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**GENETIC VARIABILITY OF 11 LOCAL CACAO CLONES DERIVED FROM  
 WEST SUMATRA USING SSR MARKERS**

**VARIABILITAS GENETIK 11 KLON KAKAO LOKAL DARI SUMATERA BARAT  
 BERDASARKAN MARKA SSR**

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**ABSTRAK**

Indonesia is the third largest cacao producing-country in the world and known having many superior local clones, such as that found in Lima Puluh Kota Regency, West Sumatra. However, there is lack of information about genetic background of those local cacao clones. This study aimed to assess genetic variability of 11 local cacao clones collected from Lima Puluh Kota Regency, West Sumatra using SSR markers. The research was conducted in the Integrated Laboratory, Indonesian Industrial and Beverage Crops Research Institute (IIBCRI), Sukabumi, from August to November 2016. The genetic variabilities of local cacao studied were compared with 9 national varieties as reference genomes. Total genomic DNA of the plants was isolated using CTAB method. Cacao DNA was amplified using 18 SSR markers to determine their genetic variability. Afterward, the amplified DNA was separated using 6% non-denaturing polyacrylamide gel electrophoresis. The result exhibited that 12 markers were polymorphic. Further analysis of these polymorphic markers using PowerMarker program revealed a total of 83 alleles were obtained from all cacao clones analyzed. Meanwhile, PIC values ranged from 0.55 to 0.86 with an average of 0.70. A genetic similarity matrix based on UPGMA revealed three main groups at 68% similarity coefficients. Interestingly, all of the 11 local cacao clones were clearly distinguished each other and also from the national varieties. The result demonstrated the usefulness of SSR markers for discriminating local cacao clones. Further study is required to use these local clones in cacao breeding programs.

**Keywords:** Genetic variability, local clones, SSR markers, *Theobroma cacao* L.

**ABSTRACT**

Indonesia adalah negara produsen kakao terbesar ketiga di dunia yang memiliki banyak genotipe kakao unggul lokal, seperti yang terdapat di Kabupaten Lima Puluh Kota, Sumatera Barat. Namun demikian, hanya sedikit informasi tentang latar belakang genetik dari kakao lokal tersebut yang diketahui. Tujuan penelitian adalah untuk menilai keragaman genetik 11 klon kakao lokal yang dikoleksi dari Kabupaten Lima Puluh Kota, Sumatera Barat menggunakan marka SSR. Penelitian dilaksanakan di Laboratorium Terpadu Balai Penelitian Tanaman Industri dan Penyegar (Balittri), Sukabumi, mulai bulan Agustus sampai November 2016. Variabilitas genetik kakao lokal yang diuji dibandingkan dengan 9 varietas nasional yang digunakan sebagai referensi. DNA genom total diisolasi menggunakan metode CTAB. DNA kakao diamplifikasi dengan menggunakan 18 marka SSR untuk menentukan variabilitas genetiknya. Setelah itu, DNA yang telah diamplifikasi dielektroforesis menggunakan 6% non-denaturasi gel poliakrilamid. Hasil penelitian menunjukkan bahwa 12 marka bersifat polimorfik. Analisis lanjutan dari marka polimorfik menggunakan program PowerMarker menghasilkan 83 alel yang diperoleh dari semua genotipe kakao yang dianalisis dalam penelitian ini. Sementara itu, nilai PIC berkisar antara 0,55 sampai 0,86 dengan rata-rata 0,70. Pengelompokan berdasarkan matriks kesamaan genetik dengan metode UPGMA menghasilkan tiga kelompok utama pada koefisien kemiripan 68%. Sebelas genotipe lokal yang berasal dari Kabupaten Lima Puluh Kota dapat dibedakan dengan jelas serta berbeda dengan varietas kakao yang sudah dilepas. Hasil analisis menunjukkan kegunaan marka SSR untuk membedakan antar genotipe lokal. Diperlukan penelitian lebih lanjut untuk menggunakan genotipe lokal ini dalam program pemuliaan kakao.

