

Short Communication: Assessment of genetic diversity in Lai (*Durio kutejensis*) local cultivars of Batuah (Indonesia) using ISSR marker

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Abstract. Handayani F, Rahayu SP. 2017. Assessment of genetic diversity in Lai (*Durio kutejensis*) local cultivars of Batuah (Indonesia) using ISSR marker. *Biodiversitas* 18: 525-529. Lai is an indigenous germplasm of Kalimantan which has potency to be developed as superior tropical fruit. Lai has attractive flesh color from yellow to reddish orange which represents the high content of carotene with smooth aroma or odorless fruit, and fruit storage period which longer than durian. Unfortunately, nowadays there is limited scientific information about its genetic diversity as important basic information for genetic conservation and breeding program. Batuah village in Kutai Kartanegara District is one of the center of origin and center of diversity of Lai in East Kalimantan, Indonesia which has many local cultivars with superior traits. The aim of this research was to study the genetic diversity of eight Lai local cultivars of Batuah village using molecular marker of ISSR. The total genomic DNA of eight Lai cultivars was isolated and was then used as template in the PCR amplification reaction using 10 ISSR primers. The result shows that similarity coefficient of eight Lai cultivars ranged between 0,34 and 0,58. Seven cultivars were grouped in the same cluster with 0,44 of similarity coefficient, while Lai Durian was separated in 0,34 of similarity coefficient. Genetically, the most similar existing cultivars are Lai Kuning and Lai Belimbing with the similarity coefficient of 0,58.

Keywords: Genetic diversity, ISSR, Lai.

Abbreviations: CTAB: Cetyl Trimethyl Ammonium Bromide, ISSR: Inter Simple Sequence Repeat, NTSYS: Numerical Taxonomy and Multivariate Analysis System, PCR: Polymerase Chain Reaction, SAHN: Sequential, Agglomerative, Hierarchical and Nested, SCAR: Sequence Characterized Amplified Region, SIMQUAL: Similarity for Qualitative Data, UPGMA: Unweight Pair-group Method Arithmetic Average

INTRODUCTION

Kalimantan island is the center of origin or center of diversity of *Durio* family. Up to now, 22 *Durio* species have been found on this island (Priyanti et al. 2016), including Lai (*Durio kutejensis* (Hassk.) Becc.). Lai is an indigenous *durio* germplasm of Kalimantan which has unique characters. The fruit of Lai is odorless or has a smooth aroma, covered by blunt spines, and has attractive flesh color from yellow to reddish orange (Antarlina 2009; Santoso 2010) that represents the high content of carotene and vitamin A (Antarlina 2009). Lai has longer fruit storage period (Antarlina 2009; Santoso 2010) compared to its close relative durian (*Durio zibethinus*) (Hariyati et al. 2013) within which it becomes one of the prospective fruits for export purposes.

Batuah Village (Loa Janan Sub-district, Kutai Kartanegara District), located in Tahura Bukit Soeharto area, is one of the center of origin or center of diversity of Lai in East Kalimantan Province, Indonesia. In Batuah village, Lai becomes one of the local MPTS (multi-purpose tree species) which was generally developed in agroforestry system. Recently, three Lai local varieties of

Batuah have been released as national superior varieties, i.e. Lai Batuah, Lai Kutai, and Lai Mahakam. Interestingly, there are still many Lai local cultivars of Batuah with superior characters that have been cultivated by farmers in the village i.e; Lai Bara, Lai Besar, Lai Gincu, Lai Merah, Lai Kuning, Lai Belimbing, Lai Apel and Lai Durian. Those cultivars were found in genetic exploration held in 2004/2005. The genetic exploration was continued by fruit contest which resulted in Lai Kutai and Lai Mahakam became the winners and then released as national superior varieties in 2007 and 2009. Genetic variability of Lai population in Batuah village was categorized as high genetic diversity (Handayani 2016). Genetic variability has an important role in genetic conservation and breeding program (Montilla-Bascon et al. 2013; Jena et al. 2014; Pereira et al. 2015). Unfortunately, there is limited scientific information about the genetic diversity of Lai in both morphology and molecular characters.

