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Analysis Kinship Genetics Sugar palm (*Arenga pinnata*) Based on Leaf Anatomy

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Abstract

The sugar palm (*Arenga pinnata*) has strong potential as a plantation commodity, with significant development prospects. This study aims to determine the diversity and genetic relationships among sugar palm accessions based on leaf anatomy. The leaf samples came from propagated seedlings collected at the Experimental Garden of the Faculty of Agriculture, Andalas University. The sample included 20 sugar palm accessions, classified by operculum position. Researchers collected five leaf samples for each operculum position. Leaf anatomy observations consistently revealed patterns of diversity that complemented and reinforced one another in describing kinship relationships among the accessions. Based on anatomical analyses, the K1 and K2 populations were identified as closely related to the Parasi/Smulen variety, with dense palisade tissue, a moderate cuticle, and dense chloroplasts. They were classified as the Medium Type. The K3 population (part of Cluster II) showed intermediate characteristics, including a thick cuticle, dense spongy tissue, and high variability; therefore, its type could not be determined. Meanwhile, the K4 population was closely related to the Dalam (Tomohon) variety, which was characterized by a thin cuticle, loose palisade tissue, and sparse chloroplasts. It was therefore classified as the Dalam Type. A strength of this research is its ability to visually and practically confirm molecular genetic groupings using leaf histological characteristics. This provides an efficient method for identifying varieties at the seedling stage without waiting for the plants to mature. Based on these results, users—especially plant breeders and farmers—are encouraged to use leaf anatomical parameters, such as palisade density and cuticle thickness, as practical guides when selecting superior sugar palm seedlings of specific types (Medium Type and Dalam Type) before transferring them to the field. This may help ensure uniformity and successful production in the future.

Keywords: Genetic Relationship, *O perculum*, Population, Selection, Variability

1. Introduction

The sugar palm (*Arenga pinnata*) has great potential as a plantation commodity, with broad development prospects. Sugar palm is a plant with the botanical classification: Kingdom: Plantae, Division: Spermatophyta, Subdivision: Angiospermae, Class: Monocotyledoneae, Order: Arecales, Family: Arecaceae, Genus: Arenga, Species: Arenga pinnata. Sugar palm is one of the trees found in tropical climates. The sugar palm leaves have leaflets 1 m or longer and 100 or more on each side (Dicky, 2019)

The leaves of the sugar palm are compound or pinnate, reaching up to 8 meters in length. The leaflets are divaricate, at least one meter long, with long petioles and pinnate veins. The upper leaf surface is fleshy green, while the underside is white and powdery. Young sugar palm leaves curl up and stand upright at the top of the stem, while the leaf sheath widens at the base and tapers towards the tip. The leaf sheath is also covered with dark black fibers at the base, and there is a fine, cotton-like mass called kawul at the base of the sheath (Lempang, 2012). These leaf characteristics play an important role in identifying and characterizing sugar palm varieties across regions. Variations in leaf characters, such as petiole length and number of unproductive leaflets, are characteristics that can distinguish one sugar palm accession from another and have succeeded in grouping sugar palm populations into several clusters based on the level of phenotypic similarity. (Harahap et al., 2019)In addition, other qualitative characters such as leaf sheath color are also among the phenotypic characteristics observed in studies of the

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variability of sugar palm accessions in various regions (Suhendra et al., 2023). Plant breeding can maximize sugar palm's potential as a superior plantation crop. This requires a deep understanding of sugar palm genetic relationships in Indonesia, which show diverse patterns, and its geographical distribution across the archipelago. (Rinawati et al., 2021)Sugar palm leaves have great potential as a source of genetic material for plant relationship analysis. Extensive genetic diversity is expected to help identify the best germplasm sources and determine plant relationships using agronomic and morphological characters.