**Kata kunci:** Genotipe lokal, marka SSR, *Theobroma cacao* L., variabilitas genetik

## INTRODUCTION

Cacao (*Theobroma cacao* L.) is a diploid ( $2n=20$ ) dicotyledonous plant in the family Malvaceae (Alverson, Whitlock, Nyffeler, Bayer, & Baum, 1999) with the center of origin and diversity of the genus *Theobroma* in the Western Upper Amazon (Thomas et al., 2012). The cultivated cacao can be divided into three types, namely Criollo, Forastero, and Trinitario (Susilo, Zhang, & Motilal, 2013). Pedigree comes from Forastero type generated bulk cacao clones, whereas Trinitario and Criollo types produce fine cacao (edel cacao) (Susilo, Zhang, Motilal, Sue, & Meinhardt, 2011). One of the main benefits of cacao is the cotyledon or nib which is used to produce chocolate confectionery. The high benefits of cacao should be followed by the increase of national cacao production. One approach that can be applied to improve national cacao production is by assembling new superior varieties. Novel superior varieties could be obtained from breeding activities that utilize genetic materials with superior traits such as high yield, high bean quality, as well as resistance to biotic and abiotic stress.

Due to a long breeding cycle, new superior varieties of cacao could be achieved through exploration and observation of local superior clones available in several cacao production centers. The second largest cacao production center in Indonesia is Sumatra with a total production of 63,460 tonnes in 2016 (Direktorat Jenderal Perkebunan [Ditjenbun], 2017). West Sumatra, particularly in Lima Puluh Kota Regency, is rich in local cacao clones. One of them is BL 50 (stands for Balubus Lima Puluh Kota) that has been released as a national superior cocoa variety. Therefore, exploration and observation of local cacao clones were focused in this region. The local clones resulting from the exploration process should be clearly identified in advance to avoid duplication of the clones. However, identification through phenotypic characterization shows many limitations due to environmental influences and plasticity. Hence, another approach is required, such as identification using molecular markers.

Molecular markers are widely used as reliable tools for determining genetic variability, cultivar identification and seed purity testing in many crop plants, since they are not affected by environmental factors (Izzah et al., 2013). One type of molecular marker that is commonly used for many genetic analysis is microsatellite or simple sequence repeat (SSR) (Rubiyo, Izzah, Sulistiyorini, & Tresniawati, 2015). SSRs are highly popular genetic markers because of their codominant inheritance, high abundance, enormous extent of allelic diversity, and the ease of assessing SSR size variation by PCR with pairs of

flanking primers (Agarwal, Shrivastava, & Padh, 2008). The use of microsatellite-based markers has been reported from many estate crop species, such as tea (*Camellia sinensis* L. (O) Kuntze) (Taniguchi et al., 2014), tobacco (*Nicotiana tabacum* L.) (Fricano et al., 2012), and coffee (*Coffea arabica*) (Izzah, Randriani, & Dani, 2015). In addition, a recent study has described the advantages of SSR markers to examine the genetic identity, structure, and allelic richness of the fungus-resistant cacao variety CCN 51 based on Motamayor's new classification (Boza et al., 2014).

Previous studies have reported the application of SSR markers to analyze genetic variability in cacao. Rubiyo et al. (2015) evaluated the genetic diversity of 12 local cacao clones collected from Kolaka, Southeast Sulawesi, using 14 polymorphic SSR markers, and identified three groups with 36% similarity coefficients. Meanwhile, Dinarti et al., (2015) assessed the genetic diversity of 205 cacao clones, consisting of 53 local clones and 152 international clones, using 15 SSR markers. Clustering dendrogram results separated these clones into two major groups. Moreover, the latest study successfully clustered 28 cacao clones into three groups using 20 polymorphic SSR markers (Wicaksono, Rubiyo, Sukma, & Sudarsono, 2017). The aim of this study was to assess genetic variability of 11 local cacao clones collected from Lima Puluh Kota Regency, West Sumatra, and 9 national varieties as references using SSR markers.

## MATERIALS AND METHODS

The research was conducted at the Integrated Laboratory of Indonesian Industrial and Beverage Crops Research Institute (IIBCRI), Sukabumi, West Java, from August to November 2016.

### Plant Materials

Leaf samples of cacao were collected from farmer's cacao plantations, in Jorong Balubus Village, Nagari Sungai Talang, Lima Puluh Kota Regency, West Sumatra Province. A total of 20 cacao clones, consisting of 11 local clones and 9 national varieties as reference, were used to assess genetic variability in this study (Table 1). These 11 local cacao clones were selected by farmers through participatory plant breeding (PPB) based on fruit and seed size performance.

### DNA Isolation

DNA extraction was carried out using 3 g of clean, healthy, and young leaf tissues. Leaf samples were grounded into powder using mortar and pestle, and then DNA was extracted using the

cetyltrimethylammonium bromide (CTAB) protocol (Allen, Flores-Vergara, Krasynanski, Kumar, & Thompson, 2006; Santos et al., 2014). DNA quality and quantity were measured using NanoDrop ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE, USA). DNA was diluted to 10 ng  $\mu$ l<sup>-1</sup> concentration using TE buffer for subsequent PCR analysis.

