

# Molecular based genetic diversity of Brongkol's superior durian germplasm of Semarang, Indonesia

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**Abstract.** Prakoso CNY, Retnoningsih A. 2021. Molecular based genetic diversity of Brongkol's superior durian germplasm of Semarang, Indonesia. *Biodiversitas* 22: 5311-5316. *Durio zibethinus* is an Indonesian most popular edible durian. This species' genetic diversity is the highest compared to other durian species. Brongkol Village, Semarang, Indonesia is one of the villages that produce superior durian varieties. Brongkol durian is well-known for its sweet and bitter taste of aril with its white to yellow color. This research aimed to analyze the diversity and to identify superior durian in Brongkol using ISSR molecular markers. DNA was isolated from the leaves of 25 varieties of Brongkol superior durian. The DNA was isolated using a modified CTAB method. The genomic DNA was amplified using PCR employing 7 ISSR markers. The amplified DNA band was visualized using a 2% agarose gel electrophoresis. Data were analyzed using the NTSYS' pc version 2.02 program. The alleles produced in this study were 340-3,000 bp in size. The Polymorphic alleles were found as much as 90.2%. The results of study showed a similarity level of 0.29-0.95. All of the Brongkol superior durians were identified as different varieties. All of the superior Brongkol durians studied were identified as distinct varieties. The results of this study indicated a high diversity of superior durian in Brongkol. In addition, specific alleles were found in varieties B5 and B16.

**Keywords:** Brongkol, superior durian, ISSR

## INTRODUCTION

The diversity of species and genetics of durian in Indonesia is known to be the largest in the world. Twenty of 30 species of durian are found in Indonesia, especially in Kalimantan, where 14 species are endemic, and nine species are edible durian (Uji 2005; Santoso and Priyono 2015). *Durio zibethinus* is one of the most popular edible durians and is found in almost all archipelago regions. The genetic diversity of this durian species is known to be the highest compared to other species (Hikmah et al. 2016, Angeliena et al. 2019; Habibah et al. 2019; Songnuan et al. 2019; Hannum et al. 2020; Maranatha et al. 2020; Yursak et al. 2020). Each region in Indonesia has its own superior durian varieties identified by the aril's taste, color, and aroma. Yet, no more than 97 varieties of superior durian are registered at the Ministry of Agriculture (Balitbang Pertanian 2019). The number of registered superior durian varieties will continue to increase and new superior durian in various regions will be discovered. This durian germplasm data is one of the considerations for developing superior durian center.

Brongkol is a village in Jambu District, Semarang Regency, Central Java. The area is located at the foot of Mount Kelir at an altitude of 600-700 meters above sea level (masl). In addition to superior durian, Brongkol Village is also known for its superior coffee varieties. Superior durians from Brongkol had won several superior durian competitions in the Central Java Province (Latif 2013). The trading names for Brongkol durian generally

use the name of the tree owner, the area's name, or other names. Hence, the genetic diversity of *D. zibethinus* in the area is known only from its name. Trees of different varieties of durian are challenging to distinguish from one another because the morphology of the vegetative organs is very similar. Therefore, cultivating certain superior durian varieties on a large scale requires identity certainty of superior varieties from the existing superior genetic diversity.

The genotype of superior Brongkol durian varieties can be ascertained through diversity analysis using molecular markers. Molecular markers provide more accurate genetic information than biochemical, cytological, or morphological markers. Isozyme biochemical markers are influenced by height differences (Fitmawati et al. 2017). Meanwhile, morphological markers are considered inaccurate because genetic and environmental factors determine the phenotypes. Environmental differences can affect the phenotype even though the genotype is the same (Handayani and Ismadi 2017). Molecular markers provide more accurate information about genetic relationships within and between different species (Ibarra-Torres et al. 2014; Patel et al. 2015). The advantages of molecular markers are that they are stable and not influenced by environmental factors (Yulita, 2013) and can be detected using DNA in all plant tissues (Kumar et al. 2006; Zulfahmi 2013; Zayed et al. 2014; Dasgupta et al. 2017).

