

Cytological and Molekular Characteristics of Salak Sidempuan (*Salacca sumatrana* Becc.) at Different Elevations

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Received: December,2, 2024 | Revised: December,18, 2024 | Accepted: December,20, 2024

Abstract. Breeding is mostly done only through phenotypic observations, so information on molecular data is still limited. In addition, most studies only focus on Salak Pondoh and Salak Gading. Therefore, we want to conduct research on the study of genetic diversity of Salak Sidempuan in the South Tapanuli region. Samples were taken from 3 locations which are the largest salak production centers in Padang Sidempuan, namely East Angkola District, West Angkola District, and South Angkola District, each of which has a different altitude. The number of samples taken was 9 sample plants from all locations. Cytological observations, namely observations of leaf chlorophyll content at 3 locations tested at the Biology Laboratory of Medan State University. Genetic varieties of Salak Sidempuan were analyzed using Random Amplified Polymorphic DNA (RAPD) molecular markers at the Biology Laboratory of Medan State University. The results of the study showed that the highest chlorophyll content was found in the West Angkola District with an altitude of 1550-1700 mdpl. The results of the RAPD analysis on Salak with 3 locations, namely West Angkola District, East Angkola District and South Angkola District, obtained polymorphic presentation results of 60 - 91% with an average of 81.4%. The highest polymorphic presentation was obtained with the OPA-13 primer and the lowest with OPA-9.

Keywords: Cytological; Elevation; Molekular; *Salacca sumatrana* Becc.

INTRODUCTION

Salak plants are tropical plants native to Indonesia which are included in the Palmae group (Nadariya, 2010). Salak plants that have been cultivated in Padang Sidempuan are Salak Sidempuan (*Salacca sumatrana* Becc.). Salak Sidempuan production is famous in Padang Sidempuan City so that this city is nicknamed Salak City because it is surrounded by hills and mountains which are the Salak Plantation Area.

The problems faced in the development of Salak Sidempuan fruit production are the limited superior varieties, research on Salak Sidempuan is limited to conventional breeding and research on salak genetics. Some of the distribution is done through morphological observations so that there is a lack of information on molecular data. Moreover, most of the research is only about Salak Pondoh and Salak Gading so that studies on the genetic diversity of Salak Sidempuan are important to do.

Plant breeding program as an effort to obtain new superior varieties that have not been widely carried out in Indonesia. In the breeding program, information is needed about diversity and its classification that can be used to show that diversity.

South Tapanuli Regency is the location of Salak Sidempuan planting. The location of Salak Sidempuan research is in Three Districts, namely West Angkola District, East Angkola District and South Angkola District which have different elevations. In general, high elevations produce high plant quality, while low elevations produce low quality (Purlani

and Rachman, 2000). In addition to observing molecular characters, it is also necessary to observe the cytological characters, namely the chlorophyll content at different elevations.

Molecular markers have advantages over morphological markers because they are stable, consistent and not affected by the environment (Zulfami, 2013). Salak, one of the molecular techniques often used in analyzing genetic diversity is Random Amplified Polymorphic DNA (RAPD). RAPD is considered practical to use compared to other markers because less DNA is needed, cheaper, does not require blotting, and data can be collected quickly (Gurijala et al. 2015).

METHOD

Location

This research was conducted from August to November 2024. The research location was determined based on a survey by determining different elevations at each location located in South Tapanuli. Salak Sidempuan sampling was carried out by purposive sampling, the number of samples taken at each location was 3 sample plants so that the total number of sample plants was 9 plants. Cytological and morphological character tests were carried out at the Biology Laboratory of Universitas Negeri Medan Sumatera Utara.

