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CHARACTERIZATION OF SALAK SIDIMPUAN (*SALACCA SUMATRANA* BECC.) BASED ON PHENOTYPIC CHARACTERS AND RAPD MARKERS IN SOUTH TAPANULI REGENCY, INDONESIA

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SUMMARY

Traditional plant breeding practices have predominantly relied on phenotypic observations, limiting molecular data availability. Furthermore, previous research on salak species has mainly focused on Salak Pondoh and Salak Gading, leaving Salak Sidimpuan understudied at the molecular level. The presented study sought to examine the genetic diversity of Salak Sidimpuan (*Salacca sumatrana* Becc.) using phenotypic and molecular approaches in South Tapanuli Regency, Indonesia. Nine samples collected from three major salak-producing districts in Padangsidimpuan—East Angkola, West Angkola, and South Angkola with different altitudes—entailed analysis. The RAPD analysis revealed a polymorphism percentage ranging from 60% to 91%, averaging 81.4%. The primer OPA-13 exhibited the highest polymorphism (91%) with 11 DNA bands, whereas the primer OPA-9 showed the lowest polymorphism (60%) with five DNA bands. The identified genetic characters totaled 38, comprising 32 polymorphic and two monomorphic characteristics. The following findings provide valuable insights into the genetic diversity of Salak Sidimpuan, offering a molecular basis for breeding strategies and conservation for the future.

Keywords: Salak Sidimpuan (*S. sumatrana* Becc.), molecular markers, polymorphism, genetic diversity, phenotypic analysis

Key findings: This study provides information on phenotype and genetic diversity in Salak Sidimpuan (*S. sumatrana*) plants based on DNA banding patterns using the RAPD method. The genetic diversity indicators based on DNA bands support the diversity at the morphological level of the Salak Sidimpuan.

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INTRODUCTION

Salak (*Salacca* spp.) belongs to the family Arecaceae described as a tropical fruit native to Indonesia (Prihatini *et al.*, 2023). The discovery of 20 species of salak has occurred worldwide, with only 13 species being identified with certainty (Noor and Effendi, 2022). The most widely cultivated snake fruit species are the Salak Pondoh, Salak Bali, Salak Sidimpuan, Salak Condet, Salak Gading, Lokal Banyumas Salak, and Salak Madu. Each salak cultivar has unique physical, chemical, and sensory properties (Zubaidah *et al.*, 2018). Salak Sidimpuan (*Salacca sumatrana* Becc.) has a characteristic taste that is astringent, sweet, and slightly sour, with a pleasant aroma and flavor, which distinguishes it from Salak Pondoh and Salak Bali (Siregar*,* 2023).

Salak Sidimpuan has the advantage over other salak. Even though Salak Pondoh has the advantage of a sweet fruit flavor, its weakness is its thin flesh. Salak Bali outperforms with a thick flesh, but the flesh is too sticky, while Salak Sidimpuan stands out with a sweet fruit flavor, thick flesh, and several bunches with 3–5 fruits. Thus, Salak Sidimpuan showed the highest commercial value as its demand for export to Singapore, Hong Kong, Malaysia, China, Japan, the Netherlands, and the United States is increasing (Hadiati *et al.,* 2023).

Salak Sidimpuan cultivation has begun since 1930 and become a recognized endemic fruit of South Tapanuli, Indonesia. However, its primary production center location is in the City of Padang Sidimpuan, renowned as 'The City of Salak' due to its surrounding topography of hills and mountains serving as extensive salak plantation areas. Salak Sidimpuan is a very popular snake fruit because it combines sweet, sour, and slightly bitter flavors, which is typical of Salak Sidimpuan (Nasution, 2019). Each salak cultivar revealed distinct adaptability to varying altitudes, a trait closely associated with its temperature tolerance (Sumantra *et al.,* 2024). Higher altitudes tend to induce specific developmental adaptations in plants, reflecting their potential to optimize growth and development under diverse environmental conditions.

