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EVALUATION OF GENETIC DIVERSITY IN CACAO COLLECTED FROM KOLAKA, SOUTHEAST SULAWESI, USING SSR MARKERS

Evaluasi Keragaman Genetik Koleksi Kakao dari Kolaka, Sulawesi Tenggara, dengan Menggunakan Marka SSR

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ABSTRACT

Kolaka, which is located in Southeast Sulawesi, has long been known as one of cacao production centers in Indonesia. Therefore, many different cacao germplasms can be found in this region. The study aimed to evaluate genetic diversity and relationships of 12 cacao genotypes collected from Kolaka. Genomic DNA was extracted by using a modified CTAB method. Meanwhile, genetic diversity was analyzed based on 16 SSR markers, which then separated by 6% non-denaturing polyacrylamide gel electrophoresis. The result showed that all of those markers, 14 markers exhibited polymorphism and subsequently used for data analysis using NTSYS and PowerMarker program. About 70 different alleles were generated from 12 cacao genotypes analyzed with an average of 5 alleles per locus. Average value of polymorphism information content (PIC) resulted in this study was 0.59. The cluster analysis using UPGMA method based on the genetic similarity coefficient revealed that all cacao genotypes were separated into three major groups. The first group consisted of five cacao genotypes, the second one held four cacao genotypes, whereas the third group contained three genotypes. This result indicates that three genotypes that clustered separately from the others could be used as a good clonal candidate for cacao breeding program. The information resulted from this present study would be useful for future cacao breeding program, especially in efforts to release a new variety.

[***Keywords***: *Theobroma cacao*, genetic diversity, SSR markers, Southeast Sulawesi]

ABSTRAK

Kolaka yang terletak di Provinsi Sulawesi Tenggara telah lama dikenal sebagai salah satu sentra produksi kakao di Indonesia. Oleh karena itu, beragam jenis plasma nutfah kakao ditemukan di wilayah ini. Penelitian ini bertujuan untuk mengevaluasi keragaman genetik 12 genotipe kakao yang dikoleksi dari Kolaka. DNA genomik diekstrak menggunakan metode CTAB yang dimodifikasi. Sementara, keragaman genetik dianalisis berdasarkan pada 16 marka SSR, yang kemudian dielektroforesis menggunakan 6% non-denaturasi gel poliakrilamid. Hasil penelitian menunjukkan bahwa 14 dari 16 marka SSR yang digunakan dalam penelitian ini bersifat polimorfiks dan selanjutnya digunakan untuk analisis data menggunakan program NTSYS dan PowerMarker. Sebanyak 70 alel berhasil diamplifikasi dari 12 genotipe kakao yang dianalisis dengan nilai rata-rata 5 alel per lokus. Nilai rata-rata dari polymorphism information content *(PIC) yang diperoleh dalam penelitian ini adalah 0,59. Analisis keragaman genetik menggunakan metode UPGMA menunjukkan bahwa semua genotipe kakao dapat dikelompokkan menjadi tiga grup. Grup pertama terdiri atas lima genotipe kakao, grup kedua meliputi empat genotipe kakao, sedangkan grup ketiga terdiri atas tiga genotipe. Hasil ini menunjukkan bahwa tiga genotipe yang terkelompok secara terpisah dapat digunakan sebagai kandidat klon terpilih untuk program pemuliaan kakao. Informasi yang diperoleh dalam penelitian ini akan sangat bermanfaat untuk pemuliaan tanaman kakao di masa depan, terutama dalam upaya untuk merilis varietas baru.*

[***Kata kunci***: *Theobroma cacao*, keragaman genetik, marka SSR, Sulawesi Tenggara]

INTRODUCTION

Cacao (*Theobroma cacao* L.) is an economically important crop that is commonly known as a source of cacao beans. Cacao which is belongs to Malvaceae family (Alverson *et al.* 1999) has three main genetic groups including Criollo, Forastero and their hybrid form Trinitario (Lanaud *et al.* 1999). The origin of this species is Amazonian basin (Motamayor *et al.* 2008) which then spreads to many tropical countries. To date, Indonesia is acknowledged to be the third

largest cacao producer in the world after Cote d'Ivoire and Ghana with the total production in 2009 was 525 thousand tonnes (ICO 2010). Among cacao-producing regions in Indonesia, Sulawesi is recognized as the largest producer that covers 60% of national cacao plantation (Ditjenbun 2011). In addition, Kolaka, one of the districts in Southeast Sulawesi Province has long been known as the largest contributor to the cacao production with the total production in 2012 reached 63,172 tonnes (Ditjenbun 2013).