### DNA Amplification and Genotyping Using SSR Markers

DNA samples of 20 cacao clones were amplified using 18 SSR markers previously published by Saunders et al. (2004) and Pugh et al. (2004) (Table 2). Amplification using PCR was conducted in final volume of 15  $\mu$ l, comprising 10 ng DNA, 0.2  $\mu$ M of each primer and 1x PCR mix. The procedure of DNA amplification was done through three following steps: (1) pre-denaturation (95°C; 3 minutes), (2) denaturation (95°C; 15 seconds), annealing (53°C; 15 seconds), and extension (72°C, 15 seconds), all processes in this step were repeated 35 cycles, (3) final extension (72°C; 10 minutes). The resulting PCR products were first checked using 1% agarose gel electrophoresis to confirm amplification. The next

procedure was separation of confirmed PCR products using 6% non-denaturing polyacrylamide gel electrophoresis. The gels that went through the process of electrophoresis were stained with ethidium bromide for 20 minutes and DNA bands were visualized under UV light transilluminator using the gel documentation system.

### Data Analysis

Polymorphic DNA fragments generated in the study were scored visually in binary format, allele presence stated with 1 and allele absence stated with 0. The binary data was then used for analysis using numerical taxonomy system (NTSYS) version 2.1 (Rohlf, 2000). The grouping of 20 cacao clones was performed based on the genetic similarity matrix using unweighted pair group arithmetic mean method (UPGMA). Genetic distances between cacao clones were calculated as 1-similarity. In addition, we also conducted analysis using PowerMarker version 3.25 (Liu & Muse, 2005) to calculate alleles number, major allele frequency, gene diversity, expected heterozygosity (He), and polymorphic information content (PIC) values.

Table 1. List of 20 local cacao clones from Lima Puluh Kota Regency, West Sumatra, and their references used for genetic variability analysis

Tabel 1. Daftar 20 klon kakao dari Kabupaten Lima Puluh Kota, Sumatera Barat dan varietas nasional yang digunakan untuk analisis variabilitas genetik

Clones name	Cacao type	Origin
BL-A	Bulk	Lima Puluh Kota Regency, West Sumatra
BL-B	Bulk	Lima Puluh Kota Regency, West Sumatra
BL-C	Bulk	Lima Puluh Kota Regency, West Sumatra
BL-D	Bulk	Lima Puluh Kota Regency, West Sumatra
BL-E	Bulk	Lima Puluh Kota Regency, West Sumatra
BL-F	Bulk	Lima Puluh Kota Regency, West Sumatra
BL-G	Bulk	Lima Puluh Kota Regency, West Sumatra
BL-H	Bulk	Lima Puluh Kota Regency, West Sumatra
BL-I	Bulk	Lima Puluh Kota Regency, West Sumatra
BL 50	Bulk	Lima Puluh Kota Regency, West Sumatra
RCL	Bulk	Lima Puluh Kota Regency, West Sumatra
TSH 858	Bulk	KP.Pakuwon, West Java (reference)
Sulawesi 1	Bulk	KP.Pakuwon, West Java (reference)
ICCRI 03	Bulk	KP.Pakuwon, West Java (reference)
MCC 01	Bulk	South Sulawesi (reference)
MCC 02	Bulk	South Sulawesi (reference)
ICS 13	Bulk	KP.Pakuwon, West Java (reference)
ICS 60	Bulk	KP.Pakuwon, West Java (reference)
DR 38	Fine / edel	PTPN XII, East Java (reference)
DRC 16	Fine / edel	PTPN XII, East Java (reference)

Table 2. SSR markers used for genetic variability analysis of 20 cacao clones.  
Tabel 2. Marka SSR yang digunakan dalam analisis variabilitas genetik menggunakan 20 klon kakao.