The Salak Sidimpuan, with its phenotypic, cytological, and molecular characteristics, is crucial for establishing and conserving germplasm, as it provides valuable information for targeted breeding programs (Santos *et al.,* 2012). In salak, the germplasm improvement can succeed by selecting the genotypes with desirable traits to meet consumer demand, a process supported by the Indonesian government’s policy on food diversification (Budiyanti *et al.,* 2015). Understanding genetic diversity and classification in breeding programs is essential for assessing genetic variations. The new cultivar development mostly has influences from environmental and genetic factors. Differences and similarities in the broad morphological traits of a plant species can be useful to determine the genetic relatedness. Moreover, the wide genetic diversity observed in specific traits offers significant opportunities for selecting the genotypes with superior characteristics (Hadiati *et al.,* 2023). Plant breeding is a combination of art and science that explores how to improve plant phenotypes in a population and make them more beneficial to humans (Friska and Daryono, 2017).

Local Banyumas Salak’s (*Salacca zalacca*) three cultivars have undergone genetic variation studies based on leaf anatomy and their genetic diversity (Herawati *et al.,* 2012). The genetic distance of Salak Pondoh and Salak Gading cultivars and their hybrids gained evaluation based on RAPD markers. No research exists yet on the molecular characteristics of Salak Sidimpuan (Nandariyah *et al.,* 2021). The characteristics that distinguish Salak Sidimpuan from Salak Pondoh, Salak Gading, and Salak Gula Pasir are the thickness of the flesh, which contains 90% water, and red and white-colored pulp (Siregar*,* 2023). Subspecies discrimination can attain further support through phenotypic, anatomical, metabolic, and molecular approaches (Zhang *et al.,* 2018). Therefore, Salak Sidimpuan presents a compelling subject for study, given its distinctive flesh thickness and desirable commercial flavor.

DNA markers offer a significant advantage over phenotypic markers in plant improvement characterization due to their stability, consistency, and independence from environmental influences (Kundan *et al.,* 2014). According to Hadi and Nurchasanah (2020), identifying phenotypic characters is often inaccurate, as distinguishing chromosomes can be highly challenging and dependent on the plant's growth stage, rendering the process impractical. A widely used molecular technique for genetic diversity analysis is the random amplified polymorphic DNA (RAPD). The introduction of DNA-based molecular techniques, such as RAPD, has facilitated the development of efficient marker systems. This approach has extensive application across various organisms, including humans, animals, and crops (Ediwirman and Jamsari*,* 2015). In genetic diversity, RAPD analysis is a robust indicator for supporting diversity assessment based on specific traits (Yuniastuti *et al.,* 2023).

The RAPD fragments' generation occurs by amplification of a single locus and two types of polymorphism: DNA bands may be present or absent, and the brightness of the bands may differ. Differences in band intensity result from the number of copies, and their use cannot distinguish homozygous dominant individuals from heterozygotes because RAPD is dominant and qualitative, with the brighter bands expected to be the major ones (Semagn *et al.,* 2006). Understanding the spatial genetic structure of a population is at the organismic level of diversity (Bouza *et al*., 2006). Selection using molecular markers depends on the genetic characteristics of the plants without the influence of environmental factors, and the molecular technique is a strategic tool that can also shorten the selection time (Slamet *et al.,* 2022). The following study aimed to examine the genetic diversity of Salak Sidimpuan (*Salacca sumatrana*) using phenotypic and molecular approaches in South Tapanuli Regency, Indonesia.

MATERIALS AND METHODS

Study sites and material

This research ran from August to October 2024. The Salak Sidimpuan plant sampling took place in the central salak plantation area of Sidimpuan, located in South Tapanuli Regency, across three subdistricts, i.e., West Angkola (1,550–1,700 masl), East Angkola (1,225–1,850 masl), and South Angkola (20–1,000 masl), North Sumatra, Indonesia (Figure 1). Molecular testing happened at the Biology Laboratory of the Medan State University, North Sumatra. Based on the Decree of the Minister of Agriculture, Indonesia, three cultivars of salak are official, namely, Salak Merah Padangsidimpuan, Salak Putih Padangsidimpuan, and Salak Sibakua.

