Research Article

Clustering of Sago Palm *Metroxylon sagu* Rottb. in Luwu District South Sulawesi Based on Morphology and Genetics Similiarity Using RAPD Markers

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Abstract | The main objective of this study is to explore the characteristics of morphology and genetic diversity with RAPD markers to clustering sago in Luwu Regency. The 17 sago plants were used from 6 villages in 4 Sub-districts and 1 City. DNA analysis using 10 RAPD primers was carried out at the Molecular Biology Laboratory, Center for Research and Development of Agricultural Biotechnology and Genetic Resources, Bogor. The genetic similarity matrix was calculated using the Simple Matching Coefficient formula, and then grouping is carried out using Unweighted Pair-Group Method Arithmetic method through the Numerical Taxonomy System version 2.1 program. The DNA concentrations of the 17 sago samples varied from 188.8 ng/µL to 1561.8 ng/µL. Purity value varied from 1.82 to 1.96. All RAPD primers used were polymorphic, with the number of DNA bands varying from 2 to 12 and positions ranging from 100 bp to more than 2000 bp. The PIC values varied from 0.76 to 0.92. The genetic similarity value varies from 0.51 to 0.89. In Luwu Regency, there are 3 variations of sago based on the presence or absence of thorns and the size of the thorns, namely thornless sago, short-thorned, and long-thorned sago, all forming clumps, and the color of the young leaves and shoots is reddish, which will change to green. The dendrogram showed that at a genetic similarity level of 0.65, the 17 sago from Luwu District form 3 groups, namely, group 1 thornless sago has 2 members, group 2 consisting of 10 short-thorned sago, and the other group 3 long thorn has 5 members.

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Introduction

Palms (Arecaceae) are a family of vascular plants with numerous genera and species distributed throughout the tropics and subtropics. People generally use the palm family as ornamental plants, food crops, oils, and industrial raw materials. The palm family includes approximately 181 genera and 2,600 species worldwide (Abbas *et al.*, 2020). Sago (*Metroxylon sagu* Rottb.) is a tree species in the Arecaceae family (palm group). This plant is native to tropical Southeast Asia, including Indonesia, Malaysia, and New Guinea. Indonesia has the largest sago forest area in the world, at 1.28 million hectares, or 51.3% of the

total global sago area. Most of Indonesia's sago areas are categorized as sago forests, meaning sago palms grow naturally without or with limited intensive human intervention and external inputs (Kadir *et al*., 2022). Indonesia has the largest sago (*Metroxylon sagu* Rottb.) forests and cultivation, which shows significant genetic diversity (Abbas *et al*., 2009).

The average annual sago productivity reaches 25 tons per hectare, making sago an important commodity and needs to be developed to meet global carbohydrate needs (Bujang, 2008). Sago is rich in carbohydrates, making it a staple food in several regions in Eastern Indonesia, such as Maluku, Papua, and parts of Sulawesi (Sidiq *et al*., 2021). Previous research has identified the locations of sago plantations in Indonesia, including Irian Jaya (1,406,469 ha), Ambon (41,949 ha), Sulawesi (45,540 ha), Kalimantan (2,795 ha), West Java (292 ha), and Sumatra (31,872 ha). The distribution of sago plantations in Indonesia is uneven, as is its diversity (Abbas *et al*., 2020). The sago palm (*Metroxylon sagu* Rottb.) is a member of the Arecaceae family. Within the three subfamilies of the Arecaceae family, fourteen genera have been identified as producing starch in their trunks, with the genus Metroxylon standing out as the most valuable and promising. The genus Metroxylon is distributed from Southeast Asia to Melanesia, Micronesia, and Polynesia (Nisar and Hussain, 2022).

In taxonomy, morphological data is crucial, as is other data. The use of morphological data in plant taxonomic research has long been practiced. Collecting this data is a necessary initial step before pursuing other approaches to data collection. Morphological characteristics play a crucial role in systematics, as although many approaches can be used to develop classification systems, all are based on morphological characteristics. Observing morphological characteristics is easy and relatively inexpensive, as it doesn't require specialized equipment and can be done with herbarium specimens. According to Mohajer *et al*. (2013), the morphological characteristics that contribute most to variability are important for sago plant improvement. Pasolon (2015) states that based on morphological characteristics, sago is divided into two groups: thorny sago and spineless sago, while according to Pratama *et al*. (2018), farmers differentiate each sago accession based on morphological characteristics, namely the presence of thorns, thorn patterns, stem shape and height, as well as starch quality and production. Al-Manar *et al*. (2023) also studied the morphological characteristics of sago in Lingga Regency and divided sago into two types, namely thornless sago and thorny sago. Iriansa and Mutmainnah, (2024), besides using morphometric and distribution pattern characteristics, also used morphological characteristics of the optimal harvest phase of sago in forest areas based on drone imagery.

Higher plants, such as sago palms, possess three distinct centers of genetic information: the nuclear genome, the chloroplast genome, and the mitochondrial genome. Mitochondria in higher plants function as energy-producing organelles within the cell. The mitochondrial (mt) genome is characterized by circular, maternally inherited DNA, ranging in size from approximately 222 to 773 kb in angiosperms (Abbas *et al*., 2019). Genetic diversity is a crucial component for effective breeding and germplasm conservation strategies (Kaljun and Jaaska, 2010; Kimaro *et al*., 2020). Genetic diversity among individuals or populations is fundamental to adaptation and evolution, thus playing a crucial role in coping with various biotic and abiotic stresses. Diverse genetic resources provide better opportunities for plant breeders to create new and improved cultivars with desirable traits (Purwoko *et al*., 2024). Low genetic diversity will increase the risk of extinction (Hoban *et al*., 2020).

