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## The Molecular Diversity-based ISSR of *Durio tanjungpurensis* Originating from West Kalimantan, Indonesia

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### Cover Page Footnote

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## The Molecular Diversity-based ISSR of *Durio tanjungpurensis* Originating from West Kalimantan, Indonesia

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### Abstract

The Durian Tengkurak (*Durio tanjungpurensis* Navia) is one of the endangered exotic species in the Malvaceae family. The species is important for conservation of the germplasm and is considered a potential genetic resource for the development of durian in the future. The objective of this research project was to assess the molecular diversity of *D. tanjungpurensis* in West Kalimantan, based on Inter Simple Sequence Repeat (ISSR) markers. We applied ten ISSR primers to reveal the genetic diversity of 60 individuals from six natural endemic *D. tanjungpurensis* populations. The genetic diversity parameters were estimated based on binary data about PCR products (present or absent bands). The results showed that the mean number of observed alleles, the mean number of effective alleles, the genetic diversity, the Shannon's Information Index score, the number of polymorphic loci, and the percentage of polymorphic loci were 1.53, 1.29, 0.17, 0.26, 77.83, and 52.59, respectively. An analysis of molecular variance (AMOVA) showed that the genetic diversity within a population (65%) was higher than that found between the populations (35%). UPGMA clustering and principal coordinate analysis, based on the DICE similarity matrix, were used to classify the populations into three groups: 1) Hutan Rejunak and Tembaga, 2) Bukit Merindang, and 3) Hutan Rawak, Bukit Sagu 1, and Bukit Sagu 2. Further analysis of the population structure using STRUCTURE software was used to classify all the individuals into two major categories, thus uniting Groups 2 and 3 as one major category. In conclusion, a high level of genetic diversity in the Durian Tengkurak was revealed utilizing the ISSR markers employed in the study.

### Abstrak

**Diversitas Molekuler Berbasis ISSR pada *Durio tanjungpurensis* Asal Kalimantan Barat, Indonesia.** Durian Tengkurak (*Durio tanjungpurensis* Navia) adalah salah satu spesies langka yang eksotis dari suku Malvaceae. Durian tersebut bernilai penting untuk konservasi plasma nutfah dan berpotensi sebagai sumber daya genetik untuk pengembangan durian di masa depan. Tujuan penelitian adalah mengetahui keragaman molekuler *D. tanjungpurensis* asal Kalimantan Barat berdasarkan penanda Inter Simple Sequence Repeat (ISSR). Sepuluh primer ISSR digunakan untuk mengetahui keragaman genetik 60 individu Durian Tengkurak dari enam populasi endemik alami *D. tanjungpurensis*. Parameter keragaman genetik didasarkan pada data biner pita DNA produk PCR, yaitu ada atau tidak ada pita. Hasil penelitian menunjukkan bahwa rata-rata jumlah alel, rata-rata jumlah efektif alel, diversitas genetik, indeks informasi Shannon, jumlah polimorfik lokus, dan persentase polimorfik lokus berturut-turut adalah 1,53, 1,29, 0,17, 0,26, 77,83 dan 52,59. Analisis ragam molekuler (AMOVA) menunjukkan keragaman genetik yang lebih tinggi di dalam populasi (65%) dibandingkan antar populasi (35%). Analisis gugus menggunakan metode UPGMA berdasarkan matriks keserupaan Dice dan analisis koordinat utama digunakan untuk mengelompokkan semua individu populasi ke dalam tiga kelompok, yaitu grup 1 (Hutan Rejunak dan Tembaga), grup 2 (Bukit Merindang), dan grup 3 (Hutan Rawak, Bukit Sagu 1 dan Bukit Sagu 2). Analisis lebih lanjut terhadap struktur populasi menggunakan program STRUCTURE menyatukan grup 2 dan 3 ke dalam satu grup utama. Penelitian ini berhasil mengungkap keragaman genetik Durian Tengkurak menggunakan penanda ISSR.

*Keywords: Durian Kura, Durian Tengkurak, endemic species, genetic diversity, ISSR marker*

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### Introduction

The durian (*Durio* spp.) is a fruit with a high economic value, which is grown in Indonesia and several other

countries in Southeast Asia, including Thailand and the Philippines. It is a popular fruit because of its unique taste and its wide availability. It varies in terms of its size and smell, the color of its skin, and the color of its

flesh (seed arillus), which depend on the species and cultivar. This plant was first found in the forests of Malaya (or Malaysia) by Murray, and was given the title the King of Fruit by Wallace [1]. Morphologically, the durian is a prickly fruit that grows on a tree branch and is classified as part of the Malvaceae Family.

Borneo Island is considered to be the epicenter of durian diversity. There are 21 species of durian tree found on the island [2], one of which is the Durian Tengkurak (*Durio tanjungpurensis* Navia), which is endemic to West Kalimantan.