Phenotyping characters

Phenotypic character data observation of Salak Sidimpuan plants relied on IPGRI's (International Plant Genetic Resources Institute) methodology. The observed traits totaled 21, categorized as follows: for leaves—shape, venation pattern, margin, color, length (cm), and width (cm); for stems—height (cm), color, and shape; and for fruits—weight (g), flesh and skin color, content, firmness, soluble sugar content (°Brix), texture, seed color, and seed shape.

Genotyping

DNA analysis using random amplified polymorphic DNA (RAPD) markers involved DNA extraction from nine snake fruit leaf samples using a CTAB-based DNA isolation kit and a PCR kit. Samples (0.5–1 g), retrieved from the freezer, entailed weighing and mixing with 500 µL of PhytoPure I reagent. The homogenized mixture, as transferred to a 1.5 mL tube, succeeded in combining with 400 µL of PhytoPure II reagent while gently mixing.

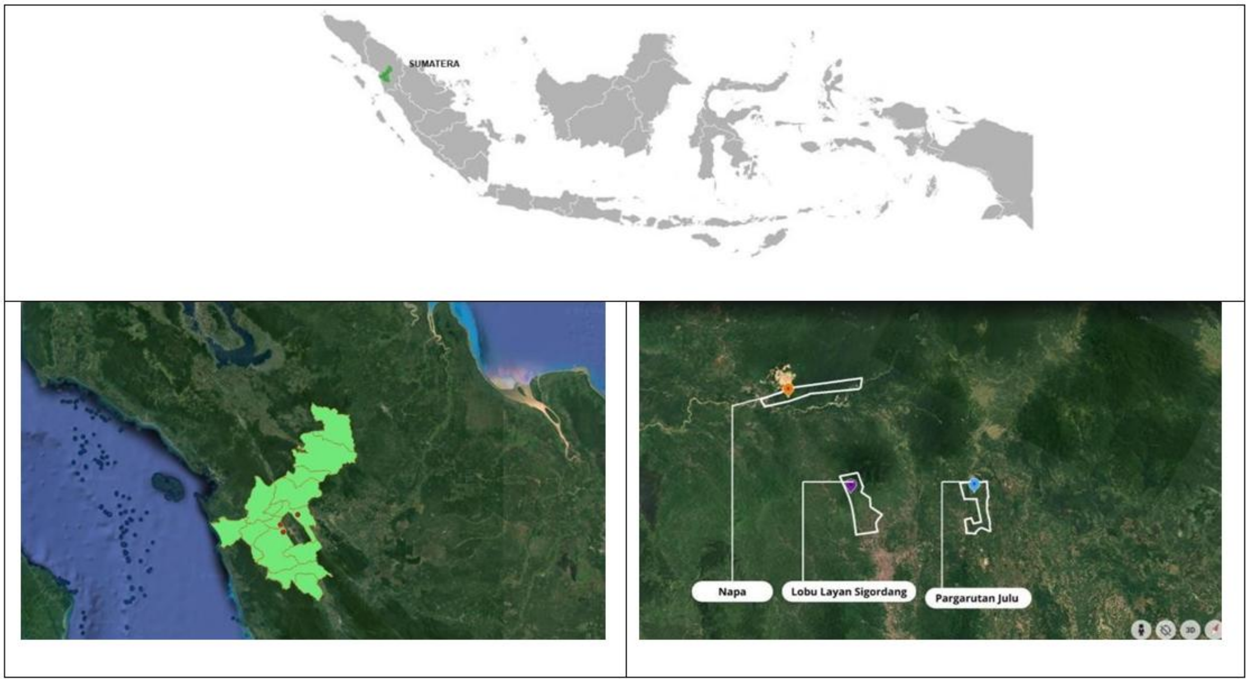

Figure 1. Locations of South Tapanuli District, North Sumatra, Indonesia, showing the sampling sites of Salak Sidimpuan (*Salacca sumatrana*).

Table 1. CR-RAPD reaction procedure.