However, fundamental problems remain in developing sugar palm commodities in Indonesia. To date, variety identification and superior seed selection still rely heavily on external, macroscopic morphological characteristics. These morphological characteristics have a major weakness: they are highly influenced by environmental factors (phenotypic plasticity) and require long observation periods, as most morphological traits emerge optimally only after the plant enters the mature or productive phase. Furthermore, the rate of land conversion and exploitation of natural sugar palm continues to increase, but the availability of standardized, superior seeds does not keep pace. As a result, breeders and farmers often fail to detect genetic uniformity early in the nursery phase, leaving the genetic quality of field plants uncertain. The high cost and limited laboratory facilities at the conventional breeding level often limit the use of molecular markers, necessitating alternative marker parameters that are fast, inexpensive, stable, and representative.

Several previous studies have been conducted to uncover the diversity patterns of sugar palm plants in Indonesia. For example, Rinawati et al. (2021) analyzed sugar palm genetic relationships using fruit morphological markers and agronomic traits across several regions of the archipelago. After re-verifying, I found concrete sources that match both parts of your narrative, including the three varieties (Parasi, Smulen, Tomohon) you mentioned. Here is a revised version:

Another study, Zebua et al. (2025), examined morphological variation in leaf organs (including leaflet/stem number, length, and width) as specific characteristics among local sugar palm accessions in West Sumatra.

This study's novelty compared with previous studies lies in integrating and visually confirming genetic characters through histological and leaf-anatomical approaches (such as cuticle thickness, palisade tissue density, and chloroplast density). This microscopic approach adds a new dimension to the early selection system, making it more accurate, stable under environmental changes, and efficient for use at the seedling stage before transfer to the planting field. Therefore, this study aims to determine sugar palm diversity and genetic relationships based on leaf anatomy to provide a robust structural database for future sugar palm breeding programs.

2. Research Methods

2.1. Research methods and procedures

The research was conducted at the Quality Testing Laboratory, Tropical Fruit Plants Standard Instrument Testing Agency (BPSI), Sumani, Solok Regency, West Sumatra and (-0.77° to -0.85° S and 100.58°) to 100.65° E 360 masl Anatomical Pathology Laboratory of Andalas University, Jati Padang City (-0.94 ° to -0.95° S and 100.36° to 100.37° E) 20 masl. The research method is descriptive quantitative, based on anatomical properties. This research began with sampling the leaves of sugar palm accessions that had been grouped based on the position of the operculum, and compared with sample leaves of sugar palm varieties of dwarf (Kutai Timur), medium (Parasi and Smulen st-1) and deep (Tomohon).

2.2. Leaf Sampling

The leaf samples analyzed were collected from various operculum positions (Figure 2). The operculum positions used were divided into four, namely:

a. Quadrant 1 (K1) (0-90 ^{O})
b. Quadrant 2 (K2) (90-180 ^{O})
c. Quadrant 3 (K3) (180 -270 ^{O})
d. Quadrant 4 (K4) (270-360 ^{O})

The leaf samples in this study came from propagated seedlings collected at the Experimental Garden of the Faculty of Agriculture, Andalas University. The sample population included 8 sugar palm accessions, selected based on operculum position, with the researcher taking 1 leaf sample from each operculum position. As a comparison to analyze the level of kinship, four superior national varieties were also used, namely the Genjah type of sugar palm (East Kutai), the Medium type (Parasi and Smulen ST-1), and the Dalam type (Tomohon). The cutting process was carried out using a microtome, which included the stages of block tidying (trimming) and thin cutting (sectioning). The resulting sections were then stretched in a water bath, dried in an oven, and stained with Haematoxylin and Eosin (H&E) to clarify cellular differentiation before being covered (mounted) with Entellan.

2.3. Leaf Anatomy Observation

Anatomical character observations were conducted using a light microscope at 40x magnification, focusing on the epidermal structure, mesophyll tissue composition, and cuticle characteristics of the 8 samples. The data obtained were analyzed descriptively and quantitatively using semi-quantitative calculations from images to evaluate differences in anatomical characters between operculum position groups in the 4 research accessions and their

comparison to comparison varieties. This data integration aims to reveal the relationship between genetic variation in operculum position and the expression of leaf anatomical structures as a form of plant adaptation. Leaf anatomical analysis was conducted through anatomical observations, supported by a series of anatomical preparation images.

