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Genetic characterization of superior durian (*Durio zibethinus* L.) accessions in Batang District, Central Java, Indonesia based on ISSR markers

MUHAMMAD ISBAT AMRULLAH[1], ENNI SUWARSI RAHAYU[1], ELING PUWANTOYO[1], RIA IKA MAHARANI[1], SOLICHIN[1], SRIYADI[1], TRI YANUAR HADIPRASETYA[2], AMIN RETNONINGSIH[1,♥]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. Jl. Raya Sekaran, Semarang 50221, Central Java, Indonesia. Tel.: +62-24-86008700, ♥email: aminretnoningsih2016@mail.unnes.ac.id

[2]Griya Mukti Indah A.34, Kecepak, Batang District, Central Java, Indonesia

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Abstract. *Amrullah MI, Rahayu ES, Puwantoyo E, Maharani RI, Solichin, Sriyadi, Hadiprasetya TY, Retnoningsih A. 2023. Genetic characterization of superior durian (*Durio zibethinus *L.) accessions in Batang District, Central Java, Indonesia based on ISSR markers. Biodiversitas 24: 4811-4819.* Durian (*Durio zibethinus* L.) has the highest level of genetic diversity in the genus of *Durio*. The genetic diversity of *D. zibethinus* in Batang District, Central Java, Indonesia needs to be explored to obtain data on superior durian accessions that are adaptive in the area. This study aimed to analyze the genetic diversity of 40 superior durian accessions from 13 locations in Batang District using 10 Inter Simple Sequence Repeat (ISSR) primers. Genomic DNA was isolated from leaves using a modified CTAB method. PCR products were electrophoresed on 2% agarose and yielded 161 ISSR alleles with a percentage of 100% polymorphic alleles. The level of genetic diversity based on Nei's gene diversity and Shannon's Information index are 0.277 and 0.434, respectively. The durian accessions studied have a similarity coefficient of 0.22-0.86 and are divided into two main clusters. Siwatu 1 and Siwatu 2 accessions have the highest genetic similarity coefficient (0.86), while Butternuts and Watu Tembogo 1 accessions show the lowest genetic similarity coefficient (0.05). Specific alleles were found in 11 durian accessions. The molecular characterization results show the high genetic diversity of superior durian accessions in Batang District and add to the list of local superior durian accessions in Indonesia.

Keywords: Alleles, Batang, genetic diversity, Inter Simple Sequence Repeat, ISSR, polymorphic, superior durian

INTRODUCTION

The genus *Durio* consists of 32 accepted species (World Flora Online 2023). One of the most popular species is *Durio zibethinus* L., known as "durian". Variations within this species are highly varied compared to other *Durio* species (Retnoningsih et al. 2016; Solikin et al. 2017). Although Indonesia is known to be the center of *Durio* species diversity (Uji 2005), Indonesia's durian production is relatively low, and its export value is far behind Thailand and Malaysia (Trade Map 2022). The export value of Indonesian durian in 2021 was only USD 155.58 thousand (Tridge 2023), even though Indonesia's average durian production is 1.3 million tons annually (BPS 2021). However, with unstable fruit quality, increasing export value is still challenging.

The Ministry of Agriculture of the Republic of Indonesia has issued certificates for 342 local superior durian varieties (Indonesian Ministry of Agriculture 2022). Only a few of these varieties are under intensive cultivation. Local durians grow naturally in gardens and forests with minimal care, producing suboptimal fruit and quality (Gaol et al. 2015). Intensive durian cultivation in Indonesia is considerably far behind Malaysia with an increase in durian export value of USD 13.7 million in 2011-2019 (International Trade Center 2020; Safari et al. 2021). Therefore, the genetic diversity of durian in the Indonesian region must be explored deeper to obtain adaptive superior accessions to develop local superior durian cultivation.

Batang District, Central Java, Indonesia is a durian-producing center which has several superior durian accessions. The criteria for superior durian include soft arilus without fiber, fluffy, thick, slightly bitter-sweet, yellowish color, high productivity, stable fruit in quantity and quality, and free of pests and diseases (FAO 2014; Indonesian Ministry of Agriculture 2021). Characterization of the Batang durian is needed to complete the database of Indonesian superior durians and to facilitate the selection of accessions developed in the region. The purpose of morphological and molecular characterization is to obtain certainty of identity. The identity of durian accessions guarantees the truth of durian clones (Limbongan and Yasin 2016; Rohman and Azizah 2021).

The use of morphological markers is cheap and easy, but the accuracy of these markers is low. The phenotype is influenced not only by genotype, but also by the interaction between genotype and environment (Wang et al. 2013; Susilawati et al. 2018). Durian reproductive organs can only be characterized during flowering and fruiting, which occurs once or twice a year. In addition, morphology is very similar among durian species, therefore, skill and

experience determine the accuracy of characterization results (Putri et al. 2019). Characterization using molecular markers is reported to be more accurate, efficient, and stable in revealing genetic diversity than morphological markers (Butiuc-Keul et al. 2019).