The Durian Tengkurak is relatively unknown, compared with the common durian, *Durio zibethinus* Murray. Interestingly, bunches of Durian Tengkurak fruit are found at the base of the tree's trunk, whereas common durian fruit is found hanging from a tree branch, as previously stated. Phenotypically, the fruit of Durian Tengkurak is slightly different from that of the common Durian Kura (*D. testudinarum* Becc.), being smaller (5-8 cm in diameter), with a brown rind, and with the white aril of the seeds being less than 1 mm thick [3].

The high rate of deforestation on Borneo Island and the conversion of much land to monocultural practices have caused the indigenous durian to become endangered. Therefore, conservation efforts in relation to the indigenous durian germplasm, including the Durian Tengkurak, must be encouraged. One of the efforts being made by researchers is in the identification of the diversity within the durian germplasm. The development of genetic tools for plants is needed in order for the genetic profile of the durian to be revealed. One current set of applications available for such a procedure are ISSR molecular markers.

ISSR markers are multilocus markers that are based on the amplification of DNA fragments, which are flanked by types of repetitive nucleotide regions (repeat sequences) with inverted orientations and are scattered throughout genome chromosomes. ISSR markers are dominant markers and have several advantages, when compared with other dominant markers, such as RAPD markers [4]. In studies, ISSRs have revealed high discrimination ratios, high genetic variability levels [5],

and high levels of polymorphism [6]. In certain circumstances, they can act as co-dominant markers that are able to identify individuals with heterozygote alleles [7-8]. Within the PCR process, oligonucleotide primers can be determined randomly based on di-, tri-, or tetra-nucleotide repeats, which, at the end of the 3' or 5', could be added with 1-3 bases. ISSR-marker analysis has been performed on many plant species, with various objectives, such as the collection and conservation of the African citrus [6], the conservation of japonica tea in China and Japan [9], the characterization of the sweet potato Brazilian germplasm [5], the sex determination of green potato plants from India [10], determination of the homogeneity of in-vitro clones in grapes [11], and the study of the evolution and speciation of the Asteraceae [12]. ISSR markers have also been used to assess the genetic diversity of *D. zibethinus* cultivars in Thailand [13], but they have not been utilized previously to reveal the genetic diversity of the Durian Tengkurak of West Kalimantan.

The objective of this research project was to analyze the genetic diversity of the Durian Tengkurak of West Kalimantan using ISSR markers. It is hoped that in future, the genetic diversity profile for the Durian Tengkurak could be used for the purpose of conserving Durian Tengkurak germplasms.

## Materials and Methods

**Plant materials.** A total of 60 samples of Durian Tengkurak leaves from West Kalimantan, spread over six locations (Table 1 and Figure 1) were examined in this research. Durian Tengkurak trees grow naturally in the indigenous forest and hill communities on the slope lands.

**DNA isolation.** DNA was isolated using the CTAB method, with some modifications [14]. Fresh leaves were weighed into sample amounts of 0.2 g and then cut up and crushed to a powder with liquid nitrogen using a mortar, before being transferred into 2 mL eppendorf tubes. 1 mL of CTAB lysis buffer was added, followed by 2 µL of 2-mercaptoethanol (Sigma Chemical Co., USA). The sample tubes were incubated in a water bath

**Table 1. Locations and Numbers of Samples of Durian Tengkurak Leaves, West Kalimantan**

Locations	Coordinates	Altitude (m asl)	Code	Samples
Hutan Rejunak, Rawak District, Sekadau Regency	0°09'16.0"S – 110°51'13.5"E	125	HRJ (H)	11
Hutan Rawak, Rawak District, Sekadau Regency	0°09'15.8"S – 110°51'13.9"E	120	HRW (R)	11
Tembaga, Nanga Mahap District, Sekadau Regency	0°30'37.4"S – 110°40'48.9"E	93	TBG (T)	10
Bukit Merindang, Nanga Taman District, Sekadau Regency	0°18'30.5"S – 110°55'49.3"E	105	MER (E)	11
Bukit Sagu 1, Nanga Silat Hilir District, Kapuas Hulu Regency	0°16'09.2"N – 111°56'11.8"E	59	BS1 (B)	8
Bukit Sagu 2, Nanga Silat Hilir District, Kapuas Hulu Regency	0°16'09.9"N – 111°56'12.1"E	74	BS2 (S)	9
Σ samples				60