One of the molecular markers often used to study genetic diversity is inter-simple sequence repeat (ISSR) (Vanijajiva 2012; Riupassa et al. 2015; Solikin et al. 2017;

Siew et al. 2018; Angeliena et al. 2019). This marker is part of a microsatellite sequence that does not code for protein (non-coding region) and comprises one, two, or three nucleotides (Ng and Tan 2015). ISSR is superior to RAPD (Cui et al. 2016; Daryono et al. 2019; Due et al. 2019). The advantages of this marker are high reproducibility, highly polymorphic, environmentally independent, cost-effective, and do not require the entire DNA sequence to design the primer (Jia et al. 2011; Thul et al. 2012; Animasaun et al. 2015; Sunar et al. 2016; Igwe et al. 2017). The ISSR sequence has high sensitivity and is easier to propagate by polymerase chain reaction (PCR) than RAPD (Due et al. 2019; Daryono et al. 2019). This study analyzed genetic diversity and identified superior Brongkol durian using ISSR molecular markers.

## MATERIALS AND METHODS

The research subjects comprised of 25 superior durian varieties (code B1-B36) obtained from exploration and cultivation in Brongkol Village, Jambu Sub-district, Semarang District, Central Java Province, Indonesia. DNA amplification was performed using the peqSTAR 2X Thermocycler (Table 2) at the Research Laboratory and Molecular Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, the University of Negeri Semarang from January to December 2020. The stages of this research were (i) isolation of genomic DNA following Vanijajiva (2011) modified by Solikin et al. (2017), (ii) DNA amplification was done

using 7 ISSR primers (Table 1), and (iii) visualization and analysis of amplified DNA fragments.

The ISSR PCR stages for Brongkol's superior durian consisted of pre-denaturation step at 95°C for 4 minutes, followed by a denaturation step at 95°C for 30 seconds, an annealing step of 44°C-53°C for 1 minute, and an extension at 72°C for 1 minute. A final extension terminated amplification at 72°C for 10 minutes. In addition, DNA multiplication by PCR was carried out for 35 cycles.

PCR results were migrated using electrophoresis on 2% agarose gel with a 100 bp marker at 100 volts for 45 minutes. Each band that appears on the gel is an ISSR allele. The relative size of the allele refers to the 100 bp marker. The allele for each variety is assigned a score of 1 if present and 0 if absent. Analysis of genetic diversity refers to the percentage of polymorphic alleles and the degree of similarity between superior durian varieties calculated using Similarity for Qualitative (SIMQUAL) with the DICE coefficient. Dendrogram construction was calculated using the UPGMA-SAHN NTSYSpc version 2.02 program (Rohlf 1998).

## RESULTS AND DISCUSSION

### Brongkol's superior durian polymorphism

DNA amplification of 25 superior durian varieties of Brongkol using 7 ISSR primers resulted in 36 alleles with a polymorphic allele percentage of 90.2% (Table 2).

**Table 1.** ISSR primer for analysis of superior durian diversity in Brongkol

Primers	Sequences	Anneling temperature (°C)
ISSR4	5'-GAGGAGGAGGAGGAGAC-3'	47.3
ISSR5	5'-GAGGAGGAGGAGGAGAT-3'	44.0
ISSR10	5'-GTGTGTGTGTGTGTGTGTA-3'	49.4
PKBT2	5'-ACACACACACACACTT-3'	53.0
PKBT7	5'-GAGAGAGAGAGAGAGAGAA-3'	49.0
PKBT9	5'-GAGAGAGAGAGAGAGAT-3'	50.9
PKBT12	5'-GTGTGTGTGTGTGTGTGTT-3'	44.9

**Table 2.** Polymorphism allele of 25 superior durian varieties of Brongkol using 7 ISSR primers

Primers	Relative size (bp)	Number of alleles	Number of polymorphic alleles	Number of monomorphic alleles	Percentage of polymorphic alleles (%)
ISSR4	500-1200	4	3	1	75.0
ISSR5	600-1500	3	3	0	100.0
ISSR10	500-940	4	4	0	100.0
PKBT2	340-1500	11	9	2	81.8
PKBT7	750-3000	6	6	0	100.0
PKBT9	370- 940	4	4	0	100.0
PKBT12	550-1500	4	3	1	75.0
Total		36	32	4	
Average					90.2