Inter-simple sequence repeat (ISSR) is a molecular marker that has been widely used to analyze the genetic diversity of plants, such as banana (Sunaryo et al. 2020), mango (Chen et al. 2022), and durian (Angeliena et al. 2019; Prakoso and Retnoningsih 2021). The advantages of ISSR markers, such that simple, efficient, cheap, and reproducible compared to other molecular markers (Ng and Tan 2015). These markers can also be used to assess the genetic stability of a new cultivar and intraspecific genetic variation. In addition, ISSR can also be developed as a specific marker to discriminate between cultivars (Son et al. 2013). Information on the genetic diversity and the certain availability of superior durian accessions in the Batang region has not been reported. This study aimed to analyze the genetic diversity of superior durian in Batang District based on ISSR markers. The analysis results can be the basis for developing the cultivation of superior durian accessions adapted to the region.

MATERIALS AND METHODS

Plant materials

Plant materials used in the study were 40 superior durian accessions based on interviews with durian farmers in 13 locations in Batang, Central Java, Indonesia (Figure 1). The list of 40 superior durian accessions in Batang is presented in Table 1.

Young durian leaves are collected from each accession as a source of genomic DNA. Young leaves are used to facilitate the isolation process, as the cell walls are easier to destroy, and the phenolic and flavonoid content is lower than the old leaves (Hanin and Pratiwi 2017). The leaves were cleaned and placed into ziplock plastics with the code Bt1-Bt40 (Table 1). ISSR allele characterization was conducted at the Laboratory of Molecular Biology and Research, Department of Biology, Faculty of Mathematics and Natural Sciences, Semarang State University, Semarang, Indonesia from August 2022 to March 2023.

Procedures

Isolation of genomic DNA

Genomic DNA isolation was done by CTAB method following Solikin et al. (2017). Dirt and scales on durian leaves were cleaned using 70% ethanol. Each leaf was then cut into small pieces and weighed 0.025 g. The sample was put into a 2 mL microtube, then added by 1 mL of extraction buffer (2% CTAB, 0.1 M Tris-HCl pH 8, 0.02 M EDTA, 1% PVP, 1.4 M NaCl, β-mercaptoethanol 0.3%). Samples were mashed using a TissueLyser, then incubated in a water bath at 60°C for 30 minutes. About 750 µL of chloroform isoamyl alcohol (CIAA) was added to the microtube and centrifuged at 10,000 rpm for 15 minutes until three layers were obtained. The supernatant was transferred into a new microtube. The addition of CIAA was repeated and centrifuged at the same speed and duration. The supernatant was transferred into a new microtube, and then 2.5 µL of RNAse was added. The solution was incubated at 37°C for 30 minutes, 1 ml of cold absolute ethanol was added to the microtube and then centrifuged at 12,000 rpm for 20 minutes at 4°C. The DNA precipitate was washed with 500 µL of 70% ethanol and centrifuged at 4°C at 12,000 rpm for 5 minutes. The washing process with 70% ethanol was carried out twice. The precipitate was air-dried, and 100 µL of TE buffer was added to dissolve the DNA.

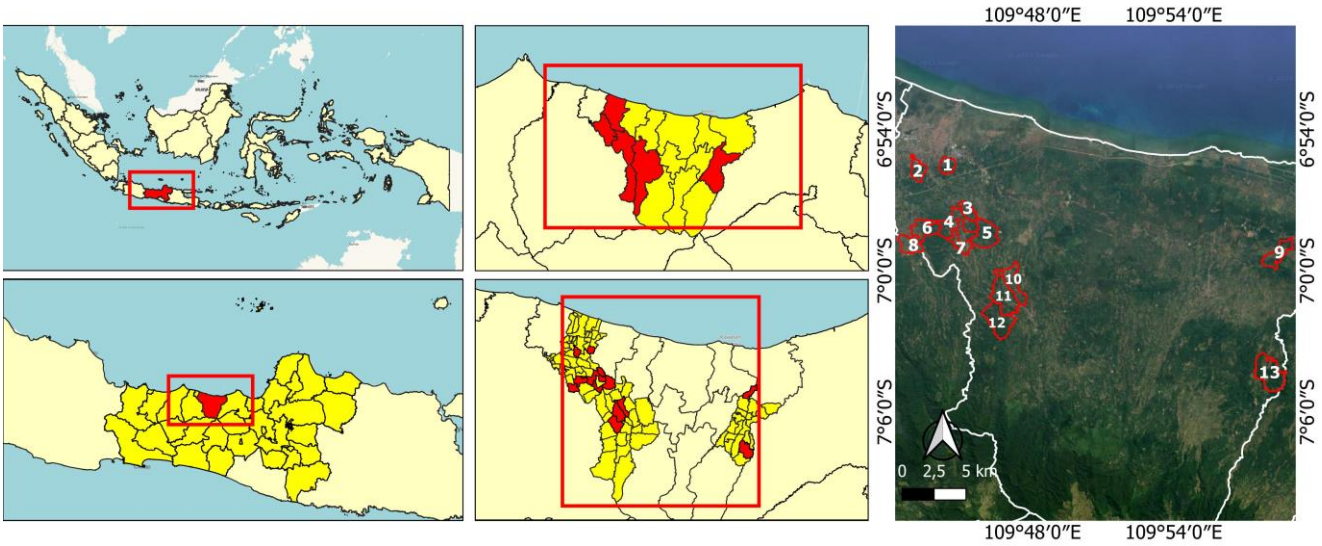

Figure 1. Source of plant materials used in the study: Distribution of 40 superior durian accessions in 13 locations in Batang District, Central Java, Indonesia. 1. Kecepak, 2. Kalisalak, 3. Penangkan, 4. Siwatu, 5. Sigayam, 6. Kaliwareng, 7. Brayo, 8. Pandansari, 9. Satriyan, 10. Pucanggading, 11. Tambahrejo, 12. Pesalakan, 13. Sidalang