Understanding the genetic diversity is vital for creating effective conservation strategies and breeding programs, as well as for optimal utilization of genetic resources. Assessment of plant genetic diversity can be conducted through analysis of morphological characteristics, biochemical, and molecular markers (Mondini *et al*., 2009; Riyanto *et al*., 2018). Genetic progress depends on the level of genetic variability within a population, and the extent of genetic diversity significantly influences the rate of genetic development (Hussein *et al*., 2023). The genetic variation observed in plants significantly influences higher levels of biodiversity. Without genetic diversity, populations cannot adapt and survive environmental changes. Examining genetic variation among different species, populations, and individuals is crucial for the assessment and conservation of germplasm (Duran *et al*., 2009).

Molecular markers are one method that can be used

to complement morphological information (Dorice *et al*., 2020). Various types of molecular markers include RFLP, RAPD, AFLP, SSR (microsatellite), and SNP, each of which has its own characteristics and advantages in analyzing genetic variation. Molecular markers are divided into two main categories: DNA-based and protein-based. DNA-based molecular markers include: RFLP (Restriction Fragment Length Polymorphism): Detects DNA variations based on the length of fragments produced after being cut by restriction enzymes. RAPD (Random Amplified Polymorphic DNA): Identifies DNA polymorphisms quickly and efficiently using random primers. SSR (Simple Sequence Repeat) or microsatellites: Generates polymorphic bands using repeats of short nucleotide sequences. AFLP (Amplified Fragment Length Polymorphism): Combines RAPD and RFLP techniques to analyze DNA variations. SNP (Single Nucleotide Polymorphism): A variation in a single nucleotide within a DNA sequence. ISSR (Inter-Simple Sequence Repeat): Uses random primers that mimic the microsatellite sequence to amplify DNA between microsatellite locations. SCAR (Sequence Characterized Amplified Region): Generates more specific markers than RAPD after the sequence has been characterized. STS (Sequence Tagged Site): A highly specific molecular marker, characterized by a unique short sequence. The main advantages of RAPD markers are that they are simple, cost-effective, and rapid, they require no prior genomic knowledge, and can be performed with small amounts of DNA. They are also non-radioactive, making them safer to use, and they are useful for a variety of applications such as gene mapping, diversity analysis, and cultivar identification (Al-Samarai and Al-Kazaz, 2015).

In an era of global environmental challenges, climate change, and rapid population growth, maintaining the availability of remaining food crop genetic resources is crucial to sustain agricultural production systems, provide healthy food for the world's population, and address significant future challenges (Panis *et al*., 2020). The research results of Ehara *et al*. (2003) revealed a correlation between genetic distance and the geographical distribution of sago palms, which indicated significant diversity in the Malay Archipelago and Papua New Guinea, as determined by RAPD markers. The increasing utilization of sago requires insights into genetic diversity, both at the species and population levels. Examination of sago genetic diversity is crucial for the advancement of future breeding and germplasm conservation initiatives. RAPD (Random Amplified Polymorphic DNA) is one of the simplest, fastest, and most cost-effective techniques commonly used in plant genetic homogeneity studies (Oliya *et al*., 2021). In Indonesia, molecular markers of sago palms showed significant variation based on RAPD levels and polymorphisms, as well as genetic diversity assessments (Abbas *et al*., 2009).

Based on the research results of several previous researchers and the advantages of using RAPD, research was conducted on the morphology of sago in Luwu Regency with a focus on the presence or absence of thorns, the size and pattern of thorns, and the genetic diversity of sago determined by RAPD molecular markers.

Materials and Methods

The plant materials utilized in this study consisted of community-owned sago plants that grow in various regions of Luwu district, South Sulawesi, using 17 sago samples (Table 1). Morphological observations were carried out at the sampling location, while genetic diversity analysis was carried out in the laboratory of Plant Molecular Biology, Center for Research and Development of Agricultural Biotechnology and Genetic Resources, Ministry of Agriculture, Bogor. The material used is in the form of young leaves that have not opened completely. To keep the sago leaf samples from being damaged, each sample bag was given silica gel and brought to the laboratory.

Research location

This research was conducted in 6 (six) locations consisting of Tampumia Radda Village, Belopa Subdistrict; Buntu Kunyi Village, Suli Subdistrict; Larompong Village, Larompong Subdistrict; Pabbaresseng Village, Bua Subdistrict; Sendana Village, Palopo City; and Botta Village, Suli Subdistrict. The map of the research location is illustrated in (Figure 1).

Morphological characterization

Morphological characterization is focused on the presence or absence of thorns, the size of the thorns, the pattern of the thorns, the color of the young leaves on the seedlings, and the growth form (forming clumps or not).