2.4. Flowchart

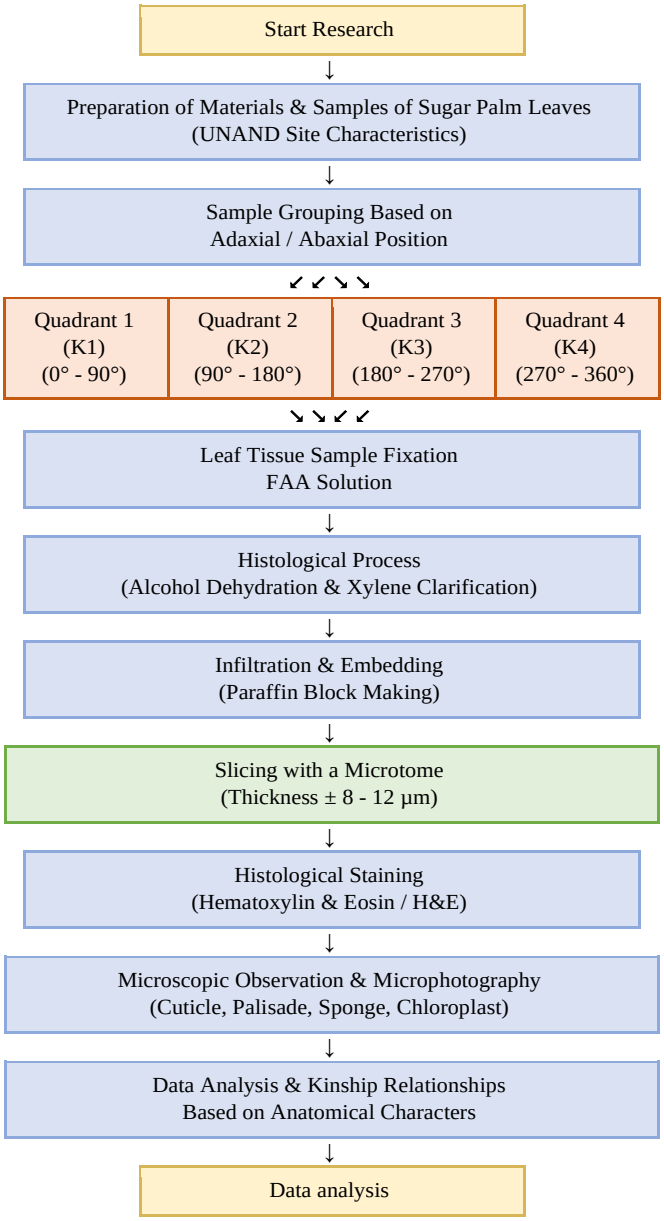


Figure 1. Research flow diagram

2.5. Fixation

The anatomical analysis procedure for sugar palm leaves begins with the fixation stage, where the leaf tissue that has been cut into small pieces based on the quadrant position treatment is immediately immersed in an FAA (*Formalin Acetic Acid-Alcohol*) solution for at least 24 hours. This fixation process is crucial to stop all cellular metabolic activity instantly and prevent cell decay (autolysis), maintaining the leaf's original microstructure in a stable condition for the next preparation stage. After fixation is complete, the sample enters the histological process (*tissue processing*), which begins with dehydration, namely the gradual withdrawal of water content from the tissue using a series of alcohols with increasing concentrations starting from 70%, 80%, 95%, to 100% absolute alcohol in order to prevent extreme cell shrinkage. Next, a clearing stage is performed by immersing the tissue in xylol, which dissolves the remaining alcohol and makes the leaf tissue transparent, preparing it to receive paraffin wax media**.**

2.6. Infiltration

The next stage is infiltration and embedding, where the clear leaf tissue is placed in an oven at 56°C-60°C containing a liquid paraffin mixture so the wax penetrates the intercellular spaces. After the paraffin is absorbed, the tissue is embedded in a paraffin block mould until it solidifies, providing the mechanical strength needed for sectioning. The solid paraffin block is then mounted on a microtome and sectioned transversely at an ultra-thin thickness of 8 to 12 μm. The resulting thin-section ribbon is then carefully attached to a glass slide using a special adhesive and a hotplate so it is stretched evenly and firmly adhered.