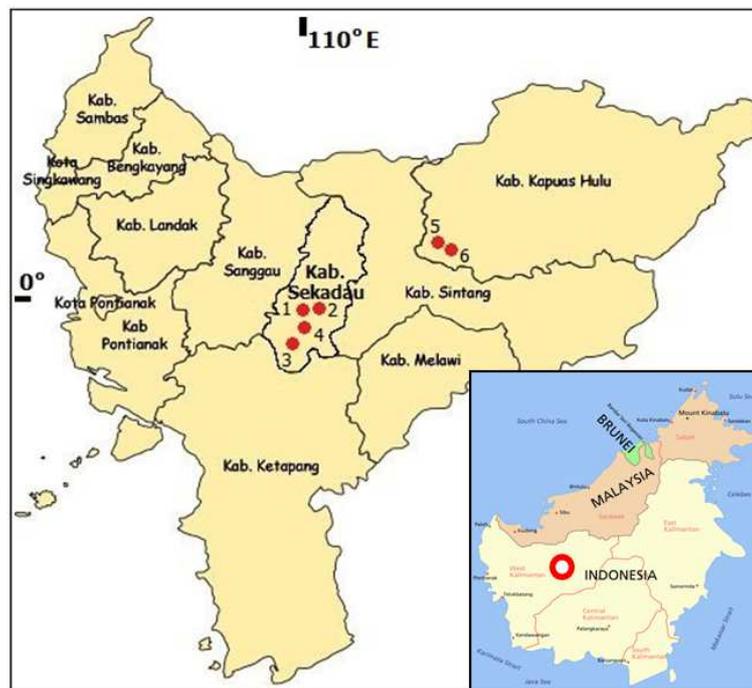


Figure 1. Distribution Map of Durian Tengkurak Populations in the West Kalimantan Province of Borneo, Indonesia (Red Circles)

Table 1. List of ISSR Primers, Primer Lengths, and Sequences, as well as the PCR annealing Temperatures used in the Study

ISSR primer	Primer sequence (5'-3')	Primer length	Annealing temperature (°C)	References
ISSR1	(AGG) <sub>5</sub>	15	49.5	[13]
ISSR3	(AGA) <sub>4</sub> AGT	15	42.5	“
ISSR4	(GAG) <sub>5</sub> AC	17	50.0	“
ISSR5	(GAG) <sub>5</sub> AT	17	51.6	“
ISSR9	(GGGGT) <sub>3</sub>	15	53.0	“
PKBT2	(AC) <sub>8</sub> TT	18	52.0	[15]
PKBT3	(AG) <sub>8</sub> T	17	47.5	“
PKBT7	(GA) <sub>9</sub> A	19	50.7	“
PKBT8	(GA) <sub>9</sub> C	19	52.8	“
PKBT12	(GT) <sub>9</sub> T	19	54.0	“

(temperature: 65 °C) for 60 min, with the tubes being inverted every 15 min. Next, the tubes was centrifuged (Microcentrifuge 16D Galaxy VWR™, IL) at 9,900 g for 10 min. The supernatants were transferred into new eppendorf tubes, to which chloroform:isoamyl alcohol (24:1) was added as the volume of the supernatant, before they were centrifuged again. The supernatants were then transferred again into new eppendorf tubes, to which cold isopropanol (as much as 0.8 of the volume of the supernatant) and sodium acetate (0.1 of the volume of the supernatant) were added, before the samples were incubated in the freezer overnight. The suspensions were then centrifuged at 16,000 x g to obtain pellets of DNA. The DNA pellets were washed with cold 500 µL 70% ethanol, then centrifuged and dried. The DNA pellets were diluted using 200 µL aquabidest distilled water as a DNA suspension. The DNA was

purified from RNA contamination using RNase A (Thermo Scientific, USA).

**DNA amplification.** The composition of the PCR reaction for a total volume of 25 µL was 12.5 µL GoTaq® Green Master Mix (Promega, USA, catalog number M7122), 0.15 µL BSA [20 mg/mL], 0.15 µL MgCl<sub>2</sub> 20 mM, 1 µL primer [10 pmol/µL], 3 µL DNA template [5 ng/µL], and 8.2 µL ddH<sub>2</sub>O nuclease-free. A total of 10 ISSR primers, originating from references, were used for the amplification of the DNA (Table 2). DNA amplification were undertaken in a T-Gradient thermocycler PCR machine (Biometra, Göttingen, Germany) with a cycle time and temperature program as follows: 5 min at 95 °C for the initial denaturation, followed by 35 cycles of 1 min at 94 °C, with a primer annealing temperature range of 42.5-54 °C (depending

on the primer –see Table 2) for 1 min, and 72 °C for 1 min, after which there was a final extension cycle of 10 min at 72 °C. Three microliters of each PCR product were electrophoresed on 0.85% (w/v) agarose gel (TopVision™ Agarose, Fermentas, USA) in 1x TBE buffer at 75 volts for 110 min. The PCR products were visualized using a UV transilluminator and documented using a digital CCD camera (Daihan WiseDoc® Portable WGD-20 Gel Documentation System, Korea).

**Data analysis.** ISSR markers were scored as having present (1) or absent (0) DNA bands in order to generate binary data. POPGENE v. 1.31 [16] was used to analyze the following population genetic parameters: the number of observed alleles ( $N_a$ ), the effective number of alleles ( $N_e$ ), the genetic diversity ( $h$ ) (or polymorphic information content; PIC), the Shannon's information index ( $I$ ), the number of polymorphic loci, the percentage of polymorphic loci, and the gene flow (total heterozygosity, average genetic diversity within populations, genetic divergence between populations, and gene flow [ $N_m$ ]). Genetic differentiation among populations was calculated using  $F_{ST} = 1/(4N_m+1)$  [17]. GenAlEx 6.5 software [18] was used to analyze the molecular variance (AMOVA) [19]. The percentage of polymorphic loci was determined by calculating the ratio of the difference between the number of bands and monomorphic bands divided by the total number of bands. The genetic diversity was calculated using POPGENE and was equivalent to polymorphic information content (PIC) for dominant markers specifically [20-21]. AMOVA was used to estimate the percentage of the genetic diversity within and among populations.