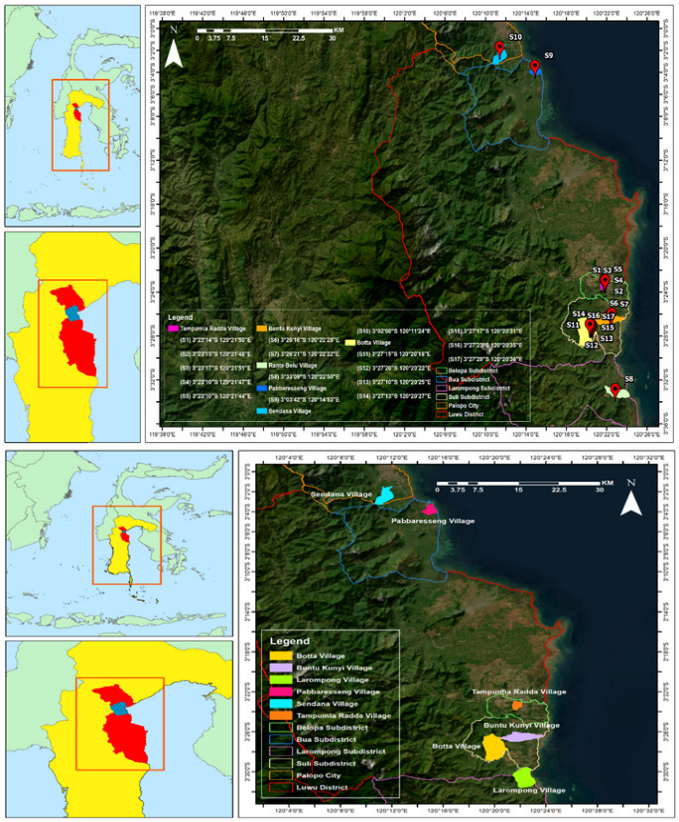

Figure 1: *Map of research locations and sample location points in luwu district, south sulawesi*

Table 1: *Sago palm and sampling locations in luwu district south sulawesi*

Sample code	Sampling location	Coordinates
S1	Tampumia radda subdistrict belopa	3°23'14"S, 120°21'50"E
S2	Tampumia radda subdistrict belopa	3°23'15"S, 120°21'48"E
S3	Tampumia radda subdistrict belopa	3°23'17"S, 120°21'51"E
S4	Tampumia radda subdistrict belopa	3°23'10"S, 120°21'47"E
S5	Tampumia radda subdistrict belopa	3°23'10"S, 120°21'44"E
S6	Buntu kunyi subdistrict suli	3°26'16"S, 120°22'28"E
S7	Buntu kunyi subdistrict suli	3°26'21"S, 120°22'22"E
S8	Rante belu subdistrict larompong	3°39'09"S, 120°22'50"E
S9	Pabbaresseng subdistrict bua	3°03'42"S, 120°14'53"E
S10	Sendana Palopo city	3°02'00"S, 120°11'24"E
S11	Botta Subdistrict Suli	3°27'15"S, 120°20'18"E
S12	Botta Subdistrict Suli	3°27'20"S, 120°20'22"E
S13	Botta Subdistrict Suli	3°27'10"S, 120°20'25"E
S14	Botta Subdistrict Suli	3°27'13"S, 120°20'27"E
S15	Botta Subdistrict Suli	3°27'17"S, 120°20'31"E
S16	Botta Subdistrict Suli	3°27'23"S, 120°20'35"E
S17	Botta Subdistrict Suli	3°27'29"S, 120°20'36"E

Genetic diversity of sago based on RAPD markers.
This activity is implemented through the following stages:

DNA exstraction
The process of isolating and extracting Sago DNA from plant leaves was conducted utilizing the modified method of Abbas *et al.* (2000), incorporating an additional 2% polyvinyl pyrrolidone (PVP). As much as 3 grams of plant leaves crushed with the help of liquid nitrogen until smooth. Then the results of the leaf grinding (leaf powder) were put into a 1.5 ml Eppendorf tube and added with 700 µl, extraction buffer (EDTA (20 mM), Tris-HCl, pH 8 (100 mM), NaCl (1.4 M), CTAB (2%), PVP (2%), and mercaptoethanol (0.2%) and incubated for 60 minutes at 650C water bath while inverting the tube every 15 minutes. Then total DNA was separated from other cell parts/contaminant components (proteins, polysaccharides, phenolic compounds, etc.) by adding a solution of phenol: chloroform: isoamylalcohol (25:24:1) (v/v/v) 700 µl, the tube was inverted for 5 minutes, and the mixture was centrifuged at 12000 rpm for 15 minutes. The supernatant was separated from the rest of the cells and transferred to a 1.5 ml microtube. To precipitate the total DNA, sodium acetate (1/10x the supernatant volume) and cold isopropanol (0.7x the supernatant volume) were added to the tube. The tube was slowly inverted and centrifuged at 12000 rpm for 10 minutes. After removing the supernatant, the total DNA precipitate was washed with 70% ethanol and centrifuged again at 12000 rpm for 5 minutes. After drying, the precipitated DNA was dissolved with TE buffer solution (1x) and stored as DNA stock. The DNA stock was stored in a freezer at -200C and ready to be used as a template in the PCR process.

