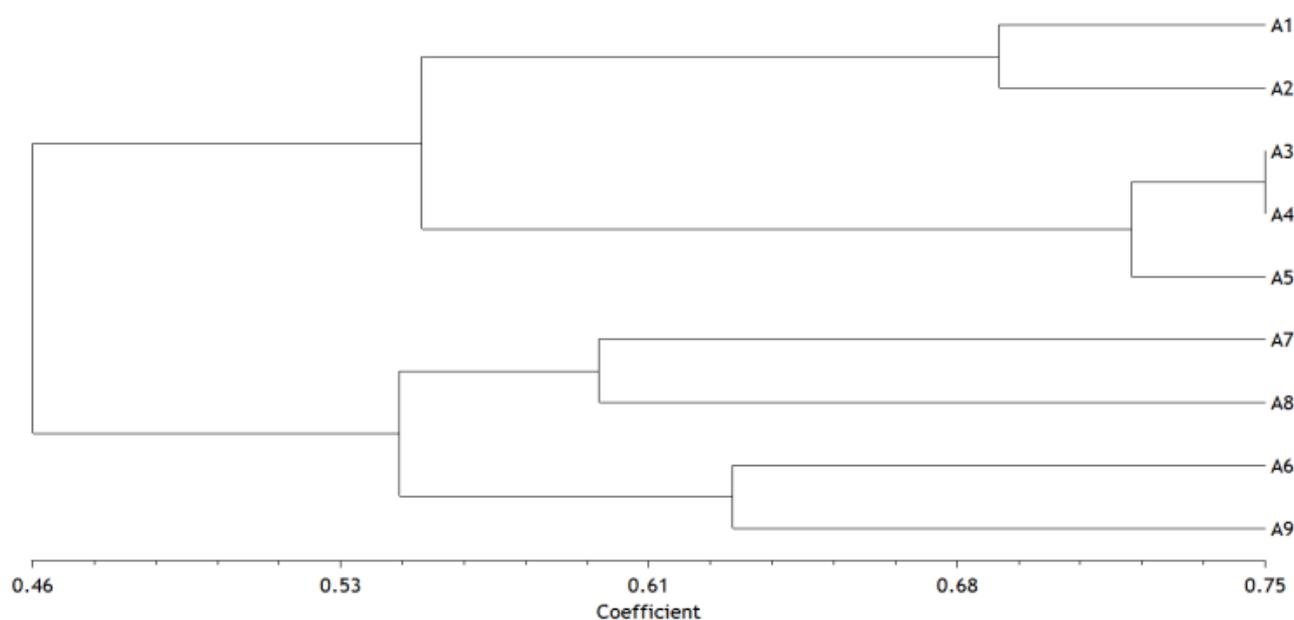


Figure 5. An UPGMA dendrogram of Salak based on morphological characters. Note: A1 = Riring I, A2 = Riring II, A3 = Riring II, A4 = Rumahsoal I, A5 = Rumahsoal II, A6 = Taniwel, A7 = Neniari I, A8 = Neniari II, A9 = Soya

Table 2. The list of primers, sequence, and number of DNA bands in RAPD analysis

Primer	Sequence 5'-3' (Nandariyah et al. 2004; Ayuningrum et al. 2012)	Size	Number of polymorphic bands	Number of monomorphic bands	Total
OPA-3	AGT CAG CCA C	50-600	13	23	36
OPA-17	GAC CGC TTG T	50-900	12	12	24
OPA-19	CAA ACG TCG G	50-1000	13	14	27
Total			38 (43.68%)	49	87

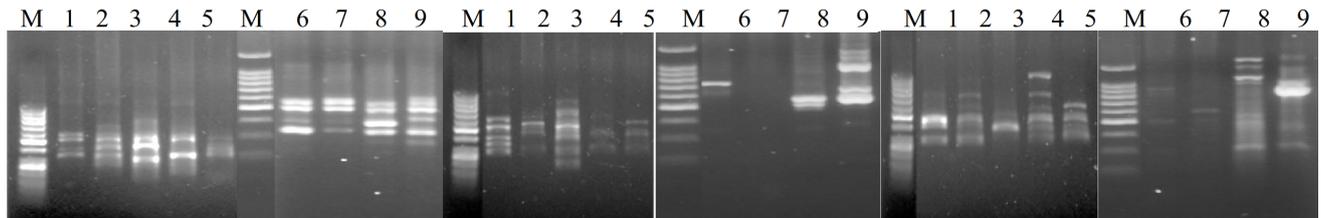


Figure 6. The profiles of DNA bands of Salak from West Seram District, Maluku, Indonesia found in each sample location. Note: A. Primer OPA-3; B. Primer OPA-17; C. Primer OPA-19; M=DNA marker, 1. Riring I, 2. Riring II, 3. Riring II, 4. Rumahsoal I, 5. Rumahsoal II, 6. Taniwel, 7. Neniari I, 8. Neniari II, 9. Soya