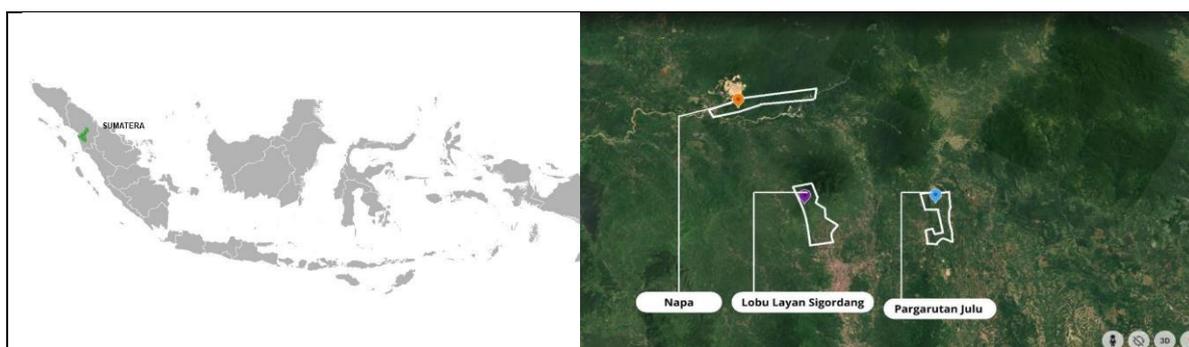


Figure 1. Location of Salak Sidempuan in South Tapanuli Regency

Table 1. Altitude of places in three sub-districts

Location	Elevation
Kecamatan Angkola Barat	1550-1700 mdpl
Kecamatan Angkola Timur	1225-1850 mdpl
Kecamatan Angkola Selatan	20-1000 mdpl

Based on Table 1. It can be seen that each sub-district has different altitudes. West Angkola sub-district has the highest altitude.

Cytological characteristics

The cytological characteristics observed were the chlorophyll content of the leaves of the Salak Sidempuan plant. Determination of chlorophyll content using the method used in the study by Friska and Daryono (2017), fresh leaves were ground as much as 0.5 grams using a mortar. The results of the grinding were then diluted with 80% acetone and filtered

using filter paper to take the clear liquid. The leaf chlorophyll solution was placed in a cuvette and inserted into a spectrophotometer device at wavelengths of 663 and 645 nm.

Molecular Characteristics

The stages of the character of Salak Sidempuan molecularly are through the stages of DNA extraction, DNA amplification, electrophoresis, RAPD band profile analysis. The markers used in this study are OPA-03, OPA-04, OPA-05, OPA-09, OPA-13.

Table 2. RAPD primers used

No	Primer	Primer Sequence 5' - 3'
1	OPA-03	5'-AGT CAG CCA C- 3'
2	OPA-04	5'-AAT CGG GCT G- 3'
3	OPA-05	5'-AGG GGT CTT G- 3'
4	OPA-09	5'-GGG TAA CGC C- 3'
5	OPA-13	5'-CAG CAC CCA C- 3'

Data Analysis

Data were analyzed descriptively based on the presence or absence of DNA fragments produced by five primers at each locus. The results of the phenetic dendrogram on Salak Sidempuan based on Jaccard's Coefficient.

RESULTS AND DISCUSSION

There are two types of classification of Salak Sidempuan plants studied, namely classification carried out cytologically, namely calculating the chlorophyll content of the leaves, and molecular classification, namely based on genetic kinship relationships.

Cytological Characteristics

The chlorophyll levels measured were chlorophyll a, chlorophyll b and total chlorophyll. The following are the results of measuring chlorophyll content presented in table form.

Table 3. Chlorophyll Content of Sidempuan Salak Plant Leaves

Location	Chlorophyll		
	a	b	Total
West Angkola District	30,58	19,37	49,37
East Angkola District	25,56	16,72	42,28
South Angkola District	28,39	18,65	47,04

The results of the study showed that the Salak Sidempuan sample in Angkola Barat District had the highest chlorophyll content, namely total chlorophyll 49.37. Chlorophyll synthesis is influenced by various factors, such as sunlight, sugar or carbohydrate content, water, temperature, genetic factors, and soil nutrients, such as N, Mg, Fe, Mn, Cu, Zn, S, and O (Hendriyani & Setiari, 2009). Plants can filter and thus reduce the level of carbon dioxide in the air through the absorption of CO₂, which is needed in the process of photosynthesis (Dolman et al., 2003).