ISSR is one of the most popular DNA-based techniques that are extensively applied in the determination of species among populations (Poyraz 2016). ISSR markers provided basic genetic knowledge among durian cultivars, and the detailed group of cultivars slightly differed from RAPD

markers and the taxonomic characters (Vanijajiva 2012). Furthermore, It does not require the knowledge of the whole DNA sequence for designing DNA primers because it amplifies the specific region of two microsatellite motifs (Cui et al. 2016). ISSR have been used to investigate many plants such as sorghum (Basahi 2015), *Magnolia wufengensis* (Chen et al. 2014), genus of *Cymbopogon* (Baruah et al. 2017), *Rhododendron trifolium* (Xu et al. 2017), genus of *Lilium* (Zhao et al. 2014), *Andrographis paniculata* (Tiwari et al. 2016), and *Aethionema* (Sunar et al. 2016). Therefore, the objective of this study was to characterize and identify Lai germplasm of Batuah using ISSR markers.

MATERIALS AND METHODS

The research was conducted at Plant Breeding and Genetic Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia from May-July 2015. The genetic materials were eight Lai local cultivars of Batuah village, in Loa Janan Sub-district, Kutai Kartanegara District, East Kalimantan, Indonesia (Table 1). DNA was extracted from fresh mature leaves using modified CTAB method (Handayani et al. 2016), then amplified using 10 ISSR primers (Table 2) obtained from Vanijajiva (2012) and Syahrudin (2012). Each 10 μ L reaction volume of DNA amplification contained 5 μ L of PCR reaction mix (Promega master mix go tag green), 2.25 μ L of nuclease-free water, 0.25 μ L of ISSR primer, and 2.5 μ L of DNA template. Thermocycler (Boeco) was programmed for an initial melting step at 94°C for 4 min, followed by 45 cycles each consisting of a denaturation step for 1 min at 94°C, an annealing step for 1 min at 38,9-52,9°C, and an extension step for 1 min 30 sec at 72°C. Amplification was terminated by a final extension of 7 min at 72°C. The ISSR products were separated by agarose gel (1,5%) electrophoresis in 1x TBE buffer for 1,5 hours at 75 Volt. The result was checked by UV transilluminator light and documented by digital camera. The bands observed from photograph were manually scored as 1 for presence and 0 for absence and then compiled in a binary matrix for statistical analysis using NTSYS 2.02. Similarity index among genotypes was calculated using SIMQUAL, while the UPGMA cluster analysis was formed using SAHN method.

RESULTS AND DISCUSSION

Polymorphism of ISSR primers and specific bands amplified

The DNA amplification of eight Lai cultivars using 10 ISSR primers resulted of 92 DNA loci (335 DNA bands in total). The number of DNA loci amplified by each primer was ranging from 6 to 14 loci, and the band size was in the range between 250-2500 bp (Table 3). The maximum number of amplified DNA loci (14 loci) was obtained using ISSR 2 primer, while the minimum number was observed in ISSR 1 and ISSR 8 (6 loci). The capability of

primer to identify its homolog sequences on DNA template will influence the number of annealing site, which it further affected the number of amplified DNA loci (Rahayu and Handayani 2010).

The effectiveness of a primer is determined by the number of amplified polymorphic loci. In this study, the number of polymorphic loci successfully amplified by 10 ISSR primers was 84 loci from the total of 92 loci (91,3%). ISSR 6 and ISSR 7 primers produced 7 DNA loci with 100% polymorphic bands. Examples of the ISSR polymorphism produced by ISSR 2 and ISSR 7 were shown in Figure 1 and Figure 2.