Polymerase Chain Reaction (PCR) and electroforesis
PCR analysis was performed with a total reaction of 20 µl containing 10 ng of template genomic DNA, each dNTP 0.1 µM (dATP, dCTP, dGTP, and dTTP), primer RAPD 0.25 pmol each, Taq DNA polymerase enzyme 0.04 units in solution 1X buffer (20 mM Tris-HCl pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween 20, 0.5% nonidet P40, and 1.5 mM MgCl2). The amplification reaction was carried out in a PCR machine (MJ Research) with the following steps, namely pre-denaturation at 94^{0}C for 2 minutes, followed by 45 cycles of denaturation at 94^{0}C for 1 minute, primer annealing

at 36^{0}C for 1 minute, and DNA elongation/synthesis at 72^{0}C for 2 minutes. The final stage of the PCR process involved a 5-minute extension at 72^{0}C. The DNA fragments resulting from PCR amplification were separated using agarose gel electrophoresis. A total of 10 µl of PCR product was separated by electrophoresis on 1.2% (w/v) agarose gel in 1X TAE buffer. Electrophoresis was carried out at 90 volts for 45 minutes. The size of the PCR amplification product was determined by comparison using standard DNA (1 kbp ladder from Invitrogen). After staining using an ethidium bromide solution (10 mg/l) for 10 minutes, the electrophoresis results were rinsed with distilled water for 20-30 minutes and visualized using the Chemidoc gel system (Biorad). In this study, 10 RAPD primers were used as shown in Table 2.

Table 2: *RAPD marker for analysis of 17 sago palm from Luwu district, South Sulawesi*

Number	Marker RAPD Code	Primer sequence
1	P01	GCG GCT GGA G
2	P02	GTG ACG CCG C
3	P04	CGT CTG CCC G
4	P06	TTC CGC GGG C
5	P17	ATG ACG ACG G
6	OPG02	GGC ATC GAG G
7	OPA04	AAT CGG GCT G
8	OPAB04	GGC ACG CGT T
9	OPAA17	GAG CCC GAC T
10	OPAB18	CTG GCG TGT C

Data analysis

Morphological character data is presented in the form of tables and explanations. Genetics Observations were made on the presence or absence of amplified DNA bands for each primer at a certain size for each sample, and then this data was translated as binary data with a value of 1 (there is a band) and 0 (no band) at a certain size. The resulting data were analyzed to determine the genetic similarity of the three sago samples tested using the NTSYS-pc version 2.1 program based on UPGMA (unweighted pairs group mathematical arithmetic).

The binary data obtained will then be calculated by the similarity coefficient using the Simple Matching Coefficient (SMC) formula Verma and Aggarwal, (2019).

$$SMC = \frac{\text{number of matching attributes}}{\text{total number of attributes}} \quad \text{or} \quad SMC = \frac{a + d}{a + b + c + d}$$

Where a : score 1, 1; b : score 1.0; c : score 0, 1; d. score 0.0

The Polymorphism Information Content (PIC) value is determined using the formula established by Anderson *et al.* (1993), as follows:

$$PIC\ j = \sum_{i=1}^{n} Pi^2$$

Where PIC j is the Polymorphism Information Content value, marker j, is the frequency of allele i at marker j, and n is the number of alleles at marker j.

Results and Discussion

Observations of morphological characteristics on 17 sago samples showed that sago plants at all research locations in Luwu District all form clumps; the young leaves are reddish in color, which in subsequent growth will change to green. The leaf sheaths and petioles are thornless, short-thorned, and long-thorned, which can be found in seedlings to mature sago. Table 3 displays the complete results.

Table 3: *Morphological characters of 17 sago palm from luwu district, south sulawesi*

Sample code	Morphological characters		
	Spines	Growth type	Color of leaf tips and young leaves
S1	thornless	clumped	reddish
S2	short spines	clumped	reddish
S3	thornless	clumped	reddish
S4	short spines	clumped	reddish
S5	short spines	clumped	reddish
S6	short spines	clumped	reddish
S7	short spines	clumped	reddish
S8	short spines	clumped	reddish
S9	short spines	clumped	reddish
S10	short spines	clumped	reddish
S11	short spines	clumped	reddish
S12	short spines	clumped	reddish
S13	long thorns	clumped	reddish
S14	long thorns	clumped	reddish
S15	long thorns	clumped	reddish
S16	long thorns	clumped	reddish
S17	long thorns	clumped	reddish

Observations of the presence of thorns on the leaf stalks and stems show that sago in Luwu Regency

has varying thorns, ranging from thornless to long and thick, with thorns reaching 19 cm in length. The results of observations on sago seedlings (the height of the seedlings watched was around 1 meter, namely in the rosette phase, or the second phase in sago growth) showed that in adult sago plants with long thorns, the seedlings had leaf sheaths and leaf stalks covered with thorns with a length of around 3 cm, while adult sago plants with short thorns had seedlings with short thorns as well, and in thornless sago plants, they were thornless from the time they were seedlings to adulthood (Figure 2).

Figure 2: *Sago seedlings (A) Spinelss (B) Short thorny (C) long thorns*

Sago palms with very short thorns (almost bald) and short thorns have variations in the color of the leaf sheath and petiole, namely reddish and green, but as they grow larger, they are all green. The position of the thorns on the leaf sheath and petiole varies; some form two grooves (the left and right sides of the leaf sheath/stem), but others form three grooves (left, right, and center) and for sago palms have long thorns the young individuals have green leaf sheaths and leaf stalks that turn yellow in older individuals, with thorns that get longer and form 2 grooves. (Figure 3).

Figure 3: *Color variations and thorn patterns on sago palm leaf sheath and petioles (A) Red with 2-groove thorns (B) Green with 3-groove thorns (C) Green and yellow with one and two groove long thorns*

Sago palm with long-thorn were only found in Botta village, Suli Sub-district. In long-thorned sago plants, the growth of thorns on the leaf sheath shows that the larger the seedling, the longer and denser the thorns grow (the size of the thorns can reach 19 cm). Even in adult plants, there are still thorns on the back of the leaf stalk; however, the sago stem does not grow thorns. (Figure 4).