SSR name	5'-3' Forward primer	5'-3' Reverse primer	LG Location	Expected size (bp)	Repeat Sequence	T <sub>m</sub> (°C)
mTcCIR1 <sup>a</sup>	F: GCAGGGCAGGCTCAGTGAAGCA	R: TGGGCAACCAGAAAACCGAT	8	128-146	(CT) <sub>14</sub>	51
mTcCIR15 <sup>a</sup>	F: CAGCCGCCTCTTGTTAG	R: TATTTGGGATCTTGATG	1	254	(TC) <sub>19</sub>	46
mTcCIR24 <sup>a</sup>	F: TTTGGGGTGATTTCTTCTGA	R: TCTGTCTCGTCTTTGGTGA	9	186-207	(AG) <sub>13</sub>	46
mTcCIR33 <sup>a</sup>	F: TGGGTTGAAGATTTGGT	R: CAACAATGAAAATAGGCA	4	265-348	(TG) <sub>11</sub>	51
mTcCIR69 <sup>b</sup>	F: TCGGTGTTCCATCAGTA	R: CATGCTATGAGATTGAAAG	5	203	(CT) <sub>20</sub>	46.8
mTcCIR90 <sup>b</sup>	F: CCACTTCAAAAACCATCTA	R: GCAACTGTCAACCATTTATCTA	9	291	(CT) <sub>10</sub>	47.6
mTcCIR109 <sup>b</sup>	F: GGAAAGTGTAGGAAAGTAGAC	R: GGACCAAAAAGAGCATA	5	162	(CT) <sub>12</sub>	46.4
mTcCIR145 <sup>b</sup>	F: CAGACTTCCAACCTCAAACT	R: TGAGAA TAGATGGACCGAT	9	117	(CT) <sub>17</sub>	49.7
mTcCIR167 <sup>b</sup>	F: GTAGAACCATAAACACATT	R: ACAATCAATAAAATACGAG	3	254	(GA) <sub>16</sub>	46.1
mTcCIR184 <sup>b</sup>	F: GGTTTTCTAGCTCCTCC	R: AGGAAAGAATGACTCATACTA	1	139	(CA) <sub>8</sub> (CT) <sub>13</sub>	48.2
mTcCIR190 <sup>b</sup>	F: AAGAAACTGAAGCACAAAT	R: CACAAAAGCATAAACTG	7	166	(TG) <sub>12</sub>	46.7
mTcCIR209 <sup>b</sup>	F: TAGGGGCTAATGGTGA	R: AGGTA TGCTGTATTTATGGT	6	259	(TG) <sub>6</sub> TAT(GA) <sub>9</sub>	47.8
mTcCIR229 <sup>b</sup>	F: ATCTCGGTAATAGCACATAA	R: CGCAATCCTACAACACA	10	307	(TC) <sub>8</sub>	47.9
mTcCIR251 <sup>b</sup>	F: TCTATGGGATTTGATGAG	R: AGATACAGCAGGAACACA	9	188	(CT) <sub>7</sub> (CA) <sub>12</sub>	46.8
mTcCIR255 <sup>b</sup>	F: TTTACCTCCACCATCTT	R: TGGCACTTATCTATTTACTGT	6	203	(AC) <sub>11</sub>	47.5
mTcCIR264 <sup>b</sup>	F: TGCTATCCACAACCAGT	R: TAACTCACTTTTGCCACTA	1	192	(CT) <sub>8</sub>	47
mTcCIR276 <sup>b</sup>	F: TCCTGGCTTTTAAATACAT	R: GTCCTATCTGCCTCACT	6	124	(GA) <sub>14</sub>	46.3
mTcCIR291 <sup>b</sup>	F: AGTCCCATAGGTTCCAAAT	R: CGAGGTTATCCCCAAA	6	218	(CT) <sub>12</sub>	50.1

**Note:** Source: <sup>a</sup> = (Saunders, Mischke, Leamy, & Hemeida, 2004); <sup>b</sup> = (Pugh *et al.*, 2004)  
**Keterangan:** Sumber: <sup>a</sup> = (Saunders *et al.*, 2004); <sup>b</sup> = (Pugh *et al.*, 2004)

## RESULTS AND DISCUSSION

### Variability of Alleles Generated from SSR Marker Genotyping

The amplification process through PCR succeeded in amplifying 20 DNA samples using 18 SSR markers. However, not all of the amplified markers produced a polymorphic pattern. Out of 18 SSR

markers, 12 markers demonstrated polymorphic, clear and scoreable bands, whereas the rest of markers produced unspecific bands. The examples of SSR markers showing the polymorphic bands are presented in Figure 1. Moreover, SSR markers that gave polymorphic patterns will be used for further analysis.