Table 1. Eight Lai local cultivars of Batuah and their specific characters

Cultivars	Specific characters
Lai Mahakam	Thick reddish orange flesh
Lai Bara	Flesh color looks like embers
Lai Besar	Big size fruit
Lai Merah	Reddish orange flesh
Lai Kuning	Yellow flesh
Lai Belimbing	Fruit locules form bulges so the shape of the fruit looks like starfruit
Lai Apel	Fruit shape looks like apple
lai Durian	Shape and color of mature fruit look like durian

Table 2. ISSR primers used in DNA amplification

Primers	Sequences
ISSR 1	5'-AGAAGAAGAAGAAGT-3'
ISSR 2	5'-AGAGAGAGAGAGAGAGT-3'
ISSR 3	5'-GAGAGAGAGAGAGAGAT-3'
ISSR 4	5'-ACACACACACACACTT-3'
ISSR 5	5'-GTGTGTGTGTGTGTGT-3'
ISSR 6	5'-AGAGAGAGAGAGAGAGTA-3'
ISSR 7	5'-AGAGAGAGAGAGAGAGAA-3'
ISSR 8	5'-GAGAGAGAGAGAGAGAA-3'
ISSR 9	5'-GAGAGAGAGAGAGAGAC-3'
ISSR 10	5'-GTGTGTGTGTGTGTGTA-3'

Table 3. Number of amplified loci, band size, and polymorphism of 10 ISSR primers

Primers	Number of amplified loci	Band size (bp)	Number of polymorphic loci	Percentage of polymorphic loci (%)
ISSR 1	6	500-1500	5	83,33
ISSR 2	14	300-1800	13	92,86
ISSR 3	12	250-1800	10	83,33
ISSR 4	13	350-1700	12	92,31
ISSR 5	10	600-2000	9	90,00
ISSR 6	7	500-1300	7	100,00
ISSR 7	7	550-1300	7	100,00
ISSR 8	6	600-1300	5	83,33
ISSR 9	8	550-1800	7	87,50
ISSR 10	9	800-2500	9	100,00
	$\Sigma=92$		$\Sigma=84$	91,30

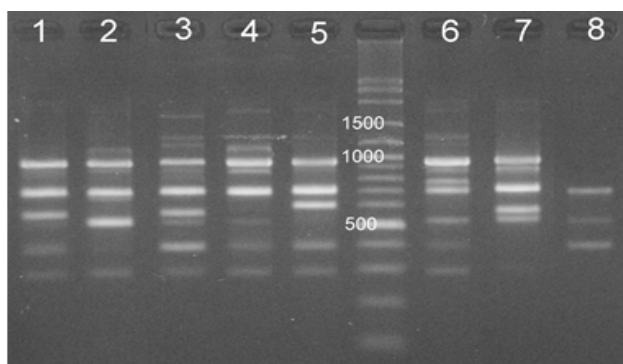


Figure 1. DNA bands produced by ISSR 2. (1) *Lai* Mahakam, (2) *Lai* Bara, (3) *Lai* Besar, (4) *Lai* Merah, (5) *Lai* Kuning, (6) *Lai* Belimbing, (7) *Lai* Apel, (8) *Lai* Durian.

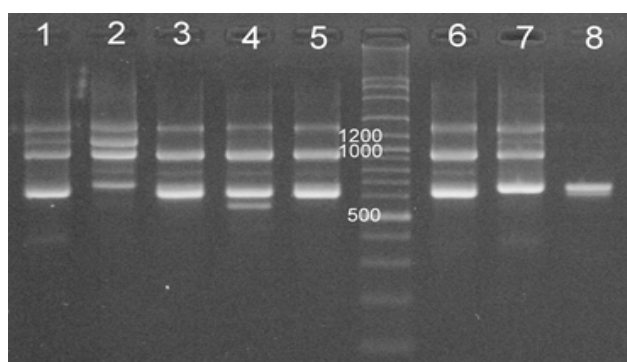


Figure 2. DNA bands produced by ISSR 7. (1) *Lai* Mahakam, (2) *Lai* Bara, (3) *Lai* Besar, (4) *Lai* Merah, (5) *Lai* Kuning, (6) *Lai* Belimbing, (7) *Lai* Apel, (8) *Lai* Durian.