The genetic similarities between the Durian Tengkurak samples, based on ISSR marker binary data, were calculated using Numerical Taxonomy Systems (NTSYSpc 2.1) [22]. The similarity matrix for the individuals was generated using the SimQual option with the DICE coefficient. Two further analyses that

utilized the matrix output were used for 1) Principal Coordinate Analysis (PCoA, nonmetric multidimensional scaling analysis), which was achieved via the MD Scale and Eigen procedures, and 2) clustering analysis, achieved via the SAHN procedure, in which an unweighted pair-group method using arithmetic averages (UPGMA) was employed to generate a dendrogram. Bootstrap analyses, which involved 1000 replicates, were carried out using Treecon for Windows v.1.3b [23] to assess the robustness of the findings, with a minimum threshold of 40%.

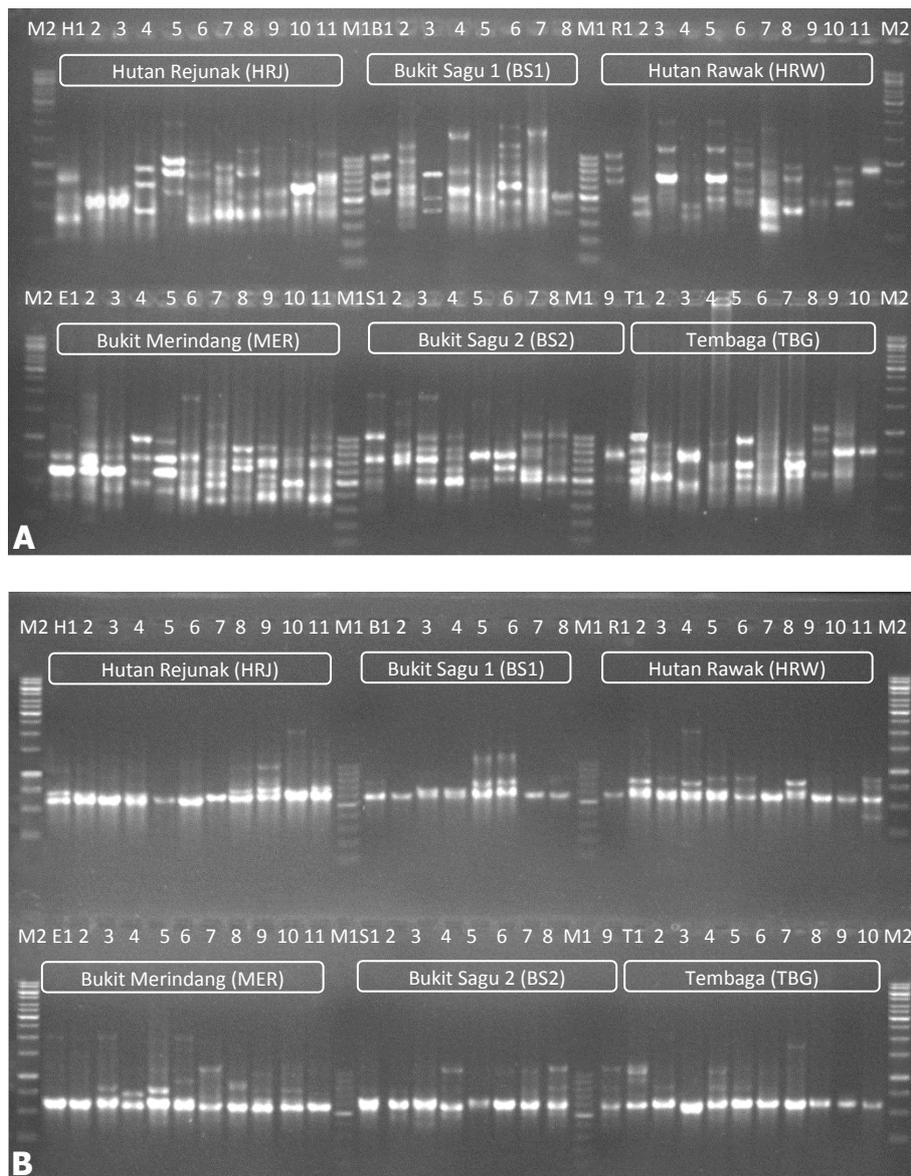
Last of all, in order to examine the population structure and/or ancestry of individuals, the admixture model was applied, specifying no *a priori* models in the subpopulation structure. All 60 individuals with adequate genotype information from all six populations were analyzed using STRUCTURE v.2.3.4 [24]. To determine correlated allele frequencies and to estimate  $K$  (the most likely number of putative populations), we performed 14 runs for the  $K$  value from 1 to 6, with 200,000 MCMC repetitions. In each case, we allowed a burn-in period of 50,000 for  $K$ . The mean and variance were plotted in "likelihood per  $K$ " using STRUCTURE HARVESTER v.0.6.94 [25] and the Evanno method [26].