In order to enhance national cacao production, the availability of good breeding materials is needed. Therefore, systematic identification, documentation and conservation of cacao genetic diversity either *ex situ* or *in situ* will be the viable approach for cacao improvement (Thondaiman *et al.* 2013). In present study, we explored cacao germplasms at Kolaka to collect local cacao genotypes. Collection of plant genetic resources is very important as a key source of genetic diversity that can be used to increase diversity in breeding materials (Roorkiwal *et al.* 2014). The genetic diversity of a species is the result of cumulative mutation, recombination and selection on individuals by the environment (Jing *et al.* 2010), and represents a rich potential source that would be useful for breeding program.

Exploitation of cacao genetic diversity can be done by using molecular markers since they consider as useful tools for assaying genetic variation and provide an efficient mean to link phenotypic and genotypic variation (Varshney *et al.* 2005). Furthermore, molecular markers help scientists to clarify the structure of genetic diversity in many plant species (Glaszmann *et al.* 2010).

In cacao, molecular markers have been used in many genetic applications, such as genetic diversity analysis, phylogenetic relationships, characterization, cultivar identification, and parentage analysis. One of molecular markers widely used for such analyses is simple sequence repeat (SSR) also known as microsatellite. SSRs are versatile genetic markers that combine the useful properties of high variability, codominant inheritance and good reproducibility (Ashworth and Clegg 2003). In addition, an international forum of cacao research project conducted in England and France in 2001 agreed that a set of standardized SSR primers should be used to characterize all *T. cacao* germplasm collections (Saunders *et al.* 2004).

Several studies reported the usefulness of SSR markers for genetic diversity analysis in cacao. For instance, De Schawe *et al.* (2013) successfully discriminated wild and cultivated cacao populations in Bolivia using nine microsatellite loci. Genetic diversity among wild cacao was lower than that of the cultivated plants. On the other hand, 105 cacao accessions collected from Huallaga and Ucayali valleys of Peru were clearly separated from each others based on 15 loci SSR profiles. The Huallaga group has lower genetic diversity, both in terms of allelic richness and gene diversity, than the Ucayali group (Zhang *et al.* 2006).

In Indonesia, evaluation of cacao genetic diversity has also been reported. Syafaruddin and Nasution (2012) analyzed the genetic diversity of 17 cacao genotypes derived from Pinrang and Luwu, South Sulawesi using eight RAPD primers. The result exhibited that all cacao genotypes can be clustered into two groups at a coefficient of similarity 4%, which indicates a narrow genetic variation. Another research done by Kurniasih *et al.* (2011) assessed genetic diversity of 29 cacao genotypes obtained from Indonesian Coffee and Cocoa Research Institute (ICCRI) using 39 SSR markers. All of 29 cacao genotypes were classified into three groups. The first group contained 12 genotypes with diversity coefficient of < 3.75, the second group composed of nine cacao genotypes with the same value of diversity coefficient (< 7.50), whereas the rest of the cacao genotypes were in the third group with diversity coefficient of > 7.50. In present study we used 16 previously reported SSR markers to genotype 12 cacao genotypes derived from Kolaka, Southeast Sulawesi. The objective was to assess genetic diversity and relationships of local cacao genotypes collected from Kolaka. The result obtained herein will improve our understanding about the diversity of local cacao genotypes in Kolaka.

MATERIALS AND METHODS

Plant Materials

Eight local cacao genotypes (Table 1) formerly screened from the farmer's fields at Kolaka by the author several years ago were used to examine their genetic diversity and relationships. Those eight cacao genotypes were selected based on some criteria including high production, weight per dried bean >1 g, fat content of dried beans >50%, and resistant to major pest and disease. All of the cacao genotypes had the fat content of dried beans >50% (data not shown). High fat content in cacao beans is one of determinants of cacao beans quality.