2.7. Coloring

Before it can be observed, the still-clear leaf section must undergo histological staining using *the Haematoxylin & Eosin* (H&E) method. This process begins with re-dissolving the paraffin (*deparaffinization*) using xylol, followed by hydration through a descending alcohol series, then stained with Haematoxylin, which will give a purple/dark blue color to acidic cell components (such as the cell nucleus) and Eosin, which gives a pink/orange color to the cytoplasm and cell walls. The stained slide is then dehydrated again, dried, and covered with a cover glass using permanent adhesive media. The final stage is microscopic observation and microphotography, where the anatomical preparations of sugar palm leaves are observed under a light microscope with gradual magnification from weak to strong magnification (100x to 400x). The focus of observation is directed at identifying dominant anatomical characters, including the thickness of the cuticle layer, the arrangement and density of the palisade tissue, the density of the spongy tissue, and the density of chloroplasts in the mesophyll cells, where the best visible structures will be documented using a digital microscopic camera for further analysis.

3. Results and Discussion

Leaf anatomy observations indicate morphological variation among the treatments and varieties tested. In

terms of leaf surface protection, the Genjah variety (E) and the K3 treatment (C) showed a thicker cuticle than other accessions, as shown in Figure 3 and the semi-quantitative scoring in Table 1. A thick cuticle serves as the leaf's primary protective layer, limiting water loss through transpiration, inhibiting pathogen penetration, and providing structural support for the underlying tissue (Yeats & Rose, 2013)

Based on Table 1, the K1 and K2 populations had the same total score (15), reflecting equivalent, optimal anatomical profiles among the research accessions. The Genjah variety had the highest total score (18), confirming its superior photosynthetic anatomy. In contrast, K4 and Tomohon obtained the lowest score (9), consistently reflecting underdeveloped leaf tissue. The Parasi and Smulen varieties ranked moderately, with scores of 14 and 13, respectively, consistent with the intermediate type they represent.

Significant variation was also observed in the palisade mesophyll tissue. The Dwarf variety (E) had the thickest palisade layer, with a very dense cell arrangement and darker colour, reflecting high chloroplast content and superior photosynthetic capacity. The densely arranged, columnar palisade cells increase the mesophyll surface area exposed to the intercellular spaces, thereby increasing the number of chloroplasts per unit leaf area and improving photosynthetic performance (Gotoh et al., 2018). This dense palisade cell arrangement also increases the mesophyll and chloroplast surface area exposed to the intercellular spaces, thereby increasing the mesophyll's CO2 conductance and ultimately increasing photosynthetic capacity. (Ren et al., 2019)A higher palisade tissue volume fraction, either through an increase in the number of cell layers or cell elongation, is generally associated with better photosynthetic capacity.(Théroux-Rancourt et al., 2023)

Treatments K1 (A) and K2 (B) also showed a dense, well-organized palisade layer. In contrast, treatment K4 (D) showed a looser, less organized palisade layer, indicating disrupted photosynthetic tissue development. The Tomohon variety (H) showed the thinnest palisade layer among all accessions observed, which may correlate with its relatively lower photosynthetic capacity ((Ren et al., 2019)

The overall results of the study showed that the position of the operculum significantly influenced the differences in each treatment, which was consistently reflected in all observed leaf anatomical parameters. The opening of the operculum in seeds is the initial stage that determines the process of water imbibition and the activation of seed metabolism, so the position and condition of the operculum during germination also determine the quality of subsequent seedling development (Farhana et al. 2013). Treatments K1 and K2 consistently showed superior anatomical characters, including thick cuticles, dense palisade mesophyll, high chloroplast density, and well-developed vascular bundles; all of these were directly correlated with better photosynthetic capacity and seedling resilience (Yeats & Rose 013). In contrast, treatment K4 showed structural anomalies in almost all leaf tissues, ranging from thin cuticles, loose palisade, to sparse chloroplasts, indicating that suboptimal germination conditions—including disturbances in the operculum opening and early imbibition stages— (Farhana et al., 2013)had a cascading impact on the overall leaf tissue architecture.