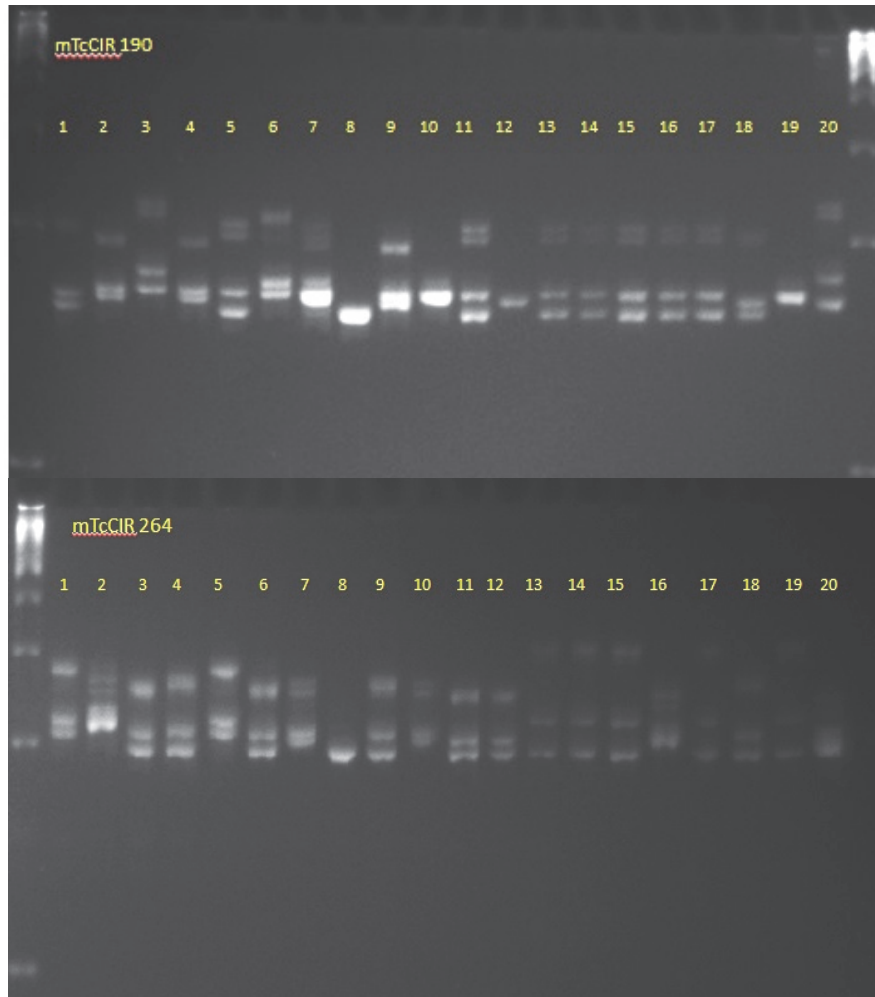


Figure 1. Two SSR markers (primer mTcCIR190 and mTcCIR264) that showed polymorphic DNA patterns of the 11 cacao clones after separation using 6% non-denaturing polyacrylamide gel electrophoresis. 1= BL-A; 2= BL-B; 3= BL-C; 4= BL-D; 5= BL-E; 6= BL-F; 7= BL-G; 8= BL-H; 9= BL-I; 10= BL-50; 11= RCL; 12= TSH 858; 13= Sulawesi 1; 14= ICCRI 03; 15= MCC 01; 16= MCC 02; 17= ICS 13; 18= ICS 60; 19= DR 38; and 20= DRC 16. Number 1–11 are local cacao clones collected from Lima Puluh Kota Regency, West Sumatra, whereas number 12–20 are national varieties as reference.

Gambar 1. Dua marka polimorfik (mTcCIR190 dan mTcCIR264) yang menunjukkan pola pita polimorfik dari DNA 11 klon kakao hasil separasi menggunakan 6% gel poliakrilamid non-denaturasi. Baris 1= BL-A; 2= BL-B; 3= BL-C; 4= BL-D; 5= BL-E; 6= BL-F; 7= BL-G; 8= BL-H; 9= BL-I; 10= BL-50; 11= RCL; 12= TSH 858; 13= Sulawesi 1; 14= ICCRI 03; 15= MCC 01; 16= MCC 02; 17= ICS 13; 18= ICS 60; 19= DR 38; and 20= DRC 16. Nomor 1–11 adalah klon kakao lokal yang berasal dari Kabupaten Lima Puluh Kota, Sumatera Barat, sedangkan nomor 12–20 adalah varietas unggul nasional sebagai referensi.