No.	Reaction	Temperature	Duration
1	Pre-denaturation	94 °C	5 minutes
2	Denaturation	94 °C	1 minute
3	Annealing	36–39 °C	1 minute
4	Elongation	72 °C	2 minutes
5	Post Elongation	72 °C	10 minutes
6	Endless	4 °C	4 minutes

The sample attained incubation at 65 °C for 10 min, then being cooled in an icebox for 20 min. Next, adding 400 µL of cold chloroform and 20 µL of PhytoPure Resin continued before centrifugation at 3,000 rpm for 10 min. The supernatant's transfer to a new tube sustained the addition of cold isopropanol. After centrifugation at 10,000 rpm for 10 min, proceed with removing the supernatant before washing the DNA pellet three times with 100 µL of 70% ethanol, centrifuging at 10,000 rpm for 5 min each time. The pellet underwent air-drying and resuspending in 50 µL of TE buffer (1X). The process comprised five RAPD primers with sequences OPA-03, OPA-04, OPA-05, OPA-09, and OPA-13; 2% agarose gel; loading dye; a 100 bp marker; PhytoPure I and II reagents; isopropanol; and 70% ethanol. The tools used included a cutter, icebox, a set of electrophoresis equipment, refrigerator, staining tray, mortar and pestle, Eppendorf tube, electric balance, measuring cup, and Erlenmeyer. Other materials were a blue tip, yellow tip, funnel, test tube, filter paper, spectrophotometer, small Petri dish, micropipette, tip, filter, small tube, and tube rack. The PCR mixture, when amplified, used a BOECO Thermal Cycler TC-PRO with specific temperature and time settings (Table 1).

Table 2. Phenotypic characters of salak sidimpuan in South Tapanuli District, Indonesia.

Phenotype characters	Location Sample		
	Angkola Barat	Angkola Timur	Angkola Selatan
Leaves			
Leaf shape	Oblongus	Oblongus	Oblongus
Leaf veins	Penninervis	Penninervis	Penninervis
Leaf margin shape	Integer	Integer	Integer
Leaf color	Dark green	Green	Green
Leaf length (cm)	65–70	62.4–65	58.2–63.9
Leaf width (cm)	4.5–7.2	4.5–6.0	4–5.6
Stems			
Stem height	7, 3–8	7–8, 3	6, 8–7, 6
Stem color	Brown	Brown	Brown
Stem shape	Thorny	Thorny	Thorny
Fruits			
Fruit shape	Round	Oval and round	Oval and round
Fruit flesh color	White, red, white with Red fibers, red with white fibers	White, red, white with Red fibers, red with white fibers	White, red, white with Red fibers, red with white fibers
Fruit skin color	Dark brown and black	Dark brown and black	Dark brown and black
Fruit weight			
Fruit content	1–3	1–3	1–3
Fruit flesh flavor	Sweet, astringent, sour Flavor	Sweet, astringent, sour flavor	Sweet, astringent, sour flavor
Soluble Sugar content (Brix)	18, 26–20, 32	17, 20–19, 32	16, 99–18, 13
Fruit weight (g)	80–120	70–90	80–100
Fruit texture	Smooth and juicy	Smooth and juicy	Smooth and juicy
Fruit seed color	Light brown, dark brown, Black	Light brown, dark brown, black	Light brown, dark brown, Black
Fruit seed weight (g)	2–4, 5	2–4	2–4
Fruit seed shape	Round	Round	Round

PCR-RAPD amplification

RAPD PCR products' preparation consisted of 2% agarose gel, a loading dye, and a 100 bp marker. Each DNA sample (5 µl) employed mixing with the loading dye (1 µl) on paraffin paper and then loading into wells, with the first well containing the marker. Running electrophoresis ensued at 50 V. The gel, stained with ethidium bromide for 20–30 min, underwent observation under a UV transilluminator. The DNA bands appeared as pink signals of varying intensity based on the DNA concentration, being photographed with a digital camera.