## Results and Discussion

**ISSR marker profiles and polymorphic loci.** During the experiment investigating the genetic diversity of 60 Durian Tengkurak plants (*D.anjungpurensis* Navia), the DNA of all the plants was amplified successfully using ten ISSR primers. Each primer produced different sizes (ranging from 200 to 2000 bp) and numbers of bands (11 to 23). The total number of bands detected was 148, with the average number of bands per primer being 14.8. All the bands produced from each primer were polymorphic bands (Table 3). Figure 2 gives examples of the two ISSR profiles achieved using the PKBT3 and ISSR4 primers.

**Table 3. Sizes of the PCR Product, Total Numbers of Bands Scored, and Numbers of Polymorphic Bands Amplified from 60 Durian Tengkurak Plants Using Ten ISSR Primers**

ISSR primer	Size range (bp)	Total number of bands scored	Number of polymorphic bands
ISSR1	250-1200	23	23
ISSR3	250-1200	11	11
ISSR4	400-2000	15	15
ISSR5	300-1500	15	15
ISSR9	400-1500	14	14
PKBT2	300-2000	14	14
PKBT3	250-1500	14	14
PKBT7	250-1400	12	12
PKBT8	200-1800	16	16
PKBT12	350-2000	14	14
Total	–	148 ( $\bar{x}=14.8$ )	148 (100%)



**Figure 2. Electropherogram of PCR Products obtained from Six Durian Tengkurak Populations Using the PKBT2 and ISSR4 Primers (A: High and B: Low Polymorphic Bands, Respectively). Marker Ladder: 100 bp (M1) (Thermo Scientific, cat. #SM0241) and 1 kb (M2) (Thermo Scientific, cat. #SM0311). The Letters followed by the Numbers Written in the Boxes Indicate the Individual Samples Represented in the Population**

The distribution of the polymorphic loci produced using each primer among the six populations varied from one primer to another (Figure 3). On average, the ISSR4 primer produced the lowest percentage of polymorphic loci among all the populations, and the PKBT2 primer produced the highest percentage of polymorphic loci.

**Genetic diversity analysis.** The genetic diversity parameters for the Durian Tengkurak plants were found to be varied among the six different populations (Table 4). The polymorphic percentage loci varied from 41.89% to 62.16%, with the lowest and highest polymorphic percentages were found in the Hutan Rejunak and Bukit

Merindang populations. The mean number of observed alleles ranged from 1.42 to 1.62, the effective number of alleles varied from 1.19 to 1.34 the Shannon's information index score [27] ranged from 0.20 to 0.31, and the genetic diversity score varied from 0.12 to 0.21. It was clear that the genetic diversity parameters for Bukit Sagu were always the smallest, when compared with those of the other populations. This indicates that the Bukit Sagu population had the lowest genetic diversity among the populations of the Durian Tengkurak plant.

To examine the hierarchical genetic differentiation, we analyzed molecular variation using GenAlEx to explain

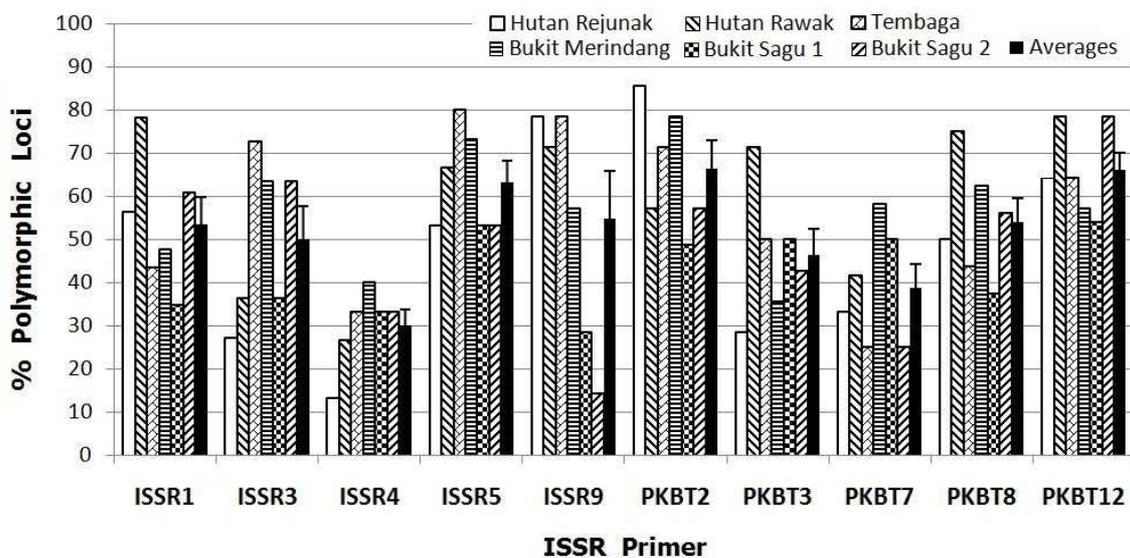


Figure 3. Distribution of Percentages of Polymorphic Loci in the Durian Tengkurak Populations, based on the PCR Products Obtained using ISSR Primers. The Average Percentage was Highest Using the PKBT2 Primer and Lowest Using the ISSR4 Primer (Averages Bars Shaded Black)