Table 4. Specific bands amplified by 10 ISSR primers

Primers	Specific bands	
	Band size (bp)	Varieties
ISSR 1	650	Bara
	800	Apel
ISSR 2	600	Kuning
	800	Belimbing
	1100	Merah
	1700	Besar
	1800	Merah
ISSR 3	300	Merah
	650	Durian
	700	Merah
	1100	Bara
ISSR 4	650	Merah
	900	Belimbing
ISSR 5	1100	Bara
	1500	Belimbing
	1700	Mahakam
	1700	Mahakam
ISSR 6	550	Besar
	600	Besar
ISSR 7	550	Merah
	550	Kuning
ISSR 9	900	Durian
	1000	Bara
	1800	Belimbing
	1800	Belimbing
ISSR 10	800	Durian
	1100	Durian

Table 4 presents specific bands of eight *Lai* cultivars amplified by 10 ISSR primers. The specific band is a band which only produced in the specific genotype so that the band could become the differentiator among other genotypes. 25 specific bands were formed by 10 ISSR primers consisted of different number of bands as follows; four bands in *Lai* Bara, one band in *Lai* Apel, two bands in *Lai* Kuning, four bands in *Lai* Belimbing, six bands in *Lai* Merah, three bands in *Lai* Besar, four bands in *Lai* Durian, and one band in *Lai* Mahakam. According to Cui et al (2016), specific bands can be used to develop the SCAR marker for a molecular identity of different varieties or lines in a further study.

ISSR is a general primer with random annealing site on each homolog sequence of DNA template. Expression of DNA sequences amplified by an ISSR primer was not directly related to genes controlling morphological traits (Handayani 2016). However, if the ISSR primer anneals at exon (protein-coding region, part of DNA which translated into protein), there is a possibility for amplifying DNA sequence which may be a gene or part of a gene controlling certain morphological trait. Therefore, the specific bands formed in this study (Table 4) might express specific morphological traits of a cultivar although the further study about the correlation between specific bands and specific morphological traits of the cultivars needs to be done.

Genetic diversity among cultivars

UPGMA method was used to construct a dendrogram based on genetic diversity among eight *Lai* local cultivars of *Batuah* (Figure 3). The similarity coefficient of eight *Lai* cultivars ranged between 0.34 and 0.58. It means that the genetic diversity of those cultivars was in the range between 42% and 66%. Genetic diversity of a species within a population is the consequence of its sexual reproduction (Hu et al. 2014; Pereira et al. 2015). Genetic diversity of *Lai* was considered in high diversity because *Lai* is a cross pollination crop and commonly propagated using open pollinated seeds derived from random mating among *Lai* genotypes. Among eight *Lai* local cultivars of *Batuah*, *Lai* Kuning and *Lai* Belimbing were genetically the closest cultivars (similarity coefficient of 0.58). Based on the similarity coefficient of 0.34, eight *Lai* cultivars divided into two clusters. The first cluster consisted of sole *Lai* durian, while the rest seven cultivars grouped in the second cluster.

Genetic diversity has an important role in the breeding program of a species, so it needs to be maintained and improved to ensure the availability of the genetic resources. A species with higher genetic diversity and more complex genetic background will endure the better adversity and more adaptable to the changes of environment conditions (Zhao et al. 2014). The divergent genetic characters of genotype relationship will improve the genetic diversity of species; thus, the information of genetic diversity can be used to select genotypes for crossing purposes. According to Rahayu and Handayani (2010), the further genetic distance between parental cultivars will result in the higher heterosis, which further affects on the progenies. However, the genetic distance was not the only factor, which was