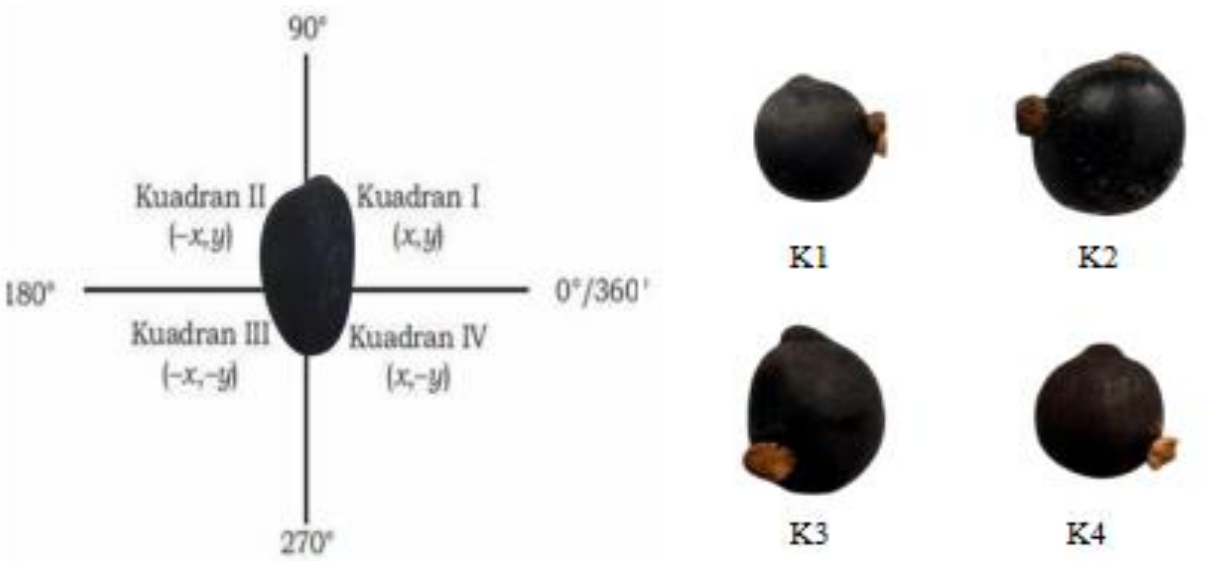


Figure 2. Quadrant Angle of Operculum Position

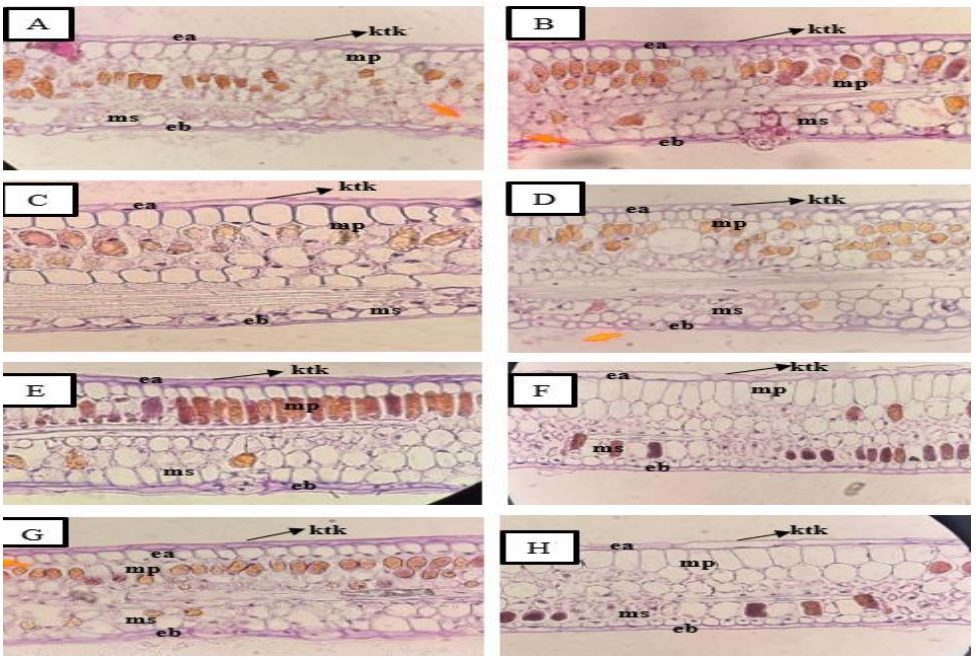


Figure 3. Anatomical structure of sugar palm leaves at 40x magnification A: K1, B: K2, C: K3, D: K4, E: genjah, F: smulen, G: parasi, H: tomohon, ea: upper epidermis, ktk: cuticle, mp: palisade mesophyll, ms: spongy mesophyll, eb: lower epidermis.

Table 1. Semi-quantitative scores of anatomical characters of sugar palm leaves per population (scale 1–4)

Population	Cuticle Thickness	Palisade Density	Chloroplast Density	Sponge Mesophyll	Thickness of Upper Epidermis	Total Score
K1	3	3	4	2	3	15
K2	3	3	4	2	3	15
K3	4	2	3	3	3	15
K4	1	1	1	4	2	9
Early	4	4	4	2	4	18
Smulen	2	3	3	3	2	13
Parasi	2	3	3	4	2	14
Tomohon	2	1	2	2	2	9

Description: 1=very low/thin/rare; 2=low/medium; 3=high/dense; 4=very high/very dense

Based on the analysis of data from the observation of leaf anatomical structure, semi-quantitative scores in Table 1, and histological visualization in Figure 3, it can be concluded that differences in the position of the operculum or apocol have a very significant effect in a cascade manner on the development of the leaf tissue architecture of the sugar palm plant. Treatments K1 and K2 showed the most optimal and equivalent anatomical development profiles, with a total score of 15; both were characterized by a medium-to-thick cuticle layer, a dense and well-organized palisade mesophyll arrangement, and very dense chloroplast density. In contrast, treatment K4 showed the most deviant and least developed anatomical characters, with the lowest total score of 9, characterized by a thin cuticle layer, loose adaxial epidermal cells, and a very loose palisade and spongy tissue arrangement with very sparse chloroplast granules due to suboptimal germination conditions.