Table 4. Genetic Diversity Parameters and Polymorphic Loci among Six Durian Tengkurak Populations in West Kalimantan, as Revealed Using ISSR Markers

Populations	Na	Ne	h	I	Number polymorphic loci	% Polymorphic loci
Hutan Rejunak	1.62	1.34	0.21	0.31	92	62.16
Hutan Rawak	1.50	1.25	0.15	0.24	74	50.00
Tembaga	1.55	1.32	0.19	0.28	82	55.41
Bukit Merindang	1.57	1.33	0.20	0.29	84	56.76
Bukit Sagu 1	1.42	1.19	0.12	0.20	62	41.89
Bukit Sagu 2	1.49	1.28	0.17	0.26	73	49.32
Averages	1.53	1.29	0.17	0.26	77.83	52.59

Na = Number of observed alleles; Ne = Number of effective alleles; h = Nei's genetic diversity; I = Shannon's information index.

the genetic variation both within populations and between populations. In the case of dominant markers such as ISSR primers, the AMOVA of dominant data were treated as haploid data (Figure 4). The percentage of molecular variance within the populations varied from 37% to 86%, with an average of 65%, while the molecular variance between populations was spread from 14% to 63%, with average of up to 35%. Statistically speaking, the AMOVA values differ significantly, based on the values obtained from the entire set of data permutations (PhiPT value of 0.349, with a probability less than 0.001 – see Table 5). The AMOVA results showed that the genetic diversity within the populations is higher than that between populations. Gene flow occurred between the populations with the lowest values, with an Nm of less than 1 (Table 6).

**Genetic relationship analysis.** Cluster analyses were performed based on 148 loci, and all the samples

grouped into three clusters (Figure 5). Two populations (Hutan Rejunak and Tembaga) were in the first cluster, one population (Bukit Merindang) was in the second cluster, and three populations (Hutan Rawak, Bukit Sagu 1 and Bukit Sagu 2) were in the third cluster. The cluster analysis was also supported by PCoA analysis (Figure 6). The Hutan Rejunak population was found to be relatively similar to the Tembaga population. Although the population Bukit Sagu 2 differed the most from the others, some individuals in the Hutan Rawak population were found to be similar to Bukit Sagu 1, and all the individuals in those three populations were clustered in the same quadrant of the PCoA. Individuals from the Hutan Rawak, Bukit Sagu 1, and Bukit Sagu 2 populations showed less genetic diversity than those in the other three populations. Following further analysis using STRUCTURE software, all the individuals were grouped into two major categories. Here, the second and the third clusters were classified into one category (Figure 7).

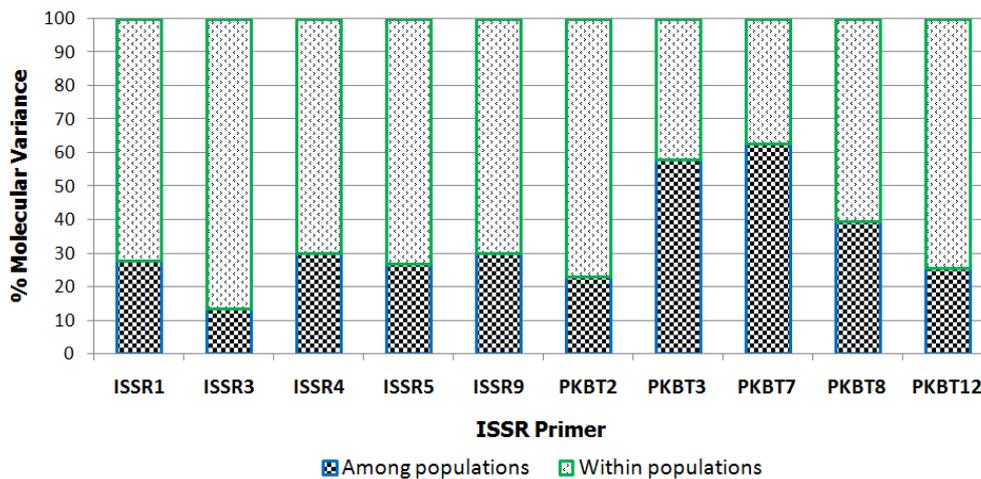


Figure 4. Percentage of Molecular Variance for Durian Tengkurak Plants, Established Using AMOVA

Table 5. Analysis of Molecular Variance (AMOVA) of Six Durian Tengkurak Populations in West Kalimantan, as Revealed Using ISSR Markers

Source of variation	df	SS	CV	TV (%)	PhiPT	P value
Between Populations	5	455.441	7.692	34.87%	0.349	<0.001
Within Populations	54	776.009	14.371	65.13%		
Total	59	1231.450	22.063			

df = degree of freedom; SS = sum of squares; CV = coefficient of variation; TV = total variation; P = probability of a variance component greater than the values observed by chance.

