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Exploring the Genetic Diversity of Superior Durian (*Durio zibethinus* L.) Accessions in Sanggau and Singkawang, West Kalimantan Indonesia

Alia Tsabitha Putricia[1]*, Amin Retnoningsih[1], Kusumadewi Sri Yulita[2], Syaiful Anwar[3], Rafdinal[4], Anton Kamaruddin[5], Mohamad Reza Tirtawinata[6]**

1 Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang City, Central Java, Indonesia
2 Ecology and Ethnobotany Research Center, Biology and Environment Organization, National Research and Innovation Agency, Bogor Regency, West Jawa, Indonesia
3 Department of Agroecotechnology, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang City, Central Java, Indonesia
4 Department of Biology, Faculty of Mathematics and Natural Science, Universitas Tanjungpura, Kubu Raya Regency, West Kalimantan , Indonesia
5 Department of Food Crops and Horticulture of West Kalimantan Province, Pontianak City, West Kalimantan, Indonesia
6 Indonesian Durian Foundation, Bogor, Indonesia

*Corresponding author's email: aliaputricia@students.unnes.ac.id **aminretnoningsih2016@mail.unnes.ac.id

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ABSTRACT

Durian (*Durio zibethinus*) is a promising tropical fruit and commodity in the domestic and international markets. Indonesia's durian production has increased over the past two years, but only one-third is suitable for export, as the fruit quality does not yet meet market standards. The regions of Sanggau and Singkawang in West Kalimantan are home to several superior durian varieties that thrive in the forests. Characterizing the genetic diversity of these superior durians is crucial for the provision of high-quality durian seedlings. Genetic diversity characterization using molecular markers is more efficient, effective, and independent of environmental factors. Inter Simple Sequence Repeat (ISSR) is a molecular marker that is relatively simple, fast and does not require prior knowledge of the target genome. This study aims to analyze the genetic diversity of superior durians from Sanggau and Singkawang using ISSR markers. The study detected the allele profiles of 21 superior durian accessions from PCR results using 11 ISSR primers. The results indicated a high polymorphic percentage of 91.73%. Specific alleles were identified in 12 accessions, with a total of 24 specific alleles. This study demonstrates that the genetic diversity of superior durians from Sanggau and Singkawang, as revealed by ISSR markers, is relatively high, because each accession originated from a seed.

Introduction

Durian is one of Indonesia's promising tropical fruit commodities in domestic and international markets. The genus *Durio* consists of 27 registered species (WFO, 2024), of which 18 are found in Kalimantan, seven species in Sumatra, and one species each in Java, Sulawesi, Maluku, and Bali (Uji, 2005). Nine species of durian are reported to have edible fruit, including *Durio dulcis* ('lahong'), *D. excelsus* ('apun'), *D. grandiflorus* ('durian munyit'), *D. graveolens*

('tuwala'), *D. kutejensis* ('lai'), *D. oxleyanus* ('kerantungan'), *D. lowianus* ('teruntung'), *D. testudinarius* ('durian sekura'), and *D. zibethinus* ('durian') (Uji, 2005). Among these, *D. zibethinus* L. is the most popular due to its taste, nutritional value, distinctive aroma, and high market value (Thorogood et al., 2022). This species also exhibits the highest genetic diversity among durians. Characterization studies of *D. zibethinus* across various regions have shown no synonyms (Amrullah et al., 2023; Angeliena et al., 2019; Mardudi et al., 2021; Prakoso and Retnoningsih, 2021). Superior durian accessions that show fruit stability for at least three years are eligible for Plant Variety Protection (PVP) certification. As of 2022, Indonesia has issued PVP for 291 durian varieties (Indonesian Ministry of Agriculture, 2022), a relatively low number compared to the available superior accessions. Intensive cultivation of these superior durians can be done efficiently, as durian seedlings can be propagated by vegetative means, allowing fruit production without reliance on a single-parent tree. Indonesia's durian production in 2023 reached approximately 1.83 million t, the highest in the past five years (BPS, 2024). However, Indonesia's durian export value cannot compete with Thailand and Malaysia's (Santoso et al., 2016). While Indonesia's durian exports have increased over the past two years, only one-third of the production is exportable (Fig. 1), as the fruit quality does not meet international market standards yet.

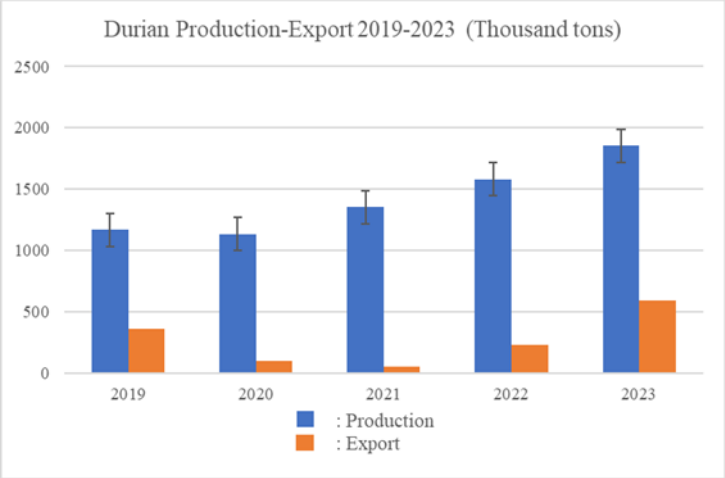


Fig. 1. National Durian Production and Export Data.