Table 3. Allelic variations of 12 polymorphic SSR markers used to evaluate genetic variability of 20 cacao clones  
 Tabel 3. Variasi tipe alel dari 12 marka SSR polimorfik yang digunakan untuk mengevaluasi keragaman genetik 20 klon kakao

Marker name	Allele number	Major allele frequencies	Gene diversity	Expected heterozygosity (He)	PIC
mTcCIR1	4	0.5	0.62	0.6	0.55
mTcCIR15	4	0.45	0.66	0.8	0.60
mTcCIR24	6	0.55	0.63	0.4	0.59
mTcCIR69	7	0.27	0.79	0.7	0.76
mTcCIR109	6	0.35	0.76	0.2	0.73
mTcCIR145	8	0.55	0.65	0.65	0.61
mTcCIR167	6	0.53	0.67	0.58	0.63
mTcCIR190	7	0.4	0.74	0.75	0.71
mTcCIR209	10	0.32	0.83	0.6	0.81
mTcCIR255	9	0.37	0.79	0.6	0.77
mTcCIR264	10	0.17	0.88	0.9	0.86
mTcCIR276	6	0.31	0.79	0.47	0.76
<b>Mean</b>	<b>6.92</b>	<b>0.39</b>	<b>0.73</b>	<b>0.60</b>	<b>0.70</b>

Allelic variations of polymorphic SSR markers can be determined through PowerMarker analysis. The result showed that polymorphic SSR markers used in this study possessed diverse alleles. All of 12 polymorphic loci generated 83 alleles with an average of 6.92 alleles per locus. Primers mTcCIR209 and mTcCIR264 had higher allele numbers compared to other primers, whereas mTcCIR1 and mTcCIR15 primers had the lowest number of alleles (Table 3). The average number of alleles per locus obtained in this study was 6.92, which is higher than average number of alleles in a set of local cacao clones collected from Kolaka, Southeast Sulawesi, which was 5 (Rubiyo et al., 2015). This finding provide evidence that local cacao clones collected in Lima Puluh Kota Regency, West Sumatra had higher levels of genetic diversity than those collected from Kolaka, Southeast Sulawesi. Allele richness found in local cacao clones in Lima Puluh Kota Regency might be related with the collection from a much wider geographical region than the Kolaka collection. The result obtained in this study is in agreement with the results of Zhang et al. (2006) that observed the genetic diversity of cacao collections in Ucayali and Huallaga regions.

We then calculated other components related to allelic diversity of SSR markers, such as major alleles frequency, gene diversity, expected heterozygosity, and PIC value. The frequency of major alleles ranged from 0.17 (mTcCIR264) to 0.55 (mTcCIR24 and mTcCIR145) with an average of 0.39. Meanwhile, an average number of gene diversity, expected heterozygosity, and PIC value calculated in the present study were 0.73, 0.60, and 0.70, respectively (Table 3). The average values of gene diversity, expected

heterozygosity and PIC were in high categories ( $> 0.5$ ), which is in accordance with Botstein et al. (1980) who stated that a locus with a PIC value  $>0.5$  can be considered as an informative locus. This result showed that SSR markers used in this study have a high level of variability. This was shown by the ability of these markers to distinguish among local cacao clones as well as between local clones and national varieties. On the other hand, high values of heterozygosity can be attributed to the cross-pollinated nature of cacao that allows mixing of multiple alleles (Wicaksono et al., 2017). Meanwhile, the high variation found in gene diversity and PIC indicated the high diversity of local cacao clones collected from Lima Puluh Kota Regency. Hence, these local clones can be utilized as genetic materials for future breeding program.

#### Genetic Variability and Relationship of 20 Cacao Clones

Genetic variability and relationships of 20 cacao clones used in this study were analyzed based on genetic similarity values implemented using the NTSYS program (Figure 2). By using a similarity coefficient of 68% as a threshold level, a dendrogram of 20 cacao clones was successfully developed which formed three major groups. Group I held most of clones evaluated in this study (16 clones), which then subdivided into four sub groups at a similarity coefficient of 70%. Seven cacao clones, consisting of six local clones (BL-A, BL 50, BL-D, BL-E, BL-G, and BL-I) and one national variety (Sulawesi 1), were placed in sub group I. The second sub group contained five cacao clones, including ICS 60, ICS 13, DRC 16, DR 38, and MCC 01. Of which, all of five clones in sub group II are national

varieties. Sub group III only held one local clone, namely BL-B, whereas sub group IV contained two local clones (BL-C and BL-H) and one national variety (TSH 858). Group II represented two local clones, namely BL-F and RCL, meanwhile two national varieties, including ICCRI 03 and MCC 02 were located in group III.