Figure 4: *Development of spines on the leaf sheath and petioles of long-spined sago plants.*

Figure 5: *All sago palms growing in Luwu District form clumps, including thorny sago palms, short-spined sago palms, and thornless sago palms*

Mohajer *et al.* (2013) stated that the morphological characters that have the greatest contribution to variability need to be known for sago plant improvement. According to Pasolon (2015), based on morphological characteristics, sago is divided into two groups, namely thorny sago and thornless sago, while according to Pratama *et al.* (2018), farmers differentiate each sago accession based on morphological characteristics, namely the presence of thorns, thorn patterns, stem shape, and height, as well as starch quality and production. Al Manar *et al.* (2023), reported result research on the morphological characteristics of Sago in Lingga Regency, Riau, also divided it into 2 types, namely thornless sago and thorny sago. All sago palms growing in Luwu Regency form clumps, including long thorny, short thorny and thornless sago palms (Figure 5).

DNA analysis showed that the DNA concentration of 17 sago samples varied between 188.8 ng/µL and 1561.8 ng/µL. The purity values varied between 1.82 and 1.96. The number of DNA bands in 10 RAPD primers varied between 2 and 12 and positions from 100 bp (base pair) to more than 2000 bp. The PIC values varied between 0.76 and 0.92. The respective data are presented in the table.

DNA extraction from the leaves was quantified using a spectrophotometer, measuring absorbance at A260, A280, and the ratio A260/A280 to determine DNA purity and concentration. Total DNA measurements obtained via a Nanodrop spectrophotometer revealed that the 17 sago samples analyzed displayed a broad spectrum of DNA concentrations, ranging from a minimum of 188.8 ng/µl (sago 3) to a maximum of 1561.8 ng/µl (sago 14). The minimum DNA purity value recorded is 1.82 (S11), while the maximum purity value is 1.96 (S13). Table 4 presents the complete concentration and purity of each sago sample.

Table 4: *DNA content and furity of 17 sago palm samples from luwu district, south sulawesi*

Number	Primer code	PIC	Polimorfisme	DNA bands	Position of DNA bands (bp)
1	P01	0.86	P	3-6	100-1000
2	P02	0.84	P	2-5	200->1000
3	P04	0.79	P	2-7	200->1000
4	P06	0.89	P	2-7	300->2000
5	P17	0.91	P	3-9	>100-1000
6	OPG02	0.92	P	7-12	100->2000
7	OPA04	0.88	P	4-8	200->2000
8	OPAB04	0.75	P	2-7	>200->2000
9	OPAA17	0.83	P	4-6	>200-1600
10	OPAB18	0.84	P	3-7	300-2000

In PCR investigations, the recommended concentration of DNA employed is typically between 10 and 100 ng/µL. According to Utaminingsih and Sophian (2022), a minimum DNA concentration of 20 ng/µL is considered good. Among the requirements for the best extraction outcomes are the purity and concentration of the extracted nucleic acid. The ideal A260/A280 ratio for DNA is around 1.8 to 2.0. Table 4 presents the DNA purity values for 17 sago samples, ranging from 1.82 to 1.96. A score below 1.7 suggests the presence of protein contamination in the extracted DNA, as noted by Abinawanto *et al.* (2019), Sophian and Syukur (2021), and Wulan *et al.* (2021). If the DNA purity measurement exceeds 2.2, it suggests the presence of RNA contamination in the DNA extraction results. According to Pangaribuan *et al.* (2022), the concentration of DNA produced during the DNA extraction process is influenced by the temperature and incubation time used. Too high an incubation temperature causes DNA damage, but if the temperature is too low, the membrane and cell tissue cannot be broken. The temperature and incubation time must be adjusted at the right time so that the concentration of the extracted DNA can be maximized.

Each DNA sample was retailed to obtain DNA with a concentration of about 10 ng for use as a template and PCR analysis. The results of PCR analysis using RAPD 10 markers are presented in the following (Figure 6).

Table 5: *Polymorphism and DNA bands of Sago plants with 10 RAPD primers from Luwu District, South Sulawesi*

Number	Primer code	PIC	Polimorfisme	DNA bands	Position of DNA bands (bp)
1	P01	0.86	P	3-6	100-1000
2	P02	0.84	P	2-5	200->1000
3	P04	0.79	P	2-7	200->1000
4	P06	0.89	P	2-7	300->2000
5	P17	0.91	P	3-9	>100-1000
6	OPG02	0.92	P	7-12	100->2000
7	OPA04	0.88	P	4-8	200->2000
8	OPAB04	0.75	P	2-7	>200->2000
9	OPAA17	0.83	P	4-6	>200-1600
10	OPAB18	0.84	P	3-7	300-2000

Figure 6 shows that the number of DNA bands produced by each marker varies (Table 4). This variation is due to differences in the DNA template binding sites recognized by the primers. Differences in the number and size of DNA bands affect the level of genetic diversity. The genomic banding profile of plants can be described by the number of polymorphic bands, which reflects the distribution of primer placement sites in the genome (Amzeri *et al.*, 2022). In genetic diversity, the PIC (Polymorphism Information Content) value is a measure of the extent of polymorphism in a genetic marker, which reflects the marker's ability to distinguish individuals or groups. Standardization of PIC values for evaluating genetic