Meanwhile, the tested varieties showed that the Genjah Variety had superior photosynthetic anatomical performance and was the best among all accessions, with the highest total score of 18. This Genjah Variety's superiority is characterized by the thickest palisade mesophyll layer with a very dense cell arrangement, high cuticle thickness, and maximum chloroplast density, reflecting photosynthetic capacity and strong adaptation to potential drought stress. On the other hand, the Parasi and Smulen Varieties occupied a moderate position as an intermediate type with total scores of 14 and 13, respectively, where the Parasi Variety showed superiority in the large intercellular space of the spongy mesophyll to support the intensity of gas exchange. In contrast, the Tomohon Variety showed the weakest anatomical characteristics with a total score of 9, which was indicated by the thinnest palisade layer and smaller and sparse chloroplast granules.

4. Conclusion

Cellularly, all treatments and varieties of sugar palm observed showed consistent single-cell arrangement in the adaxial epidermis and were exclusively hypostomatic, where stomata were found only on the lower (abaxial)

epidermis surface in accordance with the characteristics of the Arecaceae family. The chloroplast organelles appeared yellow to brown due to fixation and tissue dehydration and showed a clear gradation in quality: chloroplasts in K1, K2, and Genjah were densely and evenly distributed, while in K4 and Tomohon they were very sparse and paler. Considering that micro-anatomical characters such as cuticle thickness, palisade density, vascular bundle size, and chloroplast density have a strong positive correlation with photosynthesis rate, biomass accumulation, and potential future sap production, these findings empirically prove that leaf anatomical parameters can be used as valid, stable, and efficient early selection criteria in breeding programs and production of superior sugar palm seeds.

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