RESULTS AND DISCUSSION

Phenotypic characters

Based on the altitude, the three locations have different phenotypic characteristics of the Salak Sidimpuan plants. Phenotypic differences in salak resulted in different production centers and among the plants cultivated within the same region (Zumaidar *et al.*, 2014; Mazumdar *et al.*, 2019) (Table 2). In taxonomic identification and classification, the leaf surface phenotypic structure can help distinguish characteristics between species due to the highest structural diversity (Solangi *et al.*, 2010).

Table 3. Primary data, sequences, size, number of DNA bands, and polymorphic presentation.

Primer	Sequences (5' – 3')	Size (bp)	Number of polymorphic	Number of monomorphic	Total	Polymorphic presentation (%)
OPA-3	AGT CAG CCA C	200-700	6	1	7	86
OPA-4	AAT CGG GCT G	200-950	9	1	10	90
OPA-5	AGG GGT CTT G	200- 700	4	1	5	80
OPA-9	GGG TAA CGC C	275-1100	3	2	5	60
OPA-13	CAA ACG TCG G	185-1000	10	1	11	91
			84.21%	15.79%	100%	

Figure 2. Salak sidimpuan cultivars: A) red salak, B) white salak, and C) sibakua salak. (Personal documentation, 2024).

Nine randomly selected plants revealed distinction for 21 phenotypic qualitative and quantitative traits based on the leaf, flower, fruit, and stem traits (Table 3). Based on the altitude classification, past observations of the dissolved sugar content showed nonsignificant differences among the Salak Sidimpuan plants of the West Angkola, Hutaimbaru, and South Angkola (Adelina *et al.,* 2024). The Salak Sidimpuan plants observed belonged to farmers who had passed them down from generation to generation without cultivating them. The diversity's estimation entailed measuring the variations in the qualitative and quantitative traits. However, this approach was often limited, with the expression of quantitative traits considerably influenced by the environment (Kameswara, 2004). Ultimately, this will characterize the phenotype characters and content of active compounds in plants that affect plant growth.

According to research results, the phenotypic traits do not have distinguishing characteristics. However, a more precise assessment of phenotypic similarity can be successful through molecular analysis. The determining variation within a population requires measurement and analysis based on the biometrical principles (Budiyanti *et al.,* 2019). Aside from environmental influences, the expression of the qualitative traits in plants had genetic factors and their interactions governing them. Each gene plays a specific role in regulating individual characteristics. The genes *psbA*, *psbK*, and *psbI*, located in the chloroplasts of salak plants, encode the photosystem II proteins D1, K, and I, which are essential for photosynthesis (Chen *et al.,* 2022). According to Matatula *et al.* (2021), the altitude influences fruit skin color and plant height in salak cultivars (Figure 2). At higher altitudes, sunlight intensity and temperature fluctuate, while CO_2 concentration is lower than in lowland areas. These environmental factors directly affect photosynthesis, potentially causing variations in leaf color (Simin *et al*., 2022). However, in the presented study, despite the variations in altitude across salak