Table 1. List of eight local cacao genotypes collected from Kolaka, Southeast Sulawesi.

Clone name	Cacao type
IAARD 1	Bulk
IAARD 2	Bulk
IAARD 4	Bulk
IAARD 6	Bulk
IAARD 7	Bulk
IAARD 9	Bulk
IAARD 11	Bulk
IAARD 12	Bulk

Besides, four released cacao varieties, i.e. Sulawesi 1, Sulawesi 2, ICCRI 03 and ICCRI 04 were used as control variety. Sulawesi 1 is a high yielding varieties with potential production of 1.8-2.5 t ha^{-1} $year^{-1}$ and resistant to vascular streak dieback (VSD) disease. Sulawesi 2 is also known as high yielding variety with the production can achieve 1.8-2.75 t ha^{-1} $year^{-1}$, resistant to cacao pod borer (CPB) as well as VSD disease. These two cacao varieties that have been released by the Ministry of Agriculture are derived from Sulawesi (Susilo and Suhendi 2006; Susilo *et al.* 2009). Meanwhile, ICCRI 03 was obtained from hand pollination between cacao genotypes DR 2 x Sca 6 at Banaran plantation, Central Java. Characteristics of this variety are fat content of dried beans 55%, potential production of 2,299 kg ha^{-1} $year^{-1}$, and resistant to *Helopeltis* sp. and black pod disease (BPD). Another variety is ICCRI 04, which was also found from hand pollination between cacao genotypes ICS 60 and Sca 12 at Banaran plantation, Central Java. ICCRI 04 had a fat content of dried beans 55%, potential production of 2,266 kg ha^{-1} $year^{-1}$, and resistant to black pod disease and *Helopeltis* sp. (Suhendi 2006).

Sample Preparation for DNA Isolation

Leaf samples were collected from one individual plant of each repetition that showed good growth. The samples were then wrapped using aluminum foil and stored at -20°C until use. Prior to extraction, 3 g of healthy and young leaf tissue were cut into small pieces and then grinded using mortar and pestle. Genomic DNA of cacao was extracted following the cetyltrimethylammonium bromide (CTAB) protocol (Allen *et al.* 2006) with minor modification. DNA samples were quantified on 1% agarose gel using standard lambda DNA. The DNA concentration of a sample was then estimated by comparing the band intensity with lambda DNA. Approximately of 10 ng μl^{-1} DNA concentration was used as final working solution for PCR analysis.

SSR Marker Genotyping

Sixteen SSR loci previously established by Pugh *et al.* (2004) (Table 2) were used to amplify as well as to identify polymorphism of 12 cacao genotypes. These SSR markers were selected randomly throughout ten cacao linkage groups. PCR assays were carried out in final volume of 15 µl, containing 10 ng DNA sample,

Table 2. Characteristics of 16 SSR markers used for genetic diversity study on cacao.

Marker name	SSR motif	Forward primer	Reverse primer	LG location	Expected size (bp)
mTcCIR184	(CA)8 (CT)13	GGTTTTCTAGCTCCTCC	AGGAAAGAATGACTCATACTA	1	139
mTcCIR162	(GA)19	AAGATTGAGGTCACTCAGG	TAAGTTTTTTGCTTTACTCTTC	2	162
mTcCIR144	(TC)9	CCACTGACACGCAATGAA	CTAGGACTTAGGAAAGTGTTTG	3	254
mTcCIR82	(AG)6 AA(AG)7	ATCATGTGCCCCTTCTAA	GGCAGCTAAGTGTTCATTC	3	174
mTcCIR76	(TC)21 TTT(TC)58	AGCCAAAGAAAGGAT	TGAATCCGAGACAAAG	4	139
mTcCIR95	(TC)4 CC(TC)21	CTCCTTCCCTTTCTCTC	CATCGTCTTCCTCTCATC	4	221
mTcCIR69	(CT)20	TCGGTGTTCCATCAGTA	CATGCTATGAGATTGAAAG	5	203
mTcCIR109	(CT)12	GGAAAGTGTAGGAAAGTAGAC	GGACCAAAAAGAGCATA	5	162
mTcCIR255	(AC)11	TTTACCTCCACCATCTT	TGGCACTTATCTATTACTGT	6	203
mTcCIR291	(CT)12	