The result of the clustering dendrogram demonstrated that all local cacao clones can be distinguished from each other and also from national varieties used as references. Moreover, from the dendrogram, we can further understand the relationship between local clones and national varieties. Six local clones that located in sub group I showed a close relationship with Sulawesi 1, a national variety originating from South Sulawesi. Interestingly, BL 50, a local clone that recently has been released as a national variety is a member of sub group I, with a close relationship to Sulawesi 1. Likewise, two local clones in sub group IV, BL-C and BL-H, present a close relationship with TSH 858. This result indicates that two national varieties used as references in the current

study, Sulawesi 1 and TSH 858, might be a common ancestor for some local cacao clones found in Lima Puluh Kota Regency, West Sumatra.

Local cacao clones located in different groups are assumed to have high genetic distance values, thus they can be selected as parental clones or as candidate clones for the release of new superior varieties. We can also determine the promising parental clones based on genetic distance values that is calculated based on the formula 1 - genetic similarity value as shown in Table 4. In this study, we obtained two promising parental clone combinations, BL-B x ICCRI 03 and BL50 x BL-H, with the genetic distance value of 46% and 44%, respectively. Those combinations are expected to produce progenies with more agronomically favorable characteristics than their parents. The use of genetic distance values to determine promising parental clones for hand pollination has also been done in previous studies on cacao and coffee (Rubiyo et al., 2015; Izzah et al., 2015; Dani et al., 2016; and Wicaksono et al., 2017).

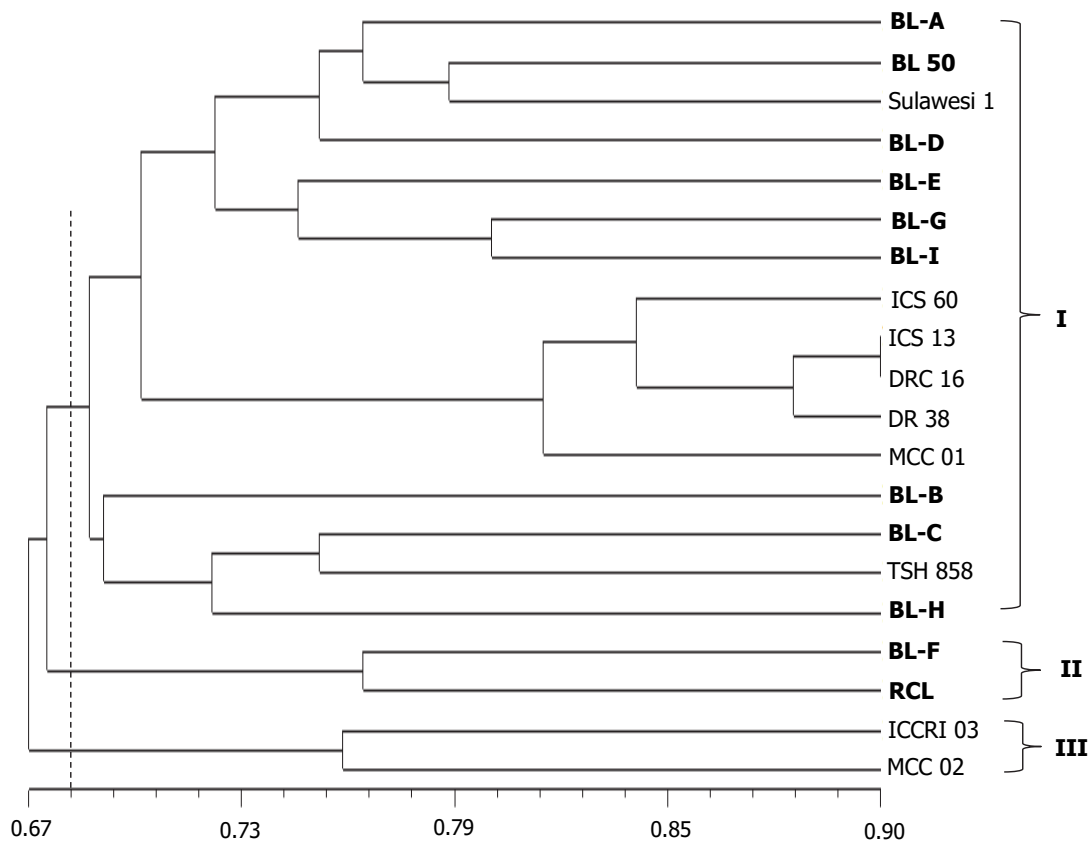


Figure 2. The grouping of 20 cacao clones based on 12 polymorphic SSR markers. Cacao clones with bold letters are collected from Lima Puluh Kota Regency, West Sumatra, while clones with normal letters are national varieties.