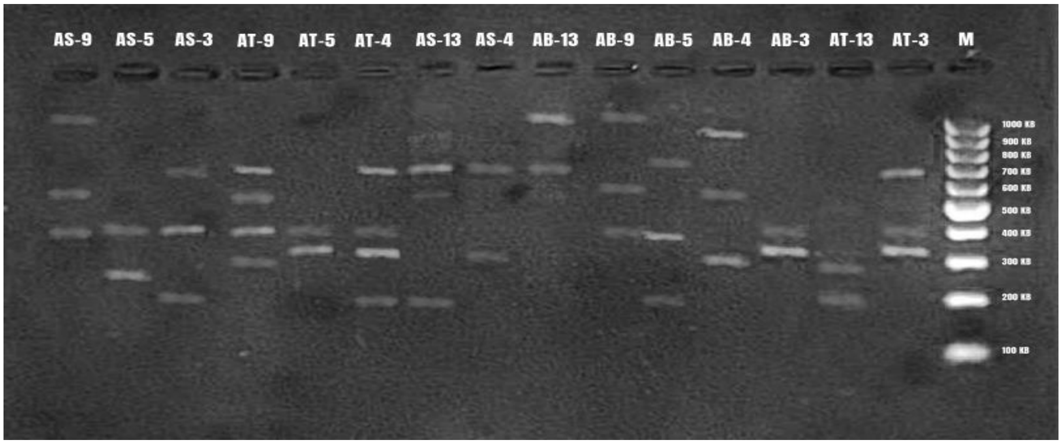

Figure 3. DNA electropherogram of RAPD PCR results with primers OPA-03, OPA-04, OPA-05, OPA-09, and OPA- 13; AS = District South Angkola, AB = District West Angkola, and AT = District East Angkola.

plantations in South Tapanuli Regency, altitudes showed no evidence of having a considerable effect. The leaf color observed at all locations remained consistently green.

Molecular characterization and DNA amplification

This study employed five primers, namely, OPA-03, OPA-04, OPA-05, OPA-09, and OPA-13, which succeeded in applying to different plants in several studies. In this study, the DNA amplification generated polymorphic bands, which authenticated that the RAPD method was effective for evaluating genetic diversity in Sidimpuan Salak plants. The RAPD method is a technique used to detect DNA polymorphisms by amplifying random DNA fragments with primers that have a nucleotide sequence of arbitrary definition, typically consisting of 10 base pairs. This method serves as a marker that is tolerant to varying DNA purity levels.

The molecular analysis using the RAPD method with five primers revealed sufficient polymorphic DNA bands, which were valuable for DNA analysis of Sidimpuan Salak plants (Figure 3). A total of 38 DNA bands amplified had sizes ranging from 200 to 1100 bp. Previous research on the local Salak Banyumas (*Salacca zalacca*) cultivar using the RAPD method identified 62 DNA bands, ranging from 300 to 2300 bp (Herawati *et al.,* 2018). According to Purnomo and Ferniah (2018), in RAPD analysis, the most informative DNA bands typically fall within the range of 300 to 3000 bp. The variation in DNA band sizes can refer to the disparities in DNA strand lengths, which the primers can further extend. However, RAPD primers generally amplify DNA fragments ranging from 100 to 1300 bp, depending on the genome and the primer's nature (Wahyudi *et al.,* 2020). According to Noor and Effendi (2022), reports of the salak plants with a similarity index of 85.4% emerged in the Tanah Grogot District, Paser Regency, East Kalimantan, Indonesia.

The RAPD analysis of salak plants from three locations revealed polymorphic representation ranging from 60% to 91%, averaging 81.4% (Table 3). The highest polymorphic percentage appeared with primer OPA-13, with 11 DNA bands and a polymorphism rate of 91%. Conversely, the lowest polymorphic percentage was evident with primer OPA-9, which enunciated five DNA bands and a polymorphism rate of 60%. The identified bands totaled 38, of which 32 were polymorphic and two were monomorphic. Similarly, Herawati *et al.* (2018) reported that 80.6% of the bands were polymorphic and 19.3% were monomorphic.

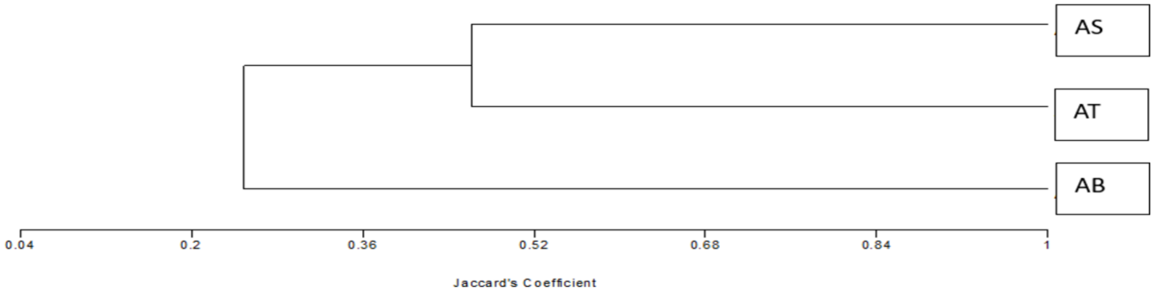

Figure 4. Phenetic dendrogram of RAPD analysis in salak sidimpuan based on Jaccard's coefficient.