Indonesia's durian genetic diversity is considered high, mainly due to its cross-pollination system (Indriyani et al., 2012). This pollination system is intrinsically outcrossing and facilitates gene flow between two unrelated durian plants, generating novel genetic recombinants amongst their progeny (Cardoso et al., 2018). Knowledge of genetic diversity is essential for selecting genotypes with superior genes (Terletskaya et al., 2023).

Kalimantan is home to various species of durian. The forests of Kalimantan harbor a diverse germplasm of durian, capable of producing new superior varieties through natural cross-pollination. Sanggau and Singkawang, cities in West Kalimantan, are among Indonesia's main durian production centers. In 2022, Sanggau produced 22,323 t of durian, while Singkawang produced 1,933 t (BPS, 2024). The potential to increase production from these regions can be realized by characterizing their durian germplasm. It has been reported that most of Sanggau's durian varieties have already received Plant Variety Protection (PVP). However, characterizing durians in this area remains crucial to expanding the national collection of superior durian varieties. Singkawang is also home to many superior durian accessions (Melaponty et al., 2019). The vegetation in Singkawang shows that *D. zibethinus* has the highest Importance Value Index (IVI) due to its strong adaptability.

Durian characterization can be performed using various methods, including morphology, isoenzymes, and DNA sequencing—the standard markers observed in durian, such as morphological traits (Habibah et al., 2019). Nonetheless, using this marker has limitations as it can be affected by environmental variability, which causes morphological discrimination to provide lower accuracy when used alone

(Chambel et al., 2005). The isoenzyme method typically detects only a small portion of genes or loci, thus providing limited information. Moreover, it is unable to detect mutations (Siddiquee, 2010).

Characterizing Indonesia's durian genetic diversity using molecular markers is still limited. Molecular markers are more efficient and effective in describing and identifying durian than morphological markers (Angeliena et al., 2019; P. Santoso et al., 2016). Unlike morphological markers, molecular markers are unaffected by environmental factors, making them more stable (Butiuc-Keul et al., 2019). Another advantage of molecular markers is that they can be characterized at all growth stages and from all tissue types (Jiang, 2013). One of the most popular molecular markers used in genetic diversity characterization is the Inter Simple Sequence Repeat (ISSR). ISSRs are regions in the genome flanked by microsatellite repeat sequences, also known as Simple Sequence Repeats (SSR) (Susanti et al., 2022). The target DNA sequence is amplified via Polymerase Chain Reaction (PCR) using a single microsatellite primer (dinucleotide, trinucleotide, tetranucleotide, or pentanucleotide repeats). This marker combines most of the advantages of previously known markers, such as SSR, Amplified Fragment Length Polymorphism (AFLP), and Random Amplified Polymorphic DNA (RAPD). ISSR is easier, faster, and does not require prior target genome information (Kaya, 2015). ISSR markers produce many polymorphic bands, making them suitable for studies on genetic diversity, gene tagging, phylogeny, genome mapping, evolutionary biology, and mutations in DNA from tissue culture (Pradeep Reddy et al., 2002; Villalobos-Olivera et al., 2022). This study aimed to analyze the genetic diversity of superior durians from Sanggau and Singkawang, West Kalimantan, using ISSR markers. Ensuring the precise identity of Indonesia's superior durian varieties is essential as a reference for seed propagation, allowing for intensive durian cultivation without relying on a single-parent tree. Furthermore, clear identification of durian varieties ensures that the planted durian meets the desired quality standards. Planting durians without knowing their identity can be costly and time-consuming, as errors in planting are only discovered when the trees bear fruit, typically after 4-5 years.

Materials and Methods

Plant materials

Leaf samples consisted of 21 superior durian accessions distributed across the Sanggau and Singkawang regions of West Kalimantan (Table 1 and Fig. 2). The durian leaves used were the third leaves from the tip of the branch. The leaves were stored by wrapping them in tissue and placing them in labeled zip-lock bags. The leaves were kept in a freezer at -20 °C. During preparation, the durian leaf samples were cleaned with 70% ethanol and tissue, and then the leaf scales were removed. Using young leaves is more efficient as they are more easily crushed due to their softer cell walls. Additionally, their phenolic and flavonoid content is lower than older leaves (Leviana et al., 2023).

Table 1. Samples and Locations of Superior Durian in Sanggau and Singkawang, West Kalimantan.