Table 1. List of 40 superior durian accessions from 13 locations in the Batang District, Central Java, Indonesia

Codes	Accessions	Villages	Coordinates (S; E)
Bt1	Cepak 1	Kecepak	6°55'17.04"; 109°44'38.04"
Bt2	Cepak 2	Kecepak	6°55'17.04"; 109°44'38.04"
Bt3	Salak	Kalisalak	6°55'29.0532"; 109°43'2.1036"
Bt4	Kentongan	Kalisalak	6°55'29.0532"; 109°43'2.1036"
Bt5	Milky 2	Kalisalak	6°55'29.0532"; 109°43'2.1036"
Bt6	Sarjana	Pesalakan	7°1'57.7236"; 109°46'26.922"
Bt7	Pagerukir	Pesalakan	7°1'4053"; 109°46'16.0644"
Bt8	Gundul Proto	Tambahrejo	7°1'6.1464"; 109°46'42.7476"
Bt9	Tangkai Panjang	Pucanggading	6°59'52.9044"; 109°46'59.6064"
Bt10	Butternuts	Pucanggading	6°59'52.9044"; 109°46'59.6064"
Bt11	Milky 1	Sigayam	6°57'54.8856"; 109°45'54.8424"
Bt12	Nangkan 1	Penangkan	6°57'12.8988"; 109°45'3.7152"
Bt13	Monlok	Penangkan	6°57'12.8988"; 109°45'3.7152"
Bt14	Pompongan	Siwatu	6°57'47.8944"; 109°45'4.8924"
Bt15	Watu tembogo 1	Siwatu	6°57'47.8944"; 109°45'4.8924"
Bt16	Siwatu 1	Siwatu	6°57'47.8944"; 109°45'4.8924"
Bt17	Siwatu 2	Siwatu	6°57'47.8944"; 109°45'4.8924"
Bt18	Siwatu 3	Siwatu	6°57'47.8944"; 109°45'4.8924"
Bt19	Susu	Siwatu	6°57'47.8944"; 109°45'4.8924"
Bt20	Puyer 16	Kaliwareng	6°58'9.2676"; 109°43'34.5432"
Bt21	Pandansari	Pandansari	6°58'9.2676"; 109°42'38.3796"
Bt22	Sookis	Satriyan	6°58'55.8912"; 109°58'44.2344"
Bt23	Mundip	Satriyan	6°58'55.8912"; 109°58'44.2344"
Bt24	Brayo 1	Brayo	6°58'57.9036"; 109°44'42.4932"
Bt25	Supermrica	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt26	Brayo 3	Brayo	6°58'57.9036"; 109°44'42.4932"
Bt27	Barno 1	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt28	Watu Tembogo 2	Siwatu	6°57'47.8944"; 109°45'4.8924"
Bt29	Muji 2	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt30	Emprit	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt31	Muji 3	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt32	Muji 4	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt33	Muji 5	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt34	Muji 6	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt35	Antik	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt36	Barno 4	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt37	Barno 5	Siwatu	6°58'23.6928"; 109°44'6.1656"
Bt38	Citotok 1	Siwatu	6°58'21.594"; 109°44'19.4388"
Bt39	Citotok 2	Siwatu	6°58'21.594"; 109°44'19.4388"
Bt40	Delisen	Sidalang	7°0'5.3496"; 109°56'53.1096"

Genomic DNA amplification

DNA amplification was performed using a Bio-Rad C1000 Touch Thermal Cycler with 10 ISSR primers (Table 2). The volume of each PCR mixture was 13.5 µL consisting of 2 µL genomic DNA samples (50 ng/ µL), 6.25µl GoTaq Green Master Mix Promega, 1 µL ISSR primer, and 4.25 µL nuclease-free water. The stages of PCR were carried the pre-denaturation stage at 95°C for 4 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 44.0-53.7°C for 30 seconds, extension at 72°C for 1 minute, final extension at 72°C for 10 minutes, and soaking at 4°C.

Electrophoresis

The PCR product was run using Cleaver Scientific multiSUB Mini electrophoresis machine on a 2% agarose gel with Florosafe DNA stain in 1x TAE buffer at 100 V for 35 minutes. The allele ISSR profile was observed using a UV transilluminator (WiseUv WUV-M20). Allele size was determined using Promega 100 bp DNA ladder.