Table 3. Similarity index of Salak from West Seram District, Maluku, Indonesia based on RAPD profiles

Location	A1	A2	A3	A4	A5	A6	A7	A8	A9
A1	1								
A2	0.8888	1							
A3	0.7963	0.7963	1						
A4	0.7639	0.7639	0.7639	1					
A5	0.8333	0.8333	0.7963	0.7639	1				
A6	0.3472	0.3472	0.3472	0.3472	0.3472	1			
A7	0.3472	0.3472	0.3472	0.3472	0.3472	0.7222	1		
A8	0.3472	0.3472	0.3472	0.3472	0.3472	0.6389	0.6389	1	
A9	0.3472	0.3472	0.3472	0.3472	0.3472	0.5	0.5	0.5	1

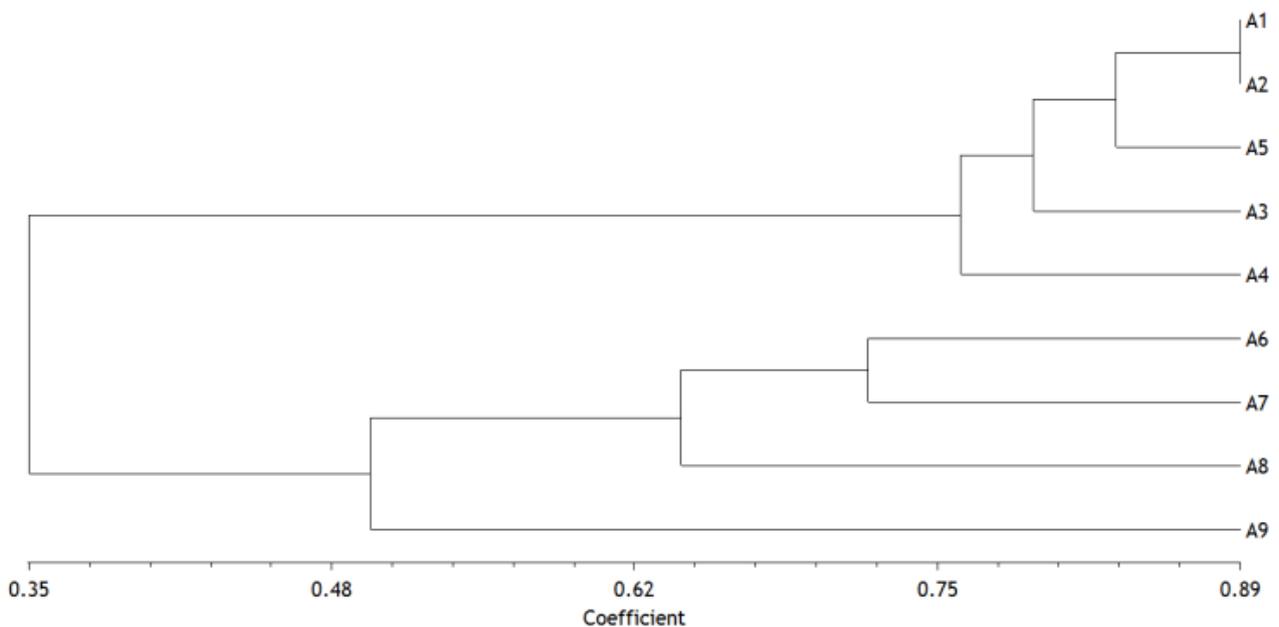


Figure 7. A UPGMA dendrogram of salak of Salak from West Seram District, Maluku, Indonesia revealed by RAPD markers. Note: A1 = Riring I, A2 = Riring II, A3 = Riring II, A4 = Rumahsoal I, A5 = Rumahsoal II, A6 = Taniwel, A7 = Neniari I, A8 = Neniari II, A9 = Soya