Code	Accession	Location
KB1	Yatim	Balai Karangan, Sanggau
KB2	Mayong	Balai Karangan, Sanggau
KB3	Kembang langsat	Balai Karangan, Sanggau
KB4	Kilman	Balai Karangan, Sanggau
KB5	Pontis	Balai Karangan, Sanggau
KB6	Raja udang	Balai Karangan, Sanggau
KB7	Dronmoy/moy	Balai Karangan, Sanggau
KB8	Setaman muda	Balai Karangan, Sanggau
KB9	Tonari	Balai Karangan, Sanggau
KB10	Si kunyit	East Singkawang
KB11	Nek tengkos	East Singkawang
KB12	Nek manto	East Singkawang
KB13	Puncak	East Singkawang

KB14	Botak	East Singkawang
KB15	Nek nombat	East Singkawang
KB16	Nek date	East Singkawang
KB17	Salak	East Singkawang
KB18	Kalang	East Singkawang
KB19	Cabang	East Singkawang
KB20	Nek ngoang	East Singkawang
KB21	Pancar	East Singkawang

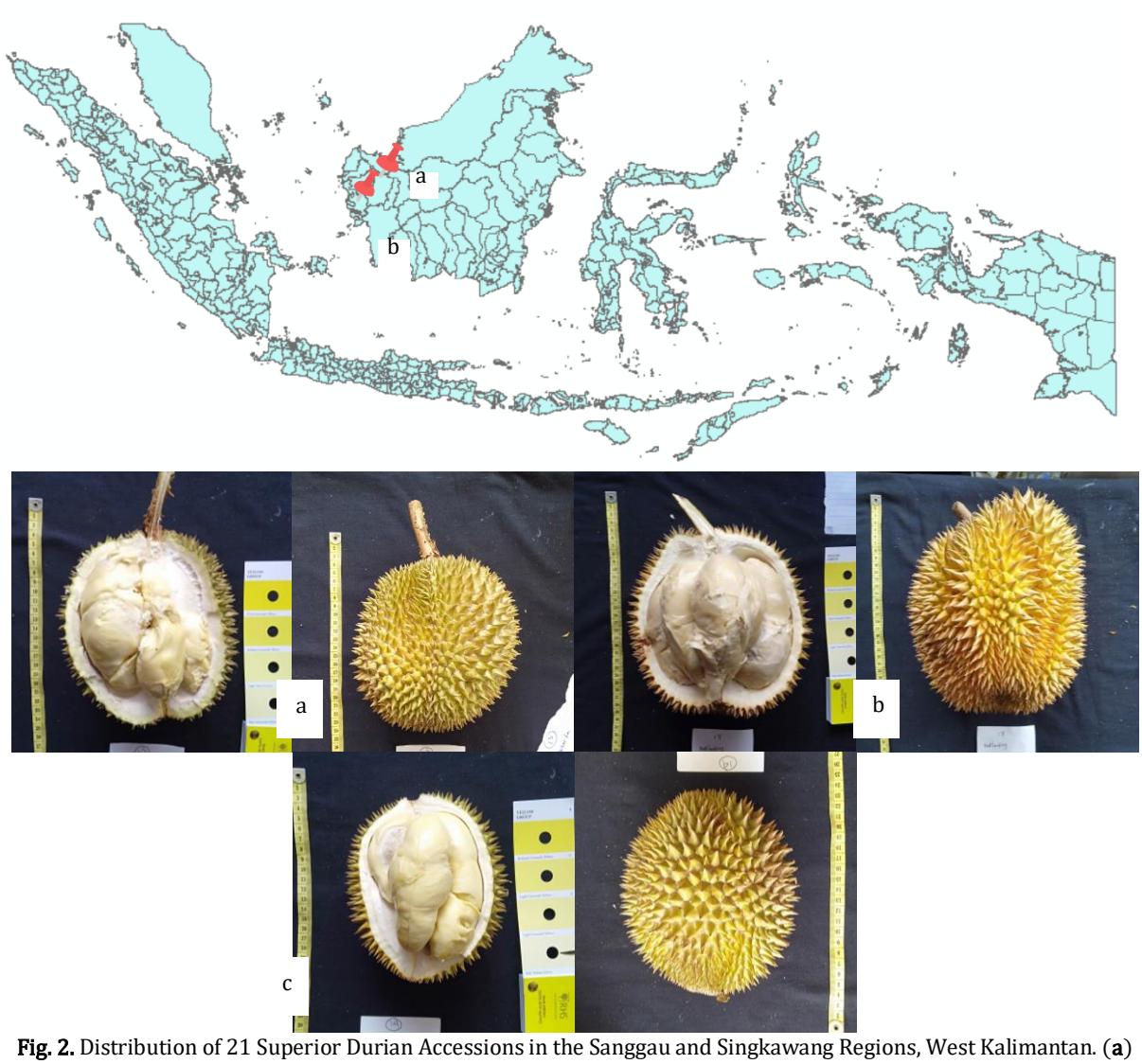


Fig. 2. Distribution of 21 Superior Durian Accessions in the Sanggau and Singkawang Regions, West Kalimantan. (**a**) Entikong; (**b**) Singkawang Timur. Morphological Diversity of Selected Superior Durian Varieties from West Kalimantan. (**a**) Puncak, (**b**) Belimbing, (**c**) Nek Ngoang.