The highest number of alleles was found in PKBT2 primers, namely 11 alleles measuring 340-1500 bp. The resulting allele size ranged from 340-3000 bp. The size of the ISSR allele was generally between 250-2000 bp (Ng and Tan 2015). Alleles of >2000 bp sizes were only found in PKBT7 primers in two varieties. Alleles of >2000 bp sizes were also found in the study by Sembiring et al. (2015). Two possible causes for the appearance of this allele are because of the contaminated DNA (Patramurti et al. 2014), and the concentration of MgCl<sub>2</sub> was too high when the PCR was conducted (Pharmawati 2009), or the accessions studied had new unique alleles. Each primer produced several alleles of varying sizes. This variation is due to differences in the nucleotide-based sequence of the primers and the interactions between the primers and DNA. The number of accessions studied can affect the number of alleles because the more accessions studied, the greater the chance for new alleles to emerge (Retnoningsih et al. 2011; 2016). The difference in the number of alleles found presumably happened due to differences in geographic areas that affect the genetic diversity of durians. Allele polymorphism in this study was higher than Thailand durian Vanijajiva (2012), but lower than Kundur Island durian (Angeliena et al. 2019). The higher number of polymorphic alleles in Brongkol durian indicates that the durian in Nonthaburi Province has more limited genetic diversity than the superior durian in Brongkol. The ISSR4 and ISSR5 primers produced almost the same number of alleles as the Vanijajiva analysis (2012), the same as PKBT7, whose results were similar to Angeliena et al. (2019).

ISSR markers have been shown to reveal and produce high polymorphic alleles (Siew et al. 2018; Due et al. 2019). The high polymorphic allele in durian is due to the open and random cross-pollination mechanism (Handayani 2016). Biological factors of pollination affect the genetic diversity of durian plants. Pollination by different tree pollen will increase the variety in the offspring. Durian flowers are hermaphrodite, which allows self-pollination. One of the barriers that prevent self-pollination is self-incompatibility (Bumrungsri et al. 2009). Several studies showed that *Durio's* pollination is assisted by a group of animals such as birds, bats, and insects (Yumoto 2000; Sheema et al. 2017; Wayo et al. 2018; Chaiyarat et al. 2019). Another factor that influences genetic variation is mutation. Mutations in repeated sequences tend to be higher (Udupa and Baum 2001). Mutations that result in the exchange of DNA base chains will cause changes in phenotypic or genotypic traits (Chahal and Gosal 2006). The environment also influences morphological changes. Different environments can produce various morphological features (Wang et al. 2012). Brongkol superior durian has high genetic diversity because each tree generally comes from seed germination, so that it can be ascertained that it has different genotypes. The genotype of the vegetatively

propagated seedlings is relatively the same as the parent because there is no gene flow.

### The molecular identity of Brongkol superior durian

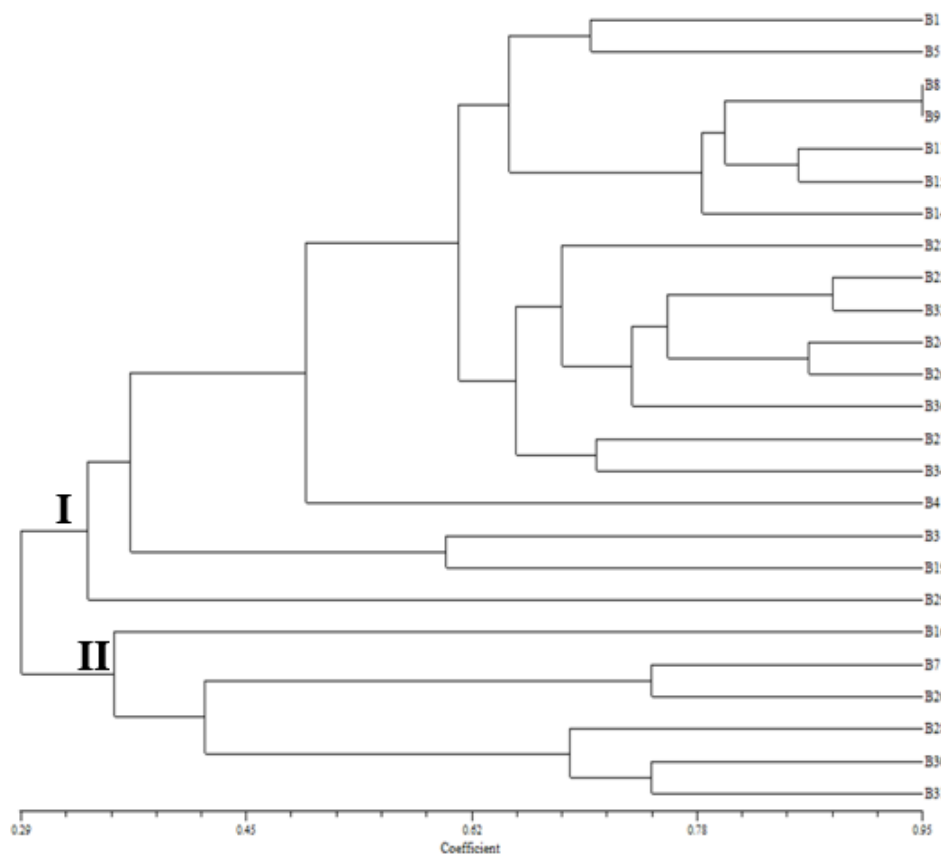
The amplification of Brongkol's superior durian DNA using ISSR primers produced several specific alleles (Table 3). The presence of specific alleles can determine plant identity. Specific alleles, if present in certain genes, allow a unique phenotype of a plant. B5 variety has the most specific alleles. Specific alleles for the B5 variety were found in ISSR4 primers measuring 1200 bp, PKBT2 primers measuring 1200 bp, and primers PKBT12 at a size of 1500 bp. Another variety that has a specific allele is B16. The B16 variety is locally known as *durian Aziz*. Specific alleles for the B16 variety were only found in PKBT2 primers measuring 1500 bp. The specific allele found in the B5 variety is not known for its unique morphological characteristics, yet it shows arilus with orange yellow color based on information from the farm owner. Specific alleles in PKBT2 primers were also found by Solikin et al. (2017), namely in *Petruk durian* on an allele measuring 450 bp, and Angeliena et al. (2019) on an allele of 410 bp. Other specific alleles were found in PKBT12 primers on HM and GT of 1500 bp size (Angeliena et al. 2019). Specific alleles are important as accurate and stable markers useful for the commercial development of durian varieties (Riupassa et al. 2015). Another use of particular alleles is in developing Sequence Characterized Amplified Region (SCAR) markers for molecular identification at the variety level (Cui et al. 2016).