Table 6. Analysis of Genetic Population Structures of Durian Tengkurak Plants in West Kalimantan

Value	H <sub>T</sub>	H <sub>S</sub>	G <sub>ST</sub>	N <sub>m</sub>	F <sub>ST</sub>
Mean	0.2759	0.1725	0.3750	0.8333	0.2308
Standard deviation	0.0238	0.0133			

H<sub>T</sub> = total heterozygosity; H<sub>S</sub> = average genetic diversity within populations; G<sub>ST</sub> = genetic divergence between populations; N<sub>m</sub> = gene flow.  $N_m = 0.5 (1 - G_{ST}) / G_{ST}$ ; F<sub>ST</sub> = genetic differentiation among populations,  $F_{ST} \sim 1 / (4N_m + 1)$ .

ISSR markers are used in this study as dominant markers, which are characterized by presence or absence in loci. Consequently, the maximum number of alleles per locus is two, and the averages indicate the abundance of alleles among individuals in a population. The allele frequency of a dominant marker is considered a genotypic characteristic. Since an ISSR marker is a dominant marker, every polymorphic band is considered to be one locus; using 10 ISSR primers, we therefore detected 148 loci across all the observed individuals.

In terms of polymorphic bands, primer ISSR1 produced the highest number, compared with other primers. All the primers revealed polymorphic bands in all the individuals, suggesting that all ten primers indicate that there is diversity in Durian Tengkurak populations. This finding was supported by the percentage polymorphic loci, which showed that each primer produced varied percentages of polymorphic loci across the populations (Figure 3). The PKBT2 and ISSR4 primers produced

the most and least polymorphic loci among the populations, respectively. All ten primers analyzed all 148 fragments produced were 100% polymorphic, indicating considerable genetic variation among the Durian Tengkurak plants studied.

The diversity found across the 60 Durian Tengkurak individuals was relatively high, compared with a previous study of a different species of durian (the common durian), for which the average polymorphic percentage was 38% [13]. We found that the averages for genetic diversity, the Shannon’s information index, and the polymorphic percentage loci were all relatively high, at 0.17, 0.26, and 52.59%, respectively.

The genetic diversity of Durian Tengkurak plants from the Sekadau Regency was higher than that found in those from the Kapuas Hulu Regency, indicating that the Sekadau Regency has more potential as a Durian Tengkurak germplasm resource. The findings show that

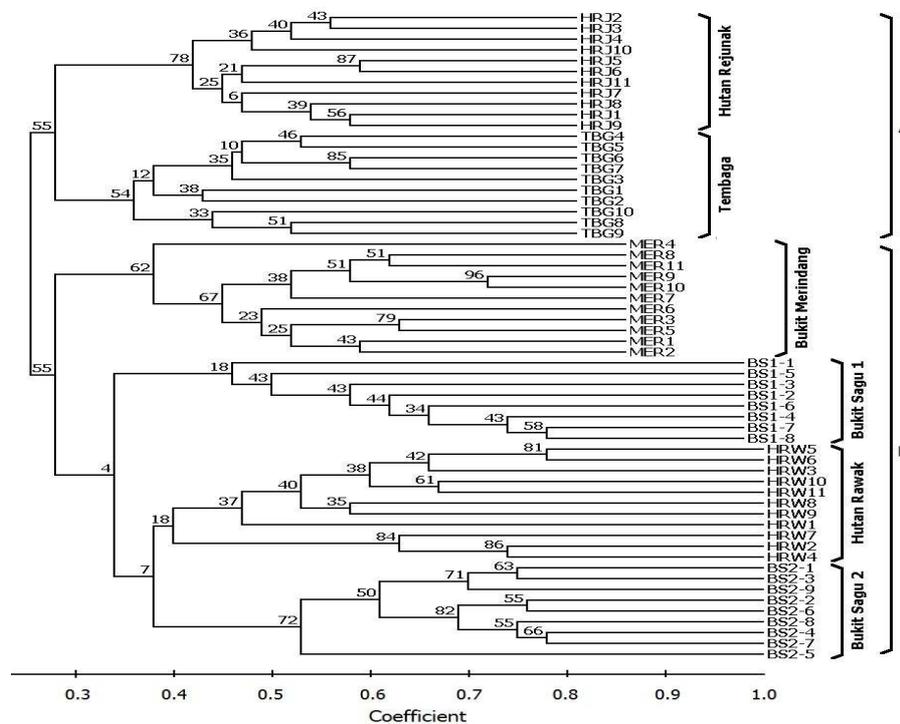
there are general spatial and temporal variations in Durian Tengkurak populations.

Analysis of molecular variance revealed that the genetic diversity within populations was higher than that between populations (Figure 4). This result was similar to previous findings in cross-pollinated plants of the rye species, which were analyzed using AFLP markers [28], implying that Durian Tengkurak plants tend to be cross-pollinated. This result was supported by the PCoA analysis (Figure 6), which showed that some individuals originating from the same regency (Sekadau Regency) had become distributed across different areas (in the Kapuas Hulu Regency, in this example).