The durian fruit exhibits a wide range of shapes and sizes, with skin colors varying from green to yellowish-brown. Its form can be round, ovoid, elliptical, elongated, or irregular. The spines also differ in shape, ranging from convex, concave, and pyramidal to sharp. Similarly, the seeds display diverse morphologies, including flattened, ellipsoid, elongated, ovoid, or rounded, with colors ranging from yellowish-brown and brown to black, and varying in size from small to large. These phenotypic variations among durian varieties are influenced by both genetic factors and environmental conditions (Brown, 1997).

Procedure

Genomic DNA isolation, PCR amplification and visualisation of PCR products

Total genomic DNA was isolated from durian leaves using the method of Sari and Murti (2015), as modified by Handayani et al. (2016). DNA amplification was performed using PCR with 11 ISSR primers at optimized annealing temperatures (Table 2) in *Thermal Cycler Bio-Rad C1000*. The optimum condition consisted of 4 min pre-denaturation at 94 degrees, followed by 35 cycles of denaturation at 94 degrees for 30s, annealing at 36.7 – 54.9°C for 30s, extension at 72 degrees for 1 min. The PCR reaction was terminated at 72 degrees for 10 min. The PCR reaction mix consisted of 1 µL genomic DNA (20 ng), 6.25 µL PCR Mix Go Taq Green Master from Promega, 1 µL ISSR primer (10 µM), and 4.25 µL nuclease-free water. The PCR reagents were prepared on an isofreeze.

Table 2. ISSR primers used in DNA amplification of superior durian from Sanggau and Singkawang, West Kalimantan.

Primer	Primer Sequence	Annealing (°C)	References
ISSR 1	5'-AGGAGGAGGAGGAGG-3'	48.4	Riupassa et al., 2015
ISSR 2	5'-AGAAGAAGAAGAAGT-3'	36.7	Angeliena et al., 2019
ISSR 4	5'-GAGGAGGAGGAGGAGAC-3'	47.3	Vanijaiva, 2012
ISSR 5	5'-GAGGAGGAGGAGGAGAT-3'	44	Riupassa et al., 2015
ISSR 10	5'-GTGTGTGTGTGTGTGTGTA-3'	49.4	Handayani et al., 2016
PKBT 2	5'-ACACACACACACACACTT-3'	53	Angeliena et al., 2019
PKBT 3	5'-AGAGAGAGAGAGAGAGT-3'	47.5	Syahruddin, 2012
PKBT 7	5'-GAGAGAGAGAGAGAGAGAA-3'	49	Angeliena et al., 2019
PKBT 8	5'-GAGAGAGAGAGAGAGAGAC-3'	53.7	Riupassa et al., 2015
PKBT 9	5'-GAGAGAGAGAGAGAGAGAT-3'	50.9	Syahruddin, 2012
PKBT 12	5'-GTGTGTGTGTGTGTGTGTT-3'	44.9	Riupassa et al., 2015

The PCR products were analyzed using electrophoresis on a 2% agarose gel, run at 100 volts for 35 min. A 100 bp ladder was used in the center wells to measure the generated DNA alleles. The electrophoresis results were visualized and recorded using Gel Documentation (Alpha Imager® EP).

Data analysis

Each DNA band represents one ISSR allele at each durian accession locus. Data analysis was conducted by scoring alleles from the PCR results, assigning a value of 1 if an allele was present and 0 if absent. Alleles of the same size as those found in other durian accessions were considered homologous, while alleles found only in specific a ccessions were termed accession-specific. DNA allele sizes were determined based on the DNA ladder size standard used. Binary data were analyzed using the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc) version 2.02. Genetic similarity was assessed using the Similarity of Qualitative Data (SYMQUAL) with the Dice coefficient, and the results were presented as a dendrogram. Clustering was based on the Sequential, Agglomerative, Hierarchical, and Nested (SAHN) - Unweighted Pair-Group Method with Arithmetic Average (UPGMA) (Rohlf, 2000). The polymorphism information content (PIC) was determined using the formula $PIC = 1 - [f^2 + (1 - f)^2]$, where f represents the frequency of the marker in the dataset (Roldán-Ruiz et al., 2000; de Riek et al., 2001). Genetic diversity was analyzed using Nei's genetic diversity index (h index) and Shannon's information index (I index) with the software POPGENE v. 1.32 (Yeh et al., 1999).