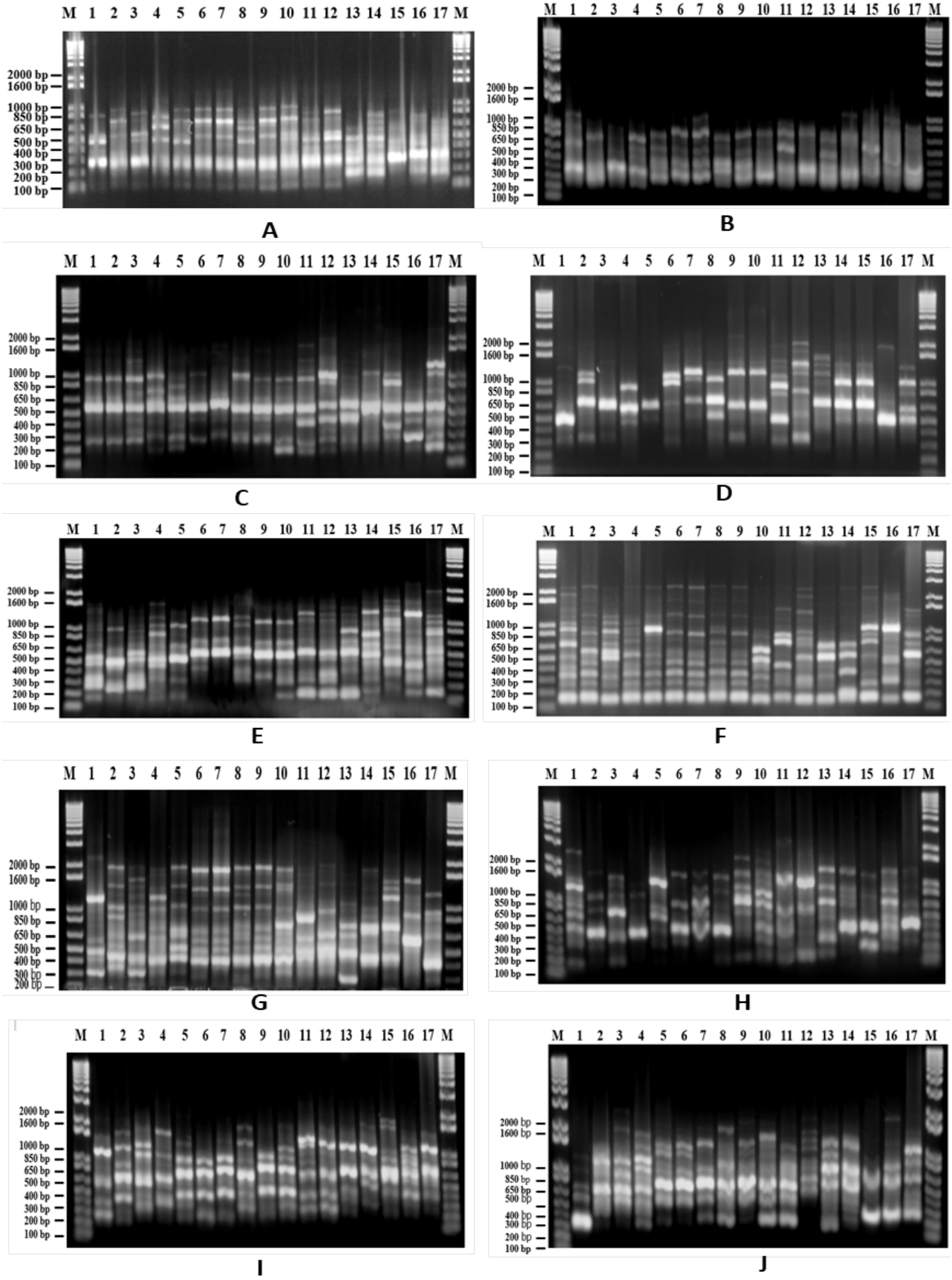

Figure 6: *DNA bands of 17 sago palm samples with 10 RAPD primers 1-17 = sago palm samples, M = Marker 1 Kb ladder (Invitrogen). (A) Primer P01, (B) Primer P02, (C) Primer P04, (D) Primer P06, (E) Primer P17, (F) Primer OPG02, (G) Primer OPA04, (H) Primer OPAB04, (I) Primer OPAA17, (J) Primer OPAB18*

Table 6: *Genetic similarity matrix of 17th Sago palm based on 10th RAPD primers from luwu district, south sulawesi*