Chlorophyll is a pigment that can dissolve in ethanol, methanol, ether, benzene,

acetone and chloroform but cannot dissolve in water (Kurniawan et al., 2010). The chlorophyll content in each Salak Sidempuan plant shows varying results, this is due to the development of pigments in the form of chlorophyll. Kusumastuty (2018) explains that the factors that affect leaf chlorophyll levels are external and internal factors. These external and internal factors include genetics, sunlight, light intensity, temperature, soil pH and nutrient levels. Altitude can also affect the chlorophyll content in Salak Sidempuan leaves. Altitude affects plant growth and production. The results of research by Qadry et al. (2017), altitude affects plant production and quality, especially in Arabica coffee plants. Furthermore, according to Istiawan & Kastono (2019), the higher the place where plants grow, especially clove plants, tends to decrease the quality and quantity of production because it is influenced by the plant's microclimate. This shows that there are differences in character or properties between plants that grow at different altitudes.

The results of Aliyaman and Asriyani's (2022) research show that cashew plants at an altitude of 0-500 m above sea level have higher chlorophyll content. Ai and Banyo (2011) said that low chlorophyll levels are also influenced by the absorption of nitrogen nutrients by plants, while nutrient absorption is determined by the N content in the soil.

Molecular Characteristics

RAPD technique is one method for phylogenetic classification, this technique has advantages because it is simple to do, easy to practice, accurate results and relatively much time to identify germplasm variations (Halley et al., 1973). RAPD analysis has been used in various types of genetic variability among plant genotypes, plant populations in plant breeding (Carvalho et al., 2004). The polymorphic results of RAPD analysis on Salak Sidempuan plants are presented in Table 4. Below.

Table 4. Primary Data, Sequence, Size, Number of DNA Bands, and Polymorphic

Primer	Sekuens (5' - 3')	Based pair	Polymorphic Number	Monomorphic Number	Total	Percentage (%)
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%	

Based on Table 4, RAPD analysis on Salak with 3 locations, namely West Angkola District, East Angkola District and South Angkola District, obtained polymorphic presentation results of 60 - 91% with an average of 81.4%. The highest polymorphic presentation was obtained with the OPA-13 primer and the lowest with OPA-9. The total characters obtained were 38, with 32 polymorphic characters, and 2 monomorphic characters.

Salak Sidempuan from three sub-districts has a sweet, sour taste and contains a lot of water, for the color of Salak Sidempuan there are four colors, namely red, red with white fibers, white with red and white fibers. Based on the results of RAPD, Salak Sidempuan is in the same group so it is estimated that the three samples of Salak Sidempuan from three sub-districts come from the same cultivar.

The polymorphism of DNA fragments produced in this study (84.21%) is in line with several previous studies using the RAPD technique. The results of Herawati et al.'s

(2018) study produced 80.6% of the number of polymorphic bands and 19.3% of the number of monomorphic bands. RAPD analysis from Nandariyah (2010) using 12 salak cultivars in Java with six random primers produced 68.4% polymorphism. Roslim et al. (2003) analyzed the genetic relationship of coconut species in the Banyuwangi, Lubuk Pakam, and Paslaten areas using ten primers resulting in 80% polymorphism. Karsinah et al. (2002) detected genetic diversity in citrus germplasm using ten primers resulting in 91.8% polymorphism. The results showed that the ten primers showed a G+C content of between 60-70%, indicating that the primers were good enough to be used for PCR-RAPD.

This is in accordance with the statement of Poerba and Yuzammi (2008) that the success of genomic DNA amplification using the PCR-RAPD technique is also determined by the primer base sequence used and the quality or content of the primers in each reaction. RAPD markers are obtained by random amplification of DNA segments from a single arbitrary primer. The primers used are generally 10 bp in size. The G+C base content in the primer sequence used for PCR-RAPD must contain more than 40% G+C bases (generally containing 50-80% G+C) and must not contain palindromic sequences.

CONCLUSION

It can be concluded that the highest chlorophyll content in Salak Sidempuan plants is found in West Angkola District with a total chlorophyll content of 49.37 while the lowest total chlorophyll content is found in East Angkola District with 42.28. The five primers are quite optimal for RAPD analysis on Salak Sidempuan to determine the phonetic dendrogram.

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