Results

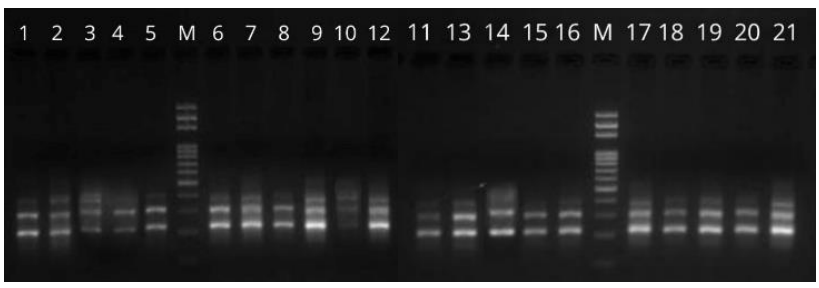
Amplification of genomic DNA from 21 superior durian accessions from Sanggau and Singkawang, West Kalimantan, at 11 ISSR loci resulted in 133 alleles (Table 3). The alleles represent variations in the sequence length of regions flanked by microsatellites or SSR. The allele sizes varied between 150-1500 bp, consistent with the use of ISSR markers to assess plant genetic diversity, which yields fragment sizes ranging from 100-3000 bp (Nurhasanah et al., 2023; Zietkiewicz et al., 1994).

The DNA amplification results for durian revealed a high level of polymorphism ($\bar{x} = 91.73\%$) (Table 3). An allele is considered polymorphic if it is present in only a few accessions within a population. Alleles found in all accessions within

a population are classified as monomorphic (Elston et al., 2012). The PCR amplification using ISSR primers gave rise to reproducible amplification products. Further, the mean PIC (polymorphism information content) was 0.253 and the lowest and highest PIC value were 0.169 (PKBT 7) and 0.346 (ISSR 10), respectively. The PIC can be classified as satisfactory (PIC $>$ 0.5), medium (0.25 $\leq$ P $\leq$ 0.5) and low (PIC $<$ 0.25) (Botstein et al., 1980). The variation in the number of alleles detected across various primers is illustrated in Figures 3a-e. Figure 3a displays the electrophoresis results for ISSR Primer 1, with estimated allele sizes of 180, 230, 330, 400, 450, 550, and 800 bp, and no specific alleles identified. ISSR Primer 2, on the other hand, shows estimated allele sizes of 100, 250, 300, 350, 500, 600, 700, 800, 900, 1000, 2000, and 3000 bp, with two specific alleles identified at sizes of 1000 and 2000 bp (Table 4).

Table 3. ISSR Primer Amplification Results for 21 Superior Durian Accessions from Sanggau and Singkawang, West Kalimantan.

Primer	Relative sizes (bp)	Number of alleles	Number of polymorphic alleles	Number of monomorphic alleles	Percentage of polymorphic alleles (%)	PIC
ISSR 1	180-800	7	3	4	42.85	0.192
ISSR 2	100-3000	12	11	1	91.66	0.209
ISSR 4	150-900	13	13	0	100	0.240
ISSR 5	180-1250	17	17	0	100	0.285
ISSR 10	200-900	8	8	0	100	0.346
PKBT 9	180-1250	13	12	1	92.30	0.305
PKBT 2	200-1500	13	13	0	100	0.282
PKBT 3	200-800	13	12	1	92.30	0.234
PKBT 7	200-800	9	5	4	55.55	0.169
PKBT 8	150-1500	14	14	0	100	0.180
PKBT 12	150-900	14	14	0	100	0.336
Total		133	122	11	Average 91.73	Average 0.253



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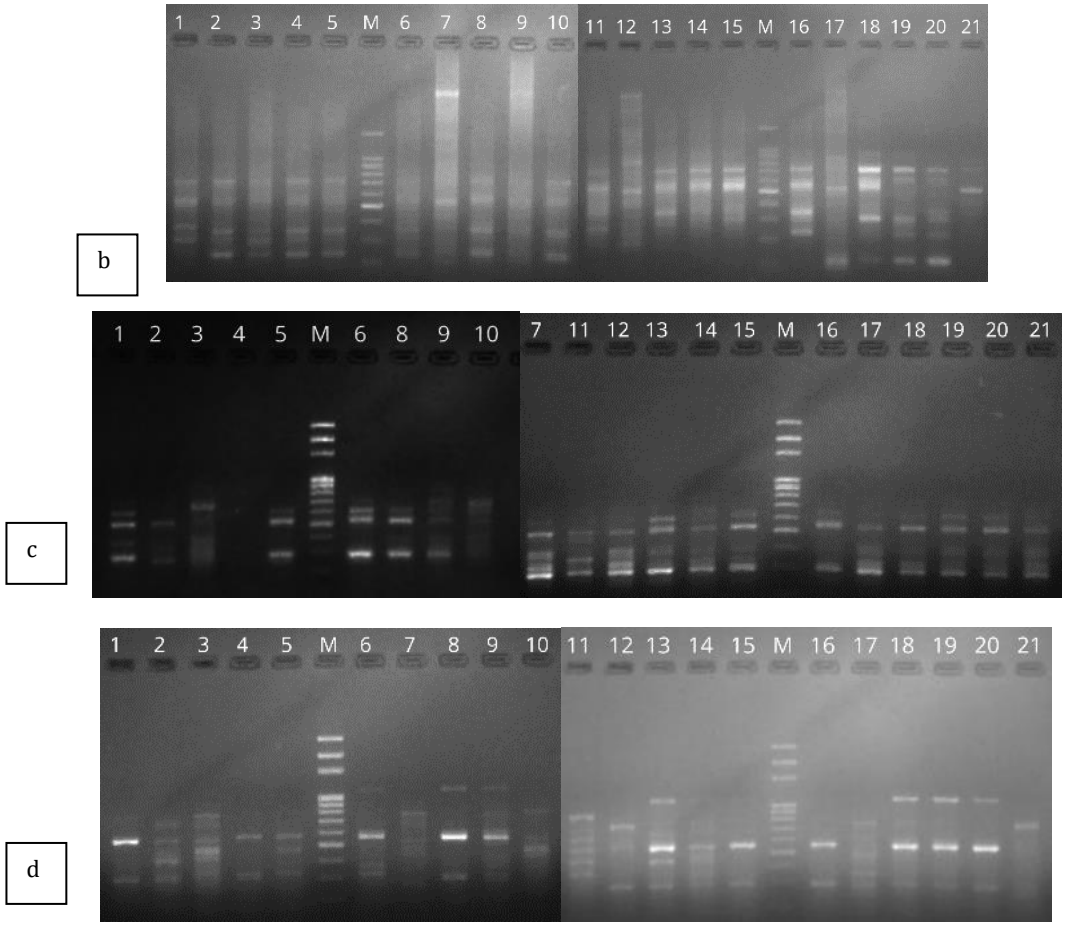