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17
S1	1.00																
S2	0.59	1.00															
S3	0.76	0.77	1.00														
S4	0.63	0.64	0.71	1.00													
S5	0.59	0.75	0.67	0.80	1.00												
S6	0.55	0.77	0.62	0.63	0.75	1.00											
S7	0.58	0.76	0.65	0.69	0.75	0.91	1.00										
S8	0.64	0.70	0.72	0.77	0.76	0.76	0.75	1.00									
S9	0.64	0.76	0.69	0.70	0.74	0.82	0.83	0.79	1.00								
S10	0.62	0.78	0.70	0.69	0.74	0.78	0.78	0.75	0.89	1.00							
S11	0.63	0.71	0.67	0.64	0.71	0.75	0.69	0.76	0.72	0.80	1.00						
S12	0.62	0.71	0.71	0.66	0.71	0.68	0.65	0.74	0.67	0.74	0.82	1.00					
S13	0.61	0.65	0.64	0.65	0.69	0.67	0.69	0.72	0.70	0.78	0.71	0.73	1.00				
S14	0.55	0.63	0.62	0.67	0.69	0.63	0.65	0.67	0.63	0.67	0.65	0.68	0.79	1.00			
S15	0.63	0.64	0.65	0.76	0.75	0.62	0.67	0.72	0.65	0.67	0.67	0.63	0.71	0.71	1.00		
S16	0.60	0.61	0.59	0.65	0.75	0.63	0.68	0.70	0.64	0.64	0.59	0.64	0.67	0.67	0.75	1.00	
S17	0.53	0.59	0.58	0.67	0.65	0.57	0.55	0.70	0.59	0.67	0.67	0.66	0.71	0.71	0.71	0.69	1.00

markers based on DNA bands from PCR amplification results is divided into 3 categories, namely: PIC > 0.5 = very informative, 0.25 > PIC > 0.5 = moderately informative, and PIC < 0.25 = low/less informative (Dalimunthe *et al.*, 2019). The PIC 10 value of RAPD primers in this study ranged from 0.75 for OPAB04 to 0.92 for OPG02, meaning that all were categorized as "very informative." Complete data is presented in Table 5.

Table 5 also shows the number and position of DNA bands in 10 RAPD primers for 17 sago samples. The number of DNA bands varied among the 10th RAPD primers. The primer P02 resulted in the least number of DNA bands (2-5 bands), whereas primer OPG02 produced the highest number of DNA bands (7-12 DNA bands). Previous research reported that primer P17 yielded the highest number of polymorphic DNA bands, totaling 12, while primers OPA04 and P06 produced the lowest, with 6 bands each (Abbas *et al.*, 2009). The results of this research are higher than those of previous research.

Information on sago genetic diversity is very useful for the use of genetic resources in breeding programsand the development of sago conservation strategies. Various studies have shown that sago genetic resources in Indonesia generally exhibit high diversity. Genome analysis of sago genetic resources based on molecular markers indicates that sago plants in Indonesia are diverse (Abbas, 2018). The high genetic diversity of sago plants detected in the genome analysis is relevant to the morphological diversity widely identified by sago researchers. Genomic molecular markers show that the genetic diversity of sago plants is relevant to the morphological diversity identified by researchers, indicating that while sago plants in Indonesia are diverse, the genetic diversity is lower than the morphological diversity (Abbas *et al.*, 2019).

Based on the DNA bands of the 17 sago samples studied, each RAPD primer was used to generate binary data (0 if the band did not appear and 1 if the band did appear) and then analyzed with the NTSYS program. The results indicate that the smallest similarity matrix is 0.53 (between sago 1 and sago 17), and the largest value is 0.91 (between sago 6 and sago 7) (Table 6).

Table 6 shows that the lowest genetic similarity coefficient based on 10 RAPD primers, namely 0.53, belongs to samples S1 and S17, and the highest genetic similarity, 0.91, belongs to S6 and S7. Based on the genetic similarity coefficient, a dendrogram was carried out, and results were obtained as in Figure 7.