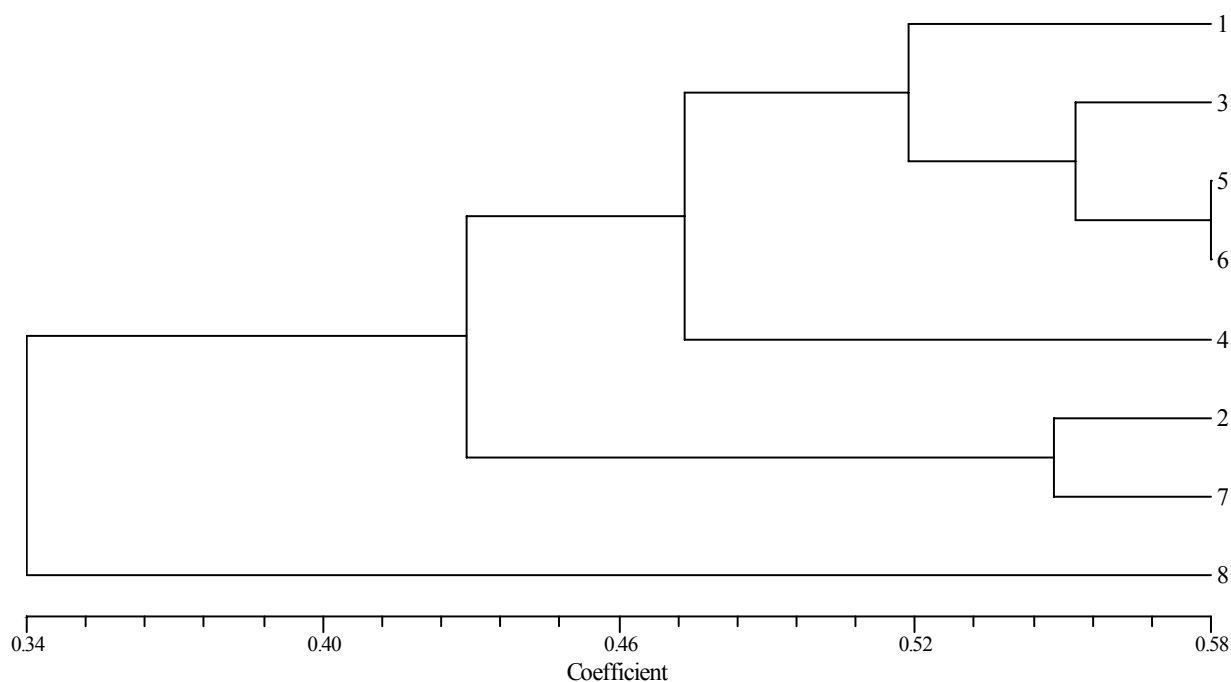


Figure 3. Dendrogram of eight Lai local cultivars of Batuah, Loa Janan Sub-district, Kutai Kartanegara District, East Kalimantan, Indonesia. Note: 1. Lai Mahakam, 2. Lai Bara, 3. Lai Besar, 4. Lai Merah, 5. Lai Kuning, 6. Lai Belimbing, 7. Lai Apel, 8. Lai Durian

considered in the parental genotype selection for crossing purposes. Superior and unique characters to produce good recombinant have also to be highly considered. In this study, it shows that seven Lai cultivars will result in the highest genetic variation of progenies when they are crossed with Lai durian (Figure 3).

The result of this study was of importance to understand the genetic relationship among eight Lai local cultivars of Batuah. The ISSR markers could be effectively used to detect the variation among Lai cultivars by producing 84 loci from the total of 92 loci (91,3%). This study was in line with Poyraz (2016) who reported that ISSR-PCR method is suitable for detecting genetic differences between closer populations in similar habitats, and its ability will decrease relatively when the location of populations become distant with each other. However, the advanced study of genetic diversity of Lai local cultivars of Batuah shall be further done by involving other molecular markers in both general or specific markers, which links to certain morphological traits prior to the comprehensive confirmation of the cultivar diversity.

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