Fig. 3. Electrophoresis result pattern of 21 superior durian accessions from Sanggau and Singkawang, (**a**) West Kalimantan, using primers ISSR 1; (**b**) ISSR 2; (**c**) ISSR 4; (**d**) ISSR 5; M:marker.

Table 4. Specific alleles found in eight variants of *Durio zibethinus* from accessions in Sanggau and Singkawang, West Kalimantan.

Accession	Code	Size (bp)							
		ISSR 2	**ISSR 4**	**ISSR 5**	**ISSR 10**	**PKBT 9**	**PKBT 2**	**PKBT 3**	**PKBT 8**	**PKBT12**
Nek manto	KB12	2000, 1000				180	1500			
Botak	KB14		800				900	750		400
Nek tengkos	KB11		480						700	850
Dronmoy/moy	KB7			850, 750, 520,					550	
Tonari	KB9			180					900	
Si kunyit	KB10			450						
Yatim	KB1				800					
Pancar	KB21					1250				
Kembang langsat	KB3							800		
Mayong	KB2								1000, 300	
Raja udang	KB6								600	

The similarity matrix with Dice coefficients for 21 superior durian accessions from Sanggau and Singkawang is presented in Table 5, ranging from 0.47 to 0.85, with an average of 0.67. The highest coefficients were found between accessions KB15 and KB16 (0.85), KB13 and KB19 (0.83), and KB19 and KB20 (0.82). The lowest similarity coefficients were observed between KB4 and

KB21, and KB4 and KB19, with values of 0.47 for each pair. A higher similarity coefficient indicates a closer genetic relationship between accessions (Mliki et al., 2003). Figure 4 shows the dendrogram generated from the UPGMA analysis using NTSYSpc. The dendrogram is divided into two clusters, Cluster I and Cluster II, with similarity coefficients ranging from 0.53 to 0.86 indicating a relatively low level of genetic variation among the accessions. Cluster I includes 20 durian accessions, further separating into two sub-clusters: Cluster IA, representing 19 accessions, and Cluster IB, representing 1 accession. Cluster II consists of only one accession, *durian Kilman*. This dendrogram illustrates the genetic proximity among the accessions. This information can serve as a foundation for plant breeding through hybridization or developing new superior varieties.

Table 5. Similarity matrix of 21 superior durian accessions from Sanggau and Singkawang, West Kalimantan.