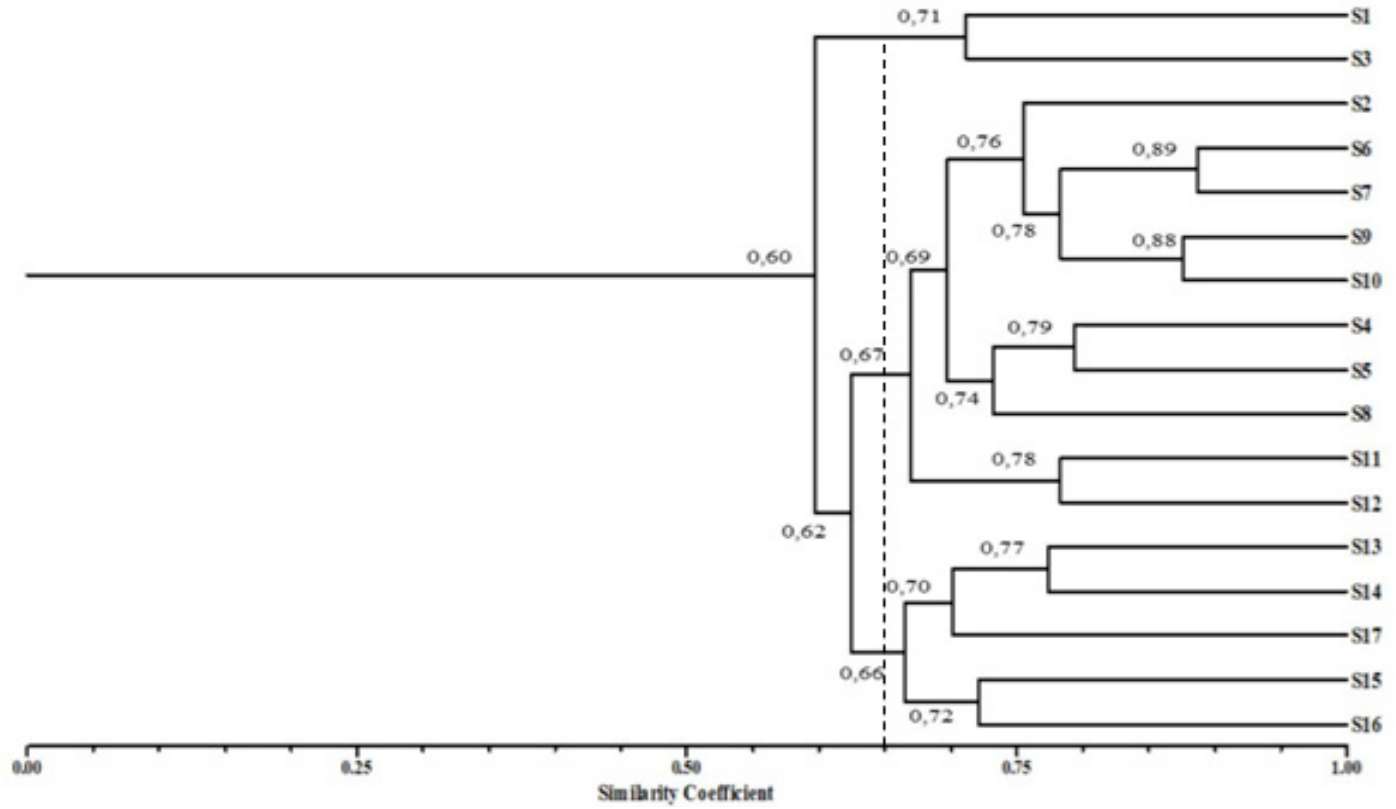

Figure 7: *Dendrogram based on the similarity of DNA bands in 17 sago palm samples in luwu district, south sulawesi with 10th RAPD markers*

Figure 7 shows that sago palms S6 and S7, with the greatest similarity (closest genetic distance), were grouped earlier than the other sago palms. At a similarity degree of around 0.65, the 17 sago form 3 groups, namely, group 1 has 2 members (sago 1 and 3), group 2 has the most members, namely 10 sago samples, while group 3 has 5 members, namely sago 13, 14, 15, 16, and 17. Group 1 is thornless sago, and 2 are short-thorn sago, while group 3 is sago with long thorns. Genetic relationships of sago palm based on RAPD markers showed that sago palm from Manokwari, Bogor, Ambon, and Palopo (Luwu) were closely related (Abbas *et al*., 2009). The results of the grouping based on genetic diversity using RAPD markers are consistent with morphological data showing that sago in Luwu Regency is mostly short-spined (10 samples), 5 samples have long-spined sago, and 2 samples are thornless. The results of this study are different from the results of research conducted by Pasalon (2015) and Al Manar *et al*. (2023), which, based on its morphological characteristics, divides sago into only two groups, namely, thorny sago and thornless sago.

Conclusions and Recommendations

In Luwu Regency, there are 3 variations of sago based on the presence or absence of thorns and the size of the thorns, namely thornless sago, short-thorned, and long-thorned sago, all forming clumps, and the color of the young leaves and shoots is reddish, which will change to green. The analysis of 17 sago samples from the Luwu district utilized 10 RAPD primers. It was concluded that 17 sago samples from the Luwu district with 10 RAPD primers showed that all primers were polymorphic; there was diversity in both the number of DNA bands (2 to 12 DNA bands) and their positions (100 to more than 2000 bp) with a PIC value of 0.76 to 0.92; and the genetic similarity coefficient varied between 0.51 and 0.89, effectively differentiating between groups of thornless sago plants, short-thorned, and long-thorned sago at a similarity distance of 0.65. The research results can be used to develop strategies for Luwu Sago development, conservation efforts, and sago cultivation in the future.

Sago palm in Luwu Regency shows morphological and genetic diversity among individuals. Morphological characteristics, especially the presence or absence of thorns and the size of the thorns, are in line with the genetic diversity of the RAPD results. The long-thorned sago is only found in one village, therefore

it is very important to carry out conservation efforts so that the wealth of sago germplasm is not eroded, which could result in the loss of this diversity in Luwu district in particular and Indonesia in general. The conservation and breeding program for sago plant germplasm in Luwu District should utilize this information on morphological characteristics and genetic diversity. The research results can be used to develop strategies for Luwu Sago development, conservation efforts, and sago cultivation in the future.

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Novelty Statement

This study presents a new investigation of the morphology and genetic similarity based on RAPD (Random Amplified Polymorphic DNA) molecular markers of sago plants, especially in Luwu Regency, South Sulawesi. In contrast to previous studies that used drone imagery to create morphological photos focusing on leaflet shape, canopy shape, sago tree trunk height, and leaflet color at the Optimal Harvest Phase (OHP) of sago in the Forest Area of North Luwu Regency, this study highlights morphology and molecularity to reveal genetic similarities and use them for grouping sago in Luwu Regency. These findings contribute significantly to the development of conservation strategies as well as for sago breeding in the future.

Author's Contributions

Mir Alam: Conceptualization, Methodology,Formal Analysis, Software, Investigation, Writing-Review and Editing, Read and approved the final manuscrip
Juhriah: Methodology, Formal Analysis, Software, Investigation, Writing-Review and Editing, Read and approved the final manuscrip.

Generative AI or AI assisted technology statement
The authors declare that no genrative AI was used in the creation of this manuscript.

Conflict of interest
The authors declare that there is no conflict of interest amongst authors of the manuscript.

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