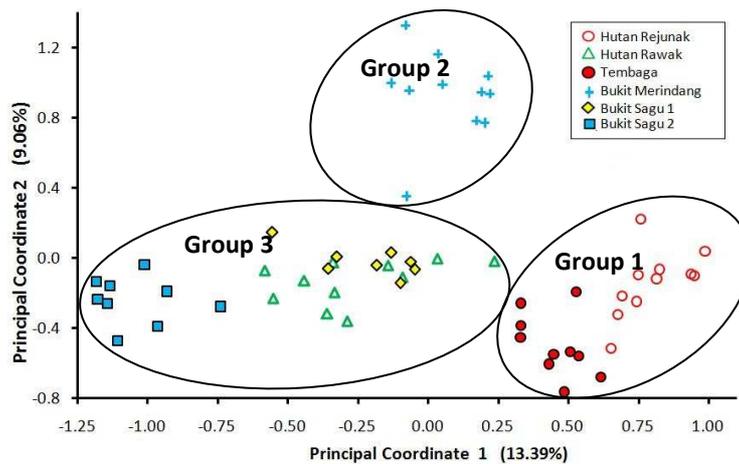
The cluster analysis grouped all individuals from one population together, indicating that individuals within populations were more similar to each other with specific loci than to plants from other populations. The cluster analysis undertaken using the UPGMA method, however, showed clear grouping and differentiation within populations. The PCoA analysis, in which the distance similarity matrix was employed, demonstrated that several individuals from Bukit Sagu 1 were mixed in with individuals from the Hutan Rawak population, which suggested that both populations might actually be part of the same group. Therefore, the genetic diversity

within populations was found to be greater than that between populations.

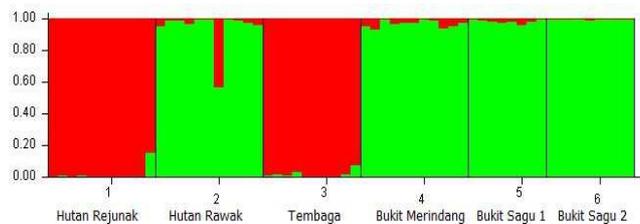
Although clustering using the UPGMA method revealed clear groups that were linked to the original sources of the populations, STRUCTURE analysis categorized the Durian Tengkurak plants into just two major groups (Figure 7). Here, the individuals from the Hutan Rejunak and Tembaga populations were grouped together in one category, and the individuals from the Hutan Rawak, Bukit Merindang, Bukit Sagu 1, and Bukit Sagu 2 populations were grouped together in the other category. Interestingly, the Hutan Rawak and Bukit Merindang populations are located far away, geographically speaking, from the Bukit Sagu 1 and 2 populations (Figure 1) but, nonetheless, they were all classified into the same category, suggesting that there is gene flow between these populations. Since the genetic diversity of Durian Tengkurak plants in West Kalimantan has been found to be relatively high and gene flow has been shown to have occurred at low levels among populations ( $N_m < 1$ ), it can be suggested that the Durian Tengkurak germplasm in West Kalimantan is still being maintained properly by the local people which the plants grow in their forest and hill. We propose that this species might be conserved *in situ* in the Sekadau and Kapuas Hulu Regencies in order



**Figure 5. Dendrogram of 60 Durian Tengkurak Individuals of from West Kalimantan, Analyzed Using the SAHN Procedure with UPGMA Methods and DICE's Similarity Coefficients as an Input Factor. Three Separate Groups were Identified Using Clustering: 1) Hutan Rejunak and Tembaga, 2) Bukit Merindang, and 3) Bukit Sagu 1, Hutan Rawak, and Bukit Sagu 2. The Major Categories A and B were Achieved via STRUCTURE analysis (Figure 7). The Letter Codes for the Populations are Listed in Table 1. The Bootstrap Values Greater than 40% from 1000 Pseudo-Replicates (Obtained Using Treecon Software) are Shown above the Branches**



**Figure 6. Matrix Plot for Principal Coordinate Analysis based on ISSR Markers, showing the Three Main Groups. The Genetic Characteristics of Individuals in the Hutan Rejunak Population tend to be Similar to those in the Tembaga Population; thus, the Two Populations Form One Group. The Bukit Sagu 1, Bukit Sagu 2, and Hutan Rawak Populations Form a Second Group, while the Bukit Merindang Population Forms the Third Group**



**Figure 7. Structure Analysis was used to Split the Population into Two Categories (Shown as Red and Green) in Terms of Genetically Distinct Ancestry. The Similarities between the Hutan Rejunak and Tembaga Populations Mean that They Form One Category, while the Hutan Rawak, Bukit Merindang, Bukit Sagu 1, and Bukit Sagu 2 belong to the Other Category**

to provide Durian Tengkurak germplasms for durian development purposes. Another finding of this research is that the ISSR markers can be used to reveal the genetic diversity of Durian Tengkurak plants, which suggests that similar studies could be carried out successfully using other members of the Malvaceae family.

The high level of genetic diversity in the Durian Tengkurak was revealed utilizing the ISSR markers employed in the study.

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