	KB 1	KB 2	KB 3	KB 4	KB 5	KB 6	KB 7	KB 8	KB 9	KB 10	KB 11	KB 12	KB 13	KB 14	KB 15	KB 16	KB 17	KB 18	KB 19	KB 20
KB 2	0.70																			
KB 3	0.76	0.77																		
KB 4	0.51	0.57	0.53																	
KB 5	0.78	0.78	0.73	0.63																
KB 6	0.76	0.65	0.67	0.53	0.75															
KB 7	0.58	0.67	0.63	0.53	0.66	0.68														
KB 8	0.69	0.75	0.71	0.58	0.75	0.79	0.70													
KB 9	0.60	0.65	0.67	0.53	0.68	0.75	0.76	0.73												
KB 10	0.70	0.75	0.73	0.56	0.78	0.68	0.67	0.69	0.74											
KB 11	0.69	0.66	0.69	0.55	0.74	0.66	0.66	0.75	0.65	0.65										
KB 12	0.62	0.66	0.58	0.49	0.61	0.67	0.69	0.70	0.68	0.65	0.67									
KB 13	0.69	0.67	0.70	0.50	0.72	0.77	0.67	0.78	0.71	0.66	0.76	0.69								
KB 14	0.67	0.64	0.64	0.48	0.66	0.71	0.69	0.71	0.70	0.67	0.72	0.74	0.78							
KB 15	0.73	0.70	0.67	0.56	0.77	0.75	0.70	0.75	0.67	0.66	0.78	0.67	0.77	0.72						
KB 16	0.72	0.65	0.64	0.57	0.78	0.77	0.69	0.77	0.69	0.69	0.75	0.66	0.76	0.70	0.86					
KB 17	0.62	0.65	0.59	0.53	0.64	0.68	0.76	0.71	0.71	0.65	0.66	0.78	0.71	0.70	0.68	0.64				
KB 18	0.67	0.62	0.62	0.50	0.70	0.74	0.71	0.68	0.66	0.69	0.71	0.66	0.71	0.75	0.80	0.79	0.76			
KB 19	0.68	0.59	0.60	0.48	0.65	0.71	0.63	0.71	0.63	0.61	0.69	0.69	0.83	0.71	0.79	0.79	0.72	0.81		
KB 20	0.66	0.61	0.62	0.56	0.73	0.73	0.67	0.73	0.63	0.66	0.71	0.67	0.76	0.70	0.82	0.82	0.67	0.78	0.83	
KB 21	0.53	0.59	0.51	0.47	0.59	0.59	0.63	0.64	0.58	0.58	0.58	0.70	0.58	0.65	0.63	0.61	0.69	0.65	0.61	0.56

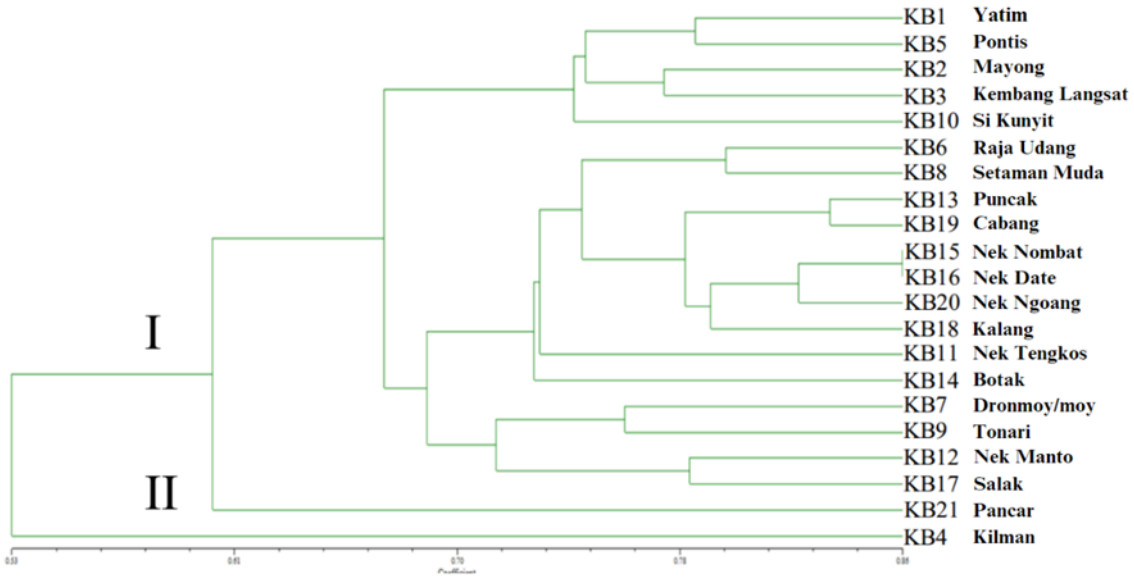

Fig. 4. Dendrogram of 21 superior durian accessions from Sanggau and Singkawang, West Kalimantan, using ISSR markers.

The genetic diversity of superior durian in Sanggau and Singkawang is relatively high (Table 6), with indicators of Nei's gene diversity (h index) and Shannon's information index (I). The h index and I index values of durian in the Sanggau area are 0.252 and 0.388, while in the Singkawang area, they are 0.216 and 0.327. Both values indicate that the genetic diversity of durian in both places is relatively high. According to Nei's gene diversity, the standard value of genetic

diversity is high > 0.15, moderate 0.05-0.15, and low < 0.05 (Nei, 1973). The high genetic diversity of a population is a potential genetic resource in optimizing adaptive durian populations (Miswarti et al., 2018; Teixeira and Huber, 2020).

Table 6. *Nei's gene diversity and Shannon's information index of superior durian from Sanggau and Singkawang.*

Population	*h ± St. Dev	*I ± St. Dev	Polymorphic alleles (%)
Sanggau	0.252 ± 0.174	0.388 ± 0.237	82.71
Singkawang	0.216 ± 0.189	0.327 ± 0.275	60.90

*h: Nei's gene diversity; *I: Shannon's information index; *St. Dev: Standard deviation.