Gambar 2. Pengelompokan 20 klon kakao berdasarkan 12 marka SSR polimorfik. Klon kakao dengan cetak tebal merupakan klon lokal hasil koleksi dari Kabupaten Lima Puluh Kota, sedangkan klon dengan cetak normal adalah varietas nasional yang sudah dilepas.

Table 4. Genetic distance values calculated from 20 cacao clones based on SSR markers  
 Tabel 4. Nilai jarak genetik yang diperoleh dari 20 klon kakao berdasarkan marka SSR

Clone	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. BL-A	0																			
2. BL-B	0,3	0																		
3. BL-C	0,29	0,35	0																	
4. BL-D	0,25	0,26	0,3	0																
5. BL-E	0,25	0,31	0,37	0,29	0															
6. BL-F	0,27	0,38	0,3	0,38	0,33	0														
7. BL-G	0,3	0,29	0,3	0,31	0,26	0,31	0													
8. BL-H	0,36	0,32	0,31	0,3	0,35	0,37	0,3	0												
9. BL-I	0,29	0,37	0,24	0,3	0,25	0,23	0,21	0,33	0											
10. BL 50	0,25	0,33	0,39	0,24	0,24	0,33	0,29	0,44	0,27	0										
11. RCL	0,39	0,41	0,25	0,33	0,36	0,24	0,36	0,37	0,27	0,33	0									
12. TSH 858	0,27	0,26	0,25	0,29	0,36	0,33	0,26	0,25	0,25	0,29	0,31	0								
13. ICS 60	0,31	0,36	0,37	0,36	0,32	0,4	0,36	0,35	0,36	0,35	0,35	0,33	0							
14. ICS 13	0,23	0,31	0,3	0,29	0,26	0,33	0,31	0,3	0,27	0,33	0,33	0,29	0,19	0						
15. DRC 16	0,21	0,31	0,3	0,33	0,29	0,26	0,31	0,32	0,27	0,31	0,29	0,31	0,17	0,1	0					
16. Sulawesi 1	0,23	0,31	0,32	0,26	0,29	0,33	0,24	0,35	0,3	0,21	0,33	0,24	0,31	0,24	0,26	0				
17. DR 38	0,23	0,36	0,32	0,36	0,33	0,26	0,36	0,35	0,32	0,33	0,31	0,29	0,13	0,14	0,1	0,26	0			
18. ICCRI 03	0,35	0,46	0,37	0,3	0,4	0,33	0,33	0,32	0,28	0,33	0,33	0,28	0,29	0,3	0,31	0,32	0,27	0		
19. MCC 01	0,25	0,33	0,3	0,31	0,31	0,29	0,26	0,3	0,3	0,31	0,31	0,24	0,21	0,21	0,17	0,29	0,17	0,27	0	
20. MCC 02	0,35	0,41	0,39	0,29	0,41	0,33	0,36	0,32	0,32	0,31	0,33	0,31	0,28	0,36	0,36	0,26	0,33	0,24	0,33	0



In addition, the grouping of cacao clones using SSR markers may help breeders in selecting promising clones as candidates for new varieties. Several local clones, such as BL-B, BL-D, BL-H, BL-F, and RCL can be selected as candidate clones for new varieties release. The results obtained in this study indicated that the use of SSR markers proved a powerful tool to assess genetic variability and relationships, to select new promising varieties, and to determine promising combination of parental clones.

## CONCLUSION

The result of the clustering dendrogram based on SSR markers successfully classified 20 cacao clones into three main groups at a similarity coefficient of 68%. Most of cacao clones examined in this study (16 clones) were located in group I, and the rest of clones clustered in group II and group III. Local cacao clones and national varieties tend to cluster separately, except for Sulawesi 1 and TSH 858 that grouped together with local clones in sub group I and sub group IV, respectively. Based on the dendrogram, we conclude that two national varieties used as references in this study, Sulawesi 1 and TSH 858, allegedly as common ancestors of several local cacao clones found in Lima Puluh Kota Regency, West Sumatra. On the other hand, SSR markers used in this study can distinguish between local cacao clones as well as between local clones and national varieties. The result obtained in present study may provide valuable information for future cacao breeding program.

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