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REFERENCES

- Ariestin Y, Kuswanto, Ashari S. 2015. Keragaman jenis salak bangkalan (*Salacca zalacca* (Gaertner) Voss) menggunakan penanda morfologi dan analisis isozim. *Jurnal Produksi Tanaman* 3 (1): 35-42. [Indonesian]
- Ayuningrum PI, Afrianto E, Mulyani Y. 2012. Keragaman rumput laut *Eucheuma* spp. dari Sukabumi Jawa Barat berdasarkan metode RAPD PCR. *Jurnal Perikanan dan Kelautan* 3 (4): 337-345. [Indonesian]
- Baig MNR, Grewal S, Dhillon S. 2009. Molecular characterization and genetic diversity analysis of citrus cultivars by RAPD markers. *J Agric For* 33: 375-384.
- Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissues. *Phytochem Bull* 19:11-15.
- Ediwirman dan Mansya, E. 2011. Characterization kapulauan (*Nephelium mutabile*) based on PCR_RAPD in West Sumatra. *Jurnal Embrio* 4 (1): 66 -73. [Indonesian]
- Fatimah S. 2013. Analisis morfologi dan hubungan kekerabatan sebelas jenis tanaman salak (*Salacca zalacca* (Gertner) Voss Bangkalan. *Agrovigor* 6 (1): 1-15. [Indonesian]
- Gurijala HK, Dileep RR, Pramoda KJ. 2015. Biodiversity of six varieties of *Mangifera indica* using RAPD. *Intl J Life Sci Biotechnol Pharma Res* 4 (2): 100 -103.
- Govarthanan M, Guruchandar A, Arunapriya S, Selvankumar T, Selvam K. 2011. Genetic variability among *Coleus* sp. studied by RAPD banding pattern analysis. *Intl J Biotechnol Mol Biol Res* 2 (12): 202-208.
- Herawati W, Kamsinah, Chasanah T. 2012. Banyumas local salak characteristics as indigenous species conservation program. Proceeding LPPM Universitas Jenderal Soedirman. Purwokerto, Banyumas. [Indonesian].
- Herawati W, Amurwanto A, Nafi'ah Z, Ningrum AM, Samiyarsih S. 2018. Variation analysis of three Banyumas local salak cultivars (*Salacca zalacca*) based on leaf anatomy and genetic diversity. *Biodiversitas* 19 (1): 119-125.
- IPGRI. 1995. Descriptors for Coconut (*Cocos nucifera* L.). International Plant Genetic Resources Institute, Rome, Italy.
- Kameswara RN. 2004. Biotechnology for Plant Resources conservation and use. Principles of seed handling in Genebanks Training course, Kampala, Uganda □
- Lee M. 1998. DNA Markers for detecting genetic relationship among germplasm revealed for establishing heterotic groups. Presented at The Maize Training Course, CIMMYT, Texcoco, Mexico, August 25, 1998. □ □
- Murti HM. 2002. Genotype variation of snake fruit landrace in Sleman. *Jurnal Habitat* 13 (1): 1-7. [Indonesian]
- Nandariyah, Soemartono, Artama WT, Taryono. 2004. Keragaman kultivar salak (*Salacca zalacca* (Gaertner) Voss). *Jurnal Agrosains* 6 (2): 75-79. [Indonesian]
- Nandariyah. 2010. Morphology and RAPD (Random Amplification of Polymorphic DNA) based classification of genetic variability of Java salacca (*Salacca zalacca* Gaertner. Voss). *Journal of Biotechnology and Biodiversity* 1 (1): 8-13. [Indonesian]
- Pattinama MJ, Boreel A, Laisina JKJ, Leimena HEP. 2007. The ethnobotany of kenari (*Canarium indicum* L) and salak (*Salacca zalacca* var *Amboinensis*) commodities to strengthen the bargaining position of the economy as well as efforts to enhance the food security program for Alune society in Seram island. Universitas Pattimura, Ambon.
- Rohlf FJ. 1998. NTSyS-p.c. Numerical Taxonomy and Multivariate Analysis System (Version 2.0). Exeter Software Publishers Ltd., Setauket.
- Semagn K, Bjørnstad Å, Ndjioudjop MN. 2006. An overview of molecular marker methods for plants. *African J Biotechnol* 5 (25): 2540-2568.
- Sudjijo. 2009. Karakterisasi dan evaluasi 10 aksesi salak di Sijunjung Sumatera Barat. *Buletin Plasma Nutfah* 15 (2): 75-79. [Indonesian]
- Suskendriyati H, Wijayati A, Hidayah N, Cahyuningdari D. 2000. Studies on morphological and phylogenetic relationship of salak pondoh varieties (*Salacca zalacca* (Gaert.) Voss.) at Sleman Highlands. *Biodiversitas* 1 (2): 59-64. [Indonesian]