Discussion

The total of 133 alleles identified in this study are fewer compared to the findings of Amrullah et al. (2023), who identified 161 alleles with only 10 ISSR primers across 40 durian accessions from Batang, Central Java. Similarly, a study on 60 durian accessions from Tengkurak, West Kalimantan, reported 148 alleles at 10 ISSR loci (Riupassa et al., 2015). However, the number of alleles in this study is greater than the 36 alleles reported by Prakoso and Retnoningsih (2021) for 25 superior Brongkol durian accessions at 7 ISSR loci. Angeliena et al. (2019) found 89 alleles at 11 ISSR loci in 55 superior Indonesian durian accessions from Kundur, Riau Islands. The variation in alleles is attributed to the number and diversity of ISSR loci studied. Durian accessions come from regions with different ecological conditions and originate from cross-pollinated seeds, resulting in diverse genotypes. Location and environment significantly contribute to genotypic diversity (Sundari and Nugrahaeni, 2017; Trustinah and Iswanto, 2013). A high percentage of polymorphic alleles indicates greater genetic diversity within a population (Probojati et al., 2019; Roldán-Ruiz et al., 2000). The higher the percentage of polymorphic alleles for a primer, the better it is for detecting genetic diversity. Several research studies have demonstrated the efficacy of ISSR markers in generating polymorphic alleles for plant genetic diversity studies. For instance, a survey of the genetic diversity of Cymbopogon in India using ISSR and RAPD markers found that ISSR markers produced a higher percentage of polymorphic alleles (90.68%) compared to RAPD markers (88.62%) (Baruah et al., 2017). Similarly, Singh et al. (2012) reported that ISSR markers generated more polymorphic alleles (95.4%) than RAPD markers (91.4%) in their study of *Curcuma longa* L. Jelvehgar et al. (2021) found that ISSR markers produced 100% polymorphic alleles, compared to 90% with SSR markers, in their analysis of *Lepidium* spp. Dinucleotide ISSR markers produced more informative genetic patterns than trinucleotide, tetranucleotide, or pentanucleotide markers (Al-Turki and Basahi, 2015; Blair et al., 1999).

Specific alleles are unique alleles found in only one accession within a population. Scientists have long studied these alleles to distinguish phenotypic differences between species (Urry et al., 2021). The highest number of specific alleles was observed with Primer PKBT 8, which identified five particular alleles, and ISSR 5, which identified three specific alleles (Table 4). Other primers yielded 1 to 2 particular alleles. The accessions Nek Manto (KB12) and Dronmoy/Moy (KB7) produced the highest number of specific alleles, with four each. Specific alleles, found exclusively in certain populations, can provide valuable information for the protection of accessions and serve as markers for further analysis of codominant markers (Srivastava et al., 2004; Verma and Singh, 2020). They can also be used to obtain Plant Variety Protection (PVP) certification for durian. Morphologically, the durian accessions studied in this research showed similarities among accessions. Specific alleles are crucial as an identity marker for these accessions. An allele can also move within the genome from one locus to another, leading to new genetic variation. This mechanism of allele movement is known as a transposon, which can induce mutations, duplications, or new genetic recombinations (Munoz-Lopez and Garcia-Perez, 2010).

Durian plants are cross-pollinated (Lim and Luders, 1998). This pollination method allows for genetic diversity because there is gene flow from two different durian individuals (Cardoso et al., 2018). Cross-pollinated durian trees have been shown to produce better-quality fruit than self-pollinated ones (Lim and Luders, 1996). The many types of Durio and the genetic diversity of *D. zibethinus* in West Kalimantan are the world's

largest sources of durian germplasm wealth. The Kalimantan durian population is a distribution source for other durians to other regions such as Sumatra, Sulawesi, Java, Papua, and Bali (Santoso et al., 2016).

Conclusion

This study provides significant genetic variation, as evidenced by the high percentage of polymorphic alleles ($\bar{x}$=91.73%) among superior *D. zibethinus* across Sanggau and Singkawang, West Kalimantan. This variation is crucial for understanding the genetic structure of durian populations in these regions and highlights the importance of preserving local accessions. Identifying specific alleles unique to certain accessions, such as those found in Nek Manto and Dronmoy, suggests potential markers for varietal protection and breeding programs. Furthermore, the clustering analysis showed clear genetic differentiation among the accessions, with two main clusters emerging. These insights underscore the role of environmental factors and cross-pollination in shaping durian's genetic diversity. Overall, the study provides essential data for future breeding programs and the conservation of genetic resources, offering opportunities to develop new superior durian varieties that can be adapted to different ecological conditions. This deep genetic diversity is a foundation for improving traits such as disease resistance, fruit quality, and yield, ensuring the sustainability of durian cultivation in the region.

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Author Contributions

Conceptualization, ATP and AR; methodology, ATP; software, AR and KSY; validation, AR, ATP, and KSY; formal analysis, AR, SA, MRT, and R; investigation, R, AK, and MRT; resources, ATP; data curation, ATP; writing—original draft preparation, ATP, AR, KSY; writing—review and editing, KSY; funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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