Table 4. Similarity Index based on Jaccard Coefficient.

Lo*cation*	AB	AT	AS
AB	1,000	-	-
AT	0.265	1,000	-
AS	0.233	0.462	1,000

Description: AB: West Angkola AT: East Angkola; AS: South Angkola

From the RAPD results, Salak Sidimpuan plants from the three subdistricts clustered within the same group, suggesting the three samples of Salak Sidimpuan were likely originating from the same cultivar. The DNA polymorphism (84.21%) observed in this study aligns with several previous studies that employed RAPD techniques. The RAPD markers, generated by random amplification of DNA segments, used a single arbitrary primer. Typically, the primers used were 10 base pairs in length. The G+C content of the primer sequence used for PCR-RAPD must exceed 40% (typically 50%–80%) and should not contain palindromic sequences.

Past findings indicated the success of primers in amplifying template DNA can be ascribable to the presence and absence of primer nucleotide sequences that are homologous to the template DNA sequences (Arsyad *et al.,* 2022). According to Ifah *et al.* (2018), the DNA fragments generated by each RAPD primer vary, making them useful for assessing the similarity characteristics of each cultivar. The presence of increasingly similar fragments can enhance the level of genetic similarity in plants. Gorji *et al.* (2011) reported an average polymorphism of approximately 20%, 31%, and 57.4%, which suggested a lower polymorphic potential than the present results.

Similarity index and dendrogram of the Sidimpuan Salak

The genetic diversity analysis in Salak Sidimpuan plants using RAPD primers comprised a dendrogram representation, illustrating the hierarchical relationship among the studied salak genotypes (Figure 4). Grouping of plant dendrograms by subdistrict displayed patterns of genetic or phenotypic diversity related to location. This approach can aid in identifying parental genotypes for future breeding programs (Slameto, 2023).

Differences observed among the studied salak genotypes depended on the similarity index and dendrogram generated using the unweighted pair group method with arithmetic mean (UPGMA) and the SIMQUAL (similarity quantitative Jaccard's index) function. Specifically, the samples obtained from different locations exhibited distinct phenotypic characteristics. The dendrogram analysis revealed two distinct clusters, namely, Cluster I and Cluster II, and AB, which showed similarity to AS. Based on the results obtained through Jaccard's coefficient method (Table 4), the similarity index ranged from 0.265 to 0.462. The molecular markers used to assess the genetic differences among the plants were crucial as they aid in grouping and provide a visual representation of genetic variability, ensuring ongoing genetic improvement (Singh *et al.*, 2021).

Dendrogram analysis grouped three genotypes of Salak Sidimpuan samples into different clusters. In plant breeding, the identification of parental lines can be according to the relationship between the resulting plant genotypes (Hanum *et al*., 2020). Furthermore, the greater the genetic distance among the parental lines, the higher the potential for producing new cultivars with broader genetic variation. In contrast, when the parental lines are closely related, the resulting cultivars exhibit less genetic variation (Slameto, 2023). The higher genetic similarity value between two cultivars indicates a closer relationship, whereas a lower similarity value suggests a greater genetic divergence among the newly developed genotypes (Hanum *et al.,* 2020).

CONCLUSIONS

The DNA amplification using five RAPD primers at the three salak plantation centers in South Tapanuli Regency resulted in polymorphism rates ranging from 60% to 91%, with an average of 81.4%. The highest polymorphism emerged with the primer OPA-13, while the lowest was with primer OPA-9. The genetic characters identified totaled 38, including 32 polymorphic and two monomorphic characters. Salak Sidimpuan obtained from the West Angkola and South Angkola exhibited the highest genetic similarity index, ranging from 0.265 to 0.462. This can be visible in the observed phenotype traits: leaves, stems, and fruits.

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