### Similarity analysis of superior Brongkol durian

The UPGMA-based dendrogram shows that 25 superior durian varieties of Brongkol have a similarity coefficient of 0.29 to 0.95 and produced two main clusters (Figure 1). Cluster I consisted of 19 varieties (B1-B29) (76%), and Cluster II consisted of 6 varieties (B16-B31) (34%). Cluster I was grouped into two sub-clusters in 18 varieties (B1, B5, B8, B9, B11, B15, B14, B22, B23, B32, B24, B26, B36, B21, B34, B4, B3, and B19) and 1 Durian belonged to variety B29. Cluster II was classified into two sub-clusters: Durian B16 and five other varieties (B7, B20, B28, B30, and B31).

**Table 3.** Specific alleles in some Brongkol superior durian varieties

Variety	Relative size (bp)		
	ISSR4	PKBT2	PKBT12
B5	1200		
B16		1500	
B5		1200	
B5			1500



**Figure 1.** Dendrogram 25 superior varieties of Brongkol durian

The highest similarity coefficient (0.95) was found between varieties B8 and B9. The similarity coefficient and dendrogram show that the higher the similarity coefficient, the lower the genetic distance. Varieties in one group show high genetic similarity (Kristantini et al. 2014). A similarity value of more than 0.70 indicates high similarity (Singh 1999), and if it is less than 0.60, it is classified as low (Trimanto 2012). The profile of durians from the same cluster tends to be similar (Yulita and Murnianjari 2010). Accessibility affects the level of genetic diversity (Alcantara et al. 2017). Some of the varieties obtained from the same area are not in the same cluster or sub-cluster; because, according to Tacca et al. (2004) and Jose et al. (2005), genotypes originating from the same area are not always in the same group. The grouping of samples into one cluster indicates high similarity between the DNA loci (Yulita 2013). Overall, the dendrogram showed no durian varieties had 100% similarity value. All Brongkol select durian varieties reviewed in this research had different genetic profiles from one another. This dendrogram strengthens the fact that the durian trees in the Brongkol area are derived from seed/sexual propagation. Brongkol durians are mostly superior, so that they have the potential to become new varieties if a distinctive marker is found; the samples studied include Brongkol's flagship durian, superior and limited in number. Other varieties generally do not have unique taste and color like the Brongkol's durian.

In conclusion, the genetic diversity of 25 superior durian varieties of Brongkol revealed by using 7 ISSR primers was high, with a percentage of polymorphic alleles of 90.2%. All the durian samples analyzed showed different varieties because there were not 100% similar to the varieties. This study also found specific alleles in B5 variety in ISSR4 primers measuring 1200 bp, PKBT2 primers measuring 1200 bp, PKBT12 primers at 1500 bp sizes, and particular alleles for B16 varieties on PKBT2 primers measuring 1500 bp.

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