Data analysis

ISSR allele profiles are converted into binary data with scoring (present = 1, absent = 0). The data used to calculate the number and percentage of polymorphic alleles, genetic diversity values based on Nei's gene diversity (h) and Shannon's information index (I) (Nei 1978) using POPGENE v. 1.31 software (Yeh 1999), specific alleles, and similarity analysis. Groupings in the form of dendrogram and principal coordinate analysis (PCoA) determined based on similarity coefficients (similarity for qualitative (SIMQUAL) with dice coefficients) between accessions using the sequential, agglomerative, hierarchical, and nested (SAHN) unweighted pair-group method, arithmetic average (UPGMA) in Numerical Taxonomy and Multivariate Analysis System (NTSYS) pc v. 2.02i (Rohlf 2000) and PAST v. 4.03 (Hammer 2020) software.

RESULTS AND DISCUSSION

Allele polymorphisms

The allele profile of 40 Batang superior durian accessions based on ISSR markers yielded as many as 161 alleles, with 10-21 alleles per primer. PCR using PKBT 7 and PKBT 12 primers produced the most alleles (21 alleles), while PKBT 2 primer produced the most minor alleles (10 alleles). The relative size range of the alleles yielded was 150-1700 bp (Table 3). ISSR alleles are typically 100-2000 bp in size (Ng and Tan 2015). The percentage of polymorphic alleles for each primer reached 100%. The genetic diversity based on Nei's gene diversity (h) and Shannon's information index (I) in superior durian populations in Batang is 0.277 and 0.434, respectively. The results of this study indicate a very high genetic variation among 40 genotypes of durian accessions. Abundant genetic variation indicates the high genetic diversity of a population (Chen et al. 2013; Aljumaili et al. 2018). The allelic profile of 40 durian accessions is shown in the PCR results using PKBT 7 and PKBT 8 primers are presented in Figure 2.

The average number of ISSR alleles per accession presented in this study was almost the same as superior durian Gunungpati, Central Java, Indonesia (Nasrika and Retnoningsih 2021), but higher than durian Tengkurak (Riupassa et al. 2015) and the durian collection of Hortimart, Central Java (Solikin et al. 2017). Each accession of Batang superior durian shows ISSR allele polymorphisms. The percentage of polymorphic alleles in this study is the same as the results of Solikin et al. (2017) on 41 accessions of Hortimart's durian collection using the same primers (PKBT 2, PKBT 3, PKBT 8, and PKBT 9).

The number of primers and accessions studied determines the number of alleles and the percentage of polymorphic alleles produced (Giang et al. 2016; Solikin et al. 2017; Siew et al. 2018; Angeliena et al. 2019). The greater number of primers and accessions used, the higher the opportunity for ISSR alleles to be revealed. The success of ISSR allele amplification highly depends on the compatibility between the DNA sequences and the primers used (Mohamad et al. 2017; Gemmill and Grierson 2021). Optimization of the annealing temperature also has an essential role in amplification success, as each primer has a specific annealing temperature. In addition, the distribution of accessions also affects the level of polymorphism in a population (Bakoumé et al. 2015; Zhang et al. 2016). Populations with a wide distribution of accessions have relatively high genetic diversity due to geographical differences (Stuessy et al. 2014).

High genetic diversity in durians has resulted from cross-pollination (Bumrungsri et al. 2009). The morphology and physiology of durian flowers affect their pollination and fertilization patterns (Torezan-Silingardi et al. 2021). The position of the stigma in durian flowers is higher than in the stalk. In addition, the ovule and pollen in a durian flower mature at different times, so the chance of self-pollination is low (Ketsa 2018). Therefore, durian is generally self-incompatible, which promotes cross-pollination (Bumrungsri et al. 2009; Wayo et al. 2018). The genotype of seeds in a durian fruit cannot be 100% similar since the pollination and fertilization involve sperm of unknown origin. Durian seeds resulting from cross-pollination carry the characteristics of both elders and determine the quality of the fruit produced. Indriyani et al. (2012) showed that cross-pollination between two superior accessions resulted in a relatively high percentage of fruit set and superior durian quality. Mutations can also cause the genetic diversity in durian. Mutations, further, change the annealing site of an accession, thus affecting the compatibility between DNA and primer sequences (El-Degwy 2013). Deletions, insertions, and substitutions at the annealing site can affect the size of the resulting PCR product, allowing for genetic variation between accessions (Singh et al. 2014).

Table 3. Relative size and number of alleles amplified from 40 superior durian accessions in Batang District using 10 ISSR primers

Primers	Relative sizes (bp)	Number of alleles	Number of polymorphic alleles
ISSR 1	180-950	15	15
ISSR 4	150-750	15	15
ISSR 5	150-1400	17	17
ISSR 10	180-1000	17	17
PKBT 2	300-900	10	10
PKBT 3	200-800	12	12
PKBT 7	150-900	21	21
PKBT 8	150-1400	18	18
PKBT 9	180-850	15	15
PKBT 12	180-1700	21	21
Total		161	161 (100%)

Table 2. List of 10 ISSR primers for analyzing genetic diversity of Batang superior durian accessions

Primers	Sequences	Annealing temperature (°C)	References
ISSR1	5'-AGGAGGAGGAGGAGG-3'	48.4	Riupassa et al. (2015)
ISSR4	5'-GAGGAGGAGGAGGAGAC-3'	47.3	Vanijajiva (2012)
ISSR5	5'-GAGGAGGAGGAGGAGAT-3'	44.0	Riupassa et al. (2015)
ISSR10	5'-GTGTGTGTGTGTGTGTGTA-3'	49.4	Riupassa et al. (2015)
PKBT2	5'-ACACACACACACACA-3'	53.0	Angeliena et al. (2019)
PKBT3	5'-AGAGAGAGAGAGAGAGT-3'	47.5	Syahruddin (2012)
PKBT7	5'-GAGAGAGAGAGAGAGAGAA-3'	49.0	Angeliena et al. (2019)
PKBT8	5'-GAGAGAGAGAGAGAGAGAC-3'	53.7	Riupassa et al. (2015)
PKBT9	5'-GAGAGAGAGAGAGAGAGAT-3'	50.9	Syahruddin (2012)
PKBT12	5'-GTGTGTGTGTGTGTGTGTT-3'	44.9	Riupassa et al. (2015)

Accessions of specific alleles

DNA amplification of Batang superior durian accessions produced several specific alleles on seven primers (ISSR 5, PKBT 2, PKBT 3, PKBT 7, PKBT 8, PKBT 9, and PKBT 12) (Table 4). Amplification with ISSR 5 and PKBT 9 primers produced the most specific alleles (3 alleles); PKBT 2 and PKBT 3 primers detected two alleles, and the other three primers detected only one allele. Specific alleles have been found in 11 accessions of durian. Pagerukir (Bt7) and Delisen (Bt40) accessions have two specific alleles, while the other nine accessions have only one allele. The presence of particular specific alleles is shown in Figure 2.

Specific alleles are found in only one individual and can be used to identify plant varieties (Lestari et al. 2016). Specific alleles amplified with dominant primers such as ISSR can be a marker for accession with a unique phenotype if the allele is located near a particular gene. It allows linkage to the gene (Amiteye 2021). Codominant molecular markers such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) are recommended to reveal the identity of an accession because linkage opportunities with specific genes are relatively common (Wu et al. 2014; Jain et al. 2015; Schlautman et al. 2015). Specific ISSR alleles can be used to develop codominant markers, such as sequence-characterized amplified regions (SCARs), which have higher reproducibility than dominant markers (Son et al. 2013). It makes the identity of an accession more accurate and stable.

Clustering analysis

The similarity matrix of the Batang superior durian accessions is shown in Table 5. Siwatu 1 and Siwatu 2 accessions have the highest similarity with a similarity coefficient of 0.86, while Butternuts and Watu Tembogo 1 accessions have the lowest similarity of 0.05. The similarity matrix and dendrogram (Figure 3) show that the 40 durian accessions analyzed in this study are distinct individuals (no synonymy among accessions) with a similarity coefficient of 0.22-0.86. The dendrogram formed two main clusters, namely clusters I and II. Cluster I contains 38 accessions, divided into two subclusters, IA and IB, with 20 and 18 accessions, respectively. Cluster II consists of only two accessions, Watu Tembogo 1 and Susu. All accessions with specific alleles are in cluster I (six accessions in subcluster IA and five accessions in subcluster IB).

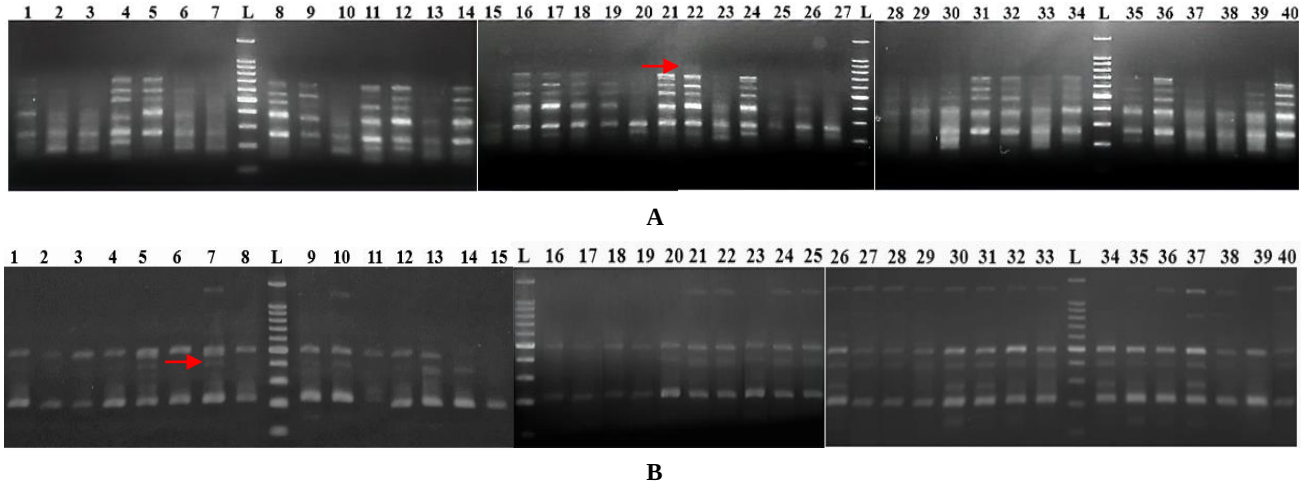

Figure 2. Allelic profile of 40 Batang superior durian accessions using PKBT 7 (A) and PKBT 8 (B) primers. Red arrow symbols indicate specific alleles

Table 4. Specific ISSR alleles found in 11 Batang superior durian accessions

Accessions	Codes	Relative sizes (bp)						
		ISSR 5	PKBT 2	PKBT 3	PKBT 7	PKBT 8	PKBT 9	PKBT 12
Brayo 3	Bt26	1400						
Siwatu 3	Bt18	800						
Muji 4	Bt32	700						
Pagerukir	Bt7		900			410		
Pandansari	Bt21		600					
Delisen	Bt40			500			550	
Milky 1	Bt11			380				
Sookis	Bt22				900			
Antik	Bt35						850	
Emprit	Bt30						180	
Citotok 1	Bt38							1700

Table 5. Similarity matrix of 40 Batang superior durian accessions based on ISSR allele profiles

Bt	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	***																																							
2	0.36	***																																						
3	0.42	0.76	***																																					
4	0.6	0.44	0.41	***																																				
5	0.63	0.4	0.3	0.79	***																																			
6	0.35	0.38	0.45	0.4	0.42	***																																		
7	0.3	0.45	0.45	0.37	0.44	0.71	***																																	
8	0.51	0.42	0.42	0.54	0.51	0.39	0.34	***																																
9	0.35	0.43	0.55	0.34	0.36	0.45	0.47	0.33	***																															
10	0.32	0.26	0.31	0.29	0.3	0.22	0.2	0.37	0.34	***																														
11	0.49	0.45	0.39	0.54	0.49	0.34	0.3	0.54	0.21	0.26	***																													
12	0.41	0.32	0.31	0.48	0.39	0.29	0.24	0.46	0.17	0.31	0.68	***																												
13	0.38	0.43	0.46	0.37	0.39	0.45	0.51	0.34	0.54	0.31	0.41	0.39	***																											
14	0.31	0.31	0.33	0.37	0.29	0.22	0.26	0.43	0.21	0.37	0.51	0.58	0.42	***																										
15	0.17	0.3	0.2	0.15	0.12	0.33	0.26	0.13	0.2	0.05	0.16	0.22	0.29	0.2	***																										
16	0.3	0.33	0.39	0.24	0.28	0.24	0.24	0.37	0.38	0.57	0.16	0.15	0.38	0.22	0.13	***																								
17	0.29	0.35	0.38	0.22	0.27	0.19	0.17	0.37	0.37	0.58	0.17	0.19	0.31	0.23	0.14	0.86	***																							
18	0.44	0.38	0.4	0.43	0.42	0.32	0.32	0.52	0.37	0.29	0.46	0.4	0.35	0.39	0.17	0.49	0.49	***																						
19	0.22	0.16	0.17	0.23	0.29	0.3	0.27	0.23	0.33	0.15	0.2	0.19	0.26	0.2	0.31	0.27	0.29	0.34	***																					
20	0.16	0.47	0.47	0.17	0.21	0.31	0.39	0.26	0.49	0.24	0.16	0.17	0.4	0.21	0.37	0.35	0.38	0.29	0.34	***																				
21	0.43	0.28	0.33	0.42	0.46	0.33	0.28	0.48	0.29	0.33	0.41	0.3	0.3	0.35	0.12	0.34	0.39	0.51	0.28	0.21	***																			
22	0.31	0.46	0.46	0.38	0.35	0.33	0.31	0.39	0.43	0.3	0.4	0.4	0.35	0.27	0.28	0.34	0.33	0.38	0.13	0.46	0.34	***																		
23	0.31	0.34	0.38	0.29	0.32	0.4	0.44	0.3	0.4	0.27	0.24	0.31	0.42	0.22	0.38	0.29	0.25	0.27	0.23	0.56	0.24	0.55	***																	
24	0.22	0.41	0.37	0.23	0.29	0.4	0.41	0.2	0.45	0.2	0.23	0.25	0.42	0.16	0.4	0.25	0.23	0.19	0.17	0.56	0.21	0.58	0.71	***																
25	0.41	0.37	0.36	0.3	0.32	0.3	0.25	0.3	0.23	0.33	0.38	0.39	0.36	0.32	0.26	0.31	0.36	0.31	0.27	0.2	0.34	0.39	0.39	0.42	***															
26	0.38	0.32	0.37	0.32	0.39	0.32	0.34	0.41	0.31	0.28	0.32	0.24	0.29	0.28	0.11	0.26	0.31	0.32	0.26	0.31	0.36	0.31	0.38	0.25	0.57	***														
27	0.57	0.33	0.39	0.42	0.44	0.29	0.3	0.4	0.38	0.29	0.32	0.26	0.29	0.25	0.13	0.33	0.29	0.44	0.27	0.26	0.34	0.31	0.23	0.25	0.44	0.62	***													
28	0.5	0.31	0.39	0.39	0.44	0.42	0.38	0.35	0.36	0.3	0.38	0.28	0.33	0.29	0.16	0.34	0.33	0.36	0.29	0.21	0.38	0.32	0.32	0.26	0.54	0.58	0.59	***												
29	0.39	0.29	0.41	0.28	0.33	0.31	0.34	0.28	0.3	0.26	0.28	0.28	0.21	0.33	0.14	0.28	0.34	0.26	0.24	0.31	0.29	0.32	0.3	0.26	0.57	0.65	0.49	0.69	***											
30	0.42	0.44	0.46	0.3	0.39	0.45	0.53	0.26	0.51	0.26	0.26	0.23	0.43	0.18	0.27	0.31	0.26	0.24	0.21	0.51	0.23	0.38	0.56	0.64	0.34	0.35	0.42	0.37	0.35	***										
31	0.59	0.46	0.42	0.42	0.44	0.28	0.28	0.46	0.33	0.27	0.31	0.22	0.25	0.29	0.16	0.34	0.37	0.44	0.12	0.3	0.52	0.4	0.25	0.26	0.41	0.44	0.53	0.59	0.52	0.42	***									
32	0.55	0.41	0.37	0.45	0.44	0.37	0.34	0.55	0.29	0.29	0.44	0.4	0.25	0.36	0.23	0.33	0.35	0.47	0.17	0.32	0.46	0.38	0.33	0.23	0.47	0.49	0.47	0.49	0.43	0.47	0.68	***								
33	0.49	0.45	0.45	0.32	0.38	0.34	0.39	0.33	0.33	0.34	0.39	0.34	0.29	0.3	0.25	0.38	0.4	0.32	0.22	0.37	0.35	0.37	0.33	0.41	0.5	0.47	0.49	0.55	0.58	0.62	0.66	0.61	***							
34	0.53	0.34	0.38	0.38	0.43	0.3	0.31	0.4	0.35	0.39	0.33	0.33	0.28	0.37	0.17	0.34	0.3	0.38	0.18	0.36	0.39	0.41	0.48	0.33	0.46	0.53	0.51	0.53	0.5	0.56	0.61	0.6	0.65	***						
35	0.33	0.36	0.35	0.33	0.34	0.24	0.28	0.44	0.43	0.32	0.3	0.3	0.24	0.26	0.15	0.39	0.45	0.33	0.27	0.32	0.37	0.36	0.24	0.23	0.46	0.43	0.39	0.54	0.57	0.38	0.57	0.51	0.67	0.57	***					
36	0.43	0.39	0.39	0.42	0.41	0.27	0.28	0.5	0.41	0.43	0.3	0.3	0.25	0.41	0.15	0.4	0.46	0.46	0.24	0.36	0.46	0.39	0.34	0.23	0.41	0.38	0.34	0.41	0.42	0.34	0.58	0.64	0.46	0.47	0.61	***				
37	0.33	0.4	0.46	0.34	0.26	0.39	0.38	0.44	0.48	0.24	0.34	0.34	0.32	0.26	0.21	0.33	0.29	0.3	0.25	0.39	0.28	0.56	0.47	0.41	0.38	0.48	0.4	0.33	0.34	0.54	0.36	0.52	0.47	0.46	0.4	0.42	***			
38	0.24	0.33	0.29	0.25	0.23	0.3	0.3	0.2	0.38	0.23	0.3	0.3	0.38	0.23	0.23	0.21	0.23	0.2	0.24	0.26	0.2	0.31	0.34	0.46	0.49	0.27	0.33	0.4	0.32	0.46	0.34	0.32	0.45	0.34	0.33	0.25	0.47	***		
39	0.37	0.42	0.42	0.44	0.38	0.31	0.26	0.52	0.36	0.36	0.45	0.45	0.36	0.35	0.18	0.4	0.42	0.34	0.15	0.36	0.35	0.53	0.37	0.29	0.38	0.44	0.4	0.38	0.27	0.37	0.41	0.56	0.48	0.49	0.39	0.43	0.6	0.45	***	
40	0.38	0.48	0.48	0.37	0.27	0.39	0.29	0.38	0.39	0.28	0.39	0.39	0.31	0.33	0.25	0.35	0.4	0.34	0.07	0.34	0.3	0.49	0.3	0.37	0.35	0.23	0.32	0.33	0.3	0.37	0.36	0.46	0.38	0.33	0.29	0.43	0.52	0.4	0.58	***

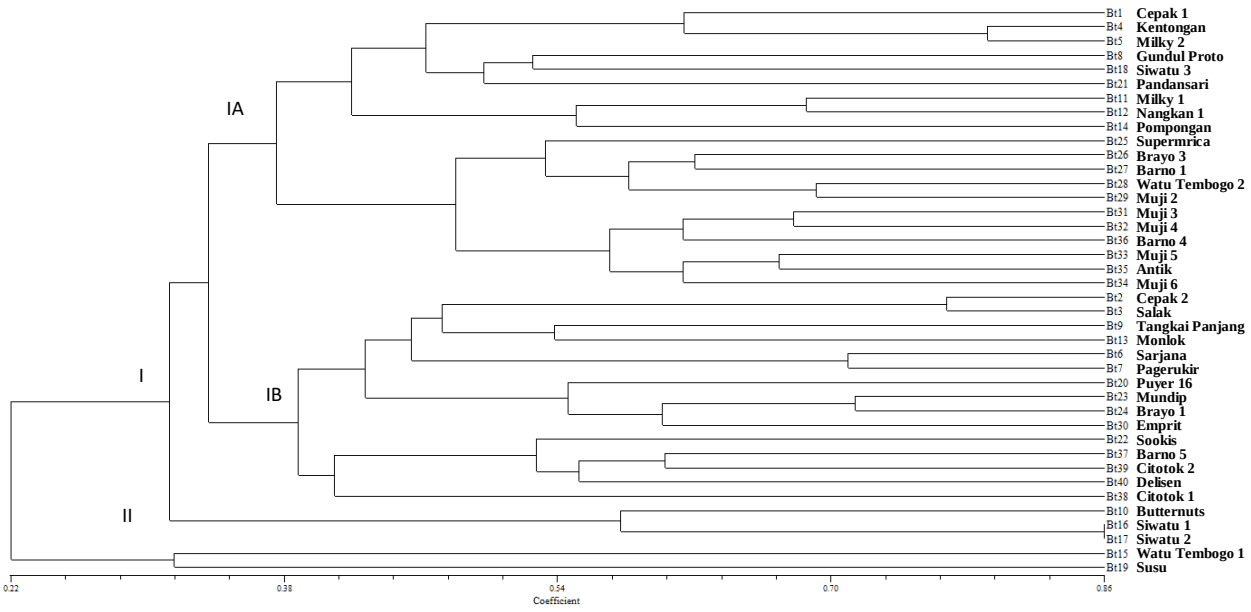

Figure 3. Dendrogram of 40 Batang durian accessions generated by the UPGMA cluster analysis

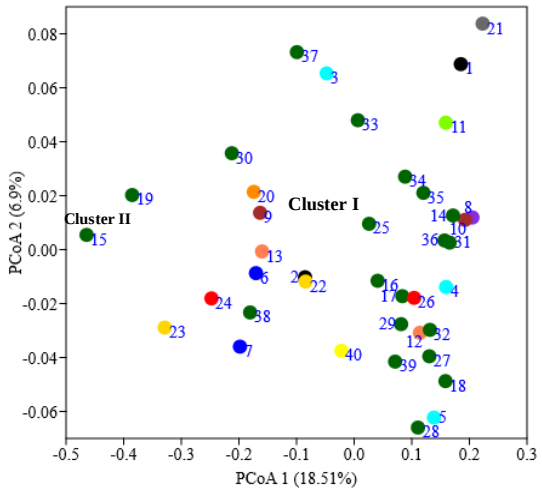

Figure 4. PCoA matrix showing two main clusters of 40 superior durian accessions from 13 locations in Batang District based on ISSR markers

The PCoA results show agreement with the dendrogram (Figure 4). Watu Tembogo 1 and Susu accessions are separated from the other 38 accessions. The PCoA matrix strengthens the dendrogram that 40 accessions of Batang superior durian based on ISSR markers produce two main clusters.

Accessions in a cluster have similar allelic profiles (Kristamtini et al. 2014). High similarity between accessions indicates low genetic distance and vice versa (Daryono et al. 2019). The higher the similarity of allelic profiles between accessions, the more likely it is that the two accessions are closely related. The high similarity between Siwatu 1 and Siwatu 2 accessions is possible because both are originated from Siwatu village. The two accessions are similar because the plants were grown from cross-pollinated seeds from the same male elders. The lowest similarity was found between Butternuts accessions from Pucanggading village and Watu Tembogo 1 from Siwatu village. The clustering results show that geographical distance does not determine whether an accession belongs to the same cluster. According to Suratman et al. (2015), the exact geographical origin does not guarantee that accessions are closely related. Accessions in the same cluster may have genetic similarities. Pollinators, such as bats, play an essential role in the cross-pollination of durian flowers (Baqi et al. 2022). Such pollination patterns allow accessions from different populations to be in the same cluster and vice versa. The similarity coefficient of 40 superior durian accessions from Batang indicates that each accession is derived from cross-pollinated seeds. Each accession has only one naturally growing tree, so the genotype is 100% different. Therefore, the 40 Batang superior durian accessions can be registered with the Center for Plant Variety Protection and Agricultural Licensing (PVP-PP) of the Republic of Indonesia and intensively developed in the region.

In conclusion, the genetic diversity of 40 Batang superior durian accessions using 10 ISSR primers includes a high category because no synonyms were found. The percentage of polymorphic alleles, h value, and I value are 100%, 0.277, and 0.434, respectively. Specific alleles found in 11 durian accessions are namely Pagerukir (Bt7), Milky 1 (Bt11), Siwatu 3 (Bt18), Pandansari (Bt21), Sookis (Bt22), Brayo 3 (Bt26), Emprit (Bt30), Muji 4 (Bt32), Antik (Bt35), Citotok 1 (Bt38), and Delisen (Bt40). All the Batang superior durian accessions studied are different accessions with a similarity coefficient of 0.22-0.86 and are divided into two main clusters. The superior Batang durian accessions can be added to the list of local superior durian accessions and become the basis for developing durian cultivation, especially in the Batang District, Central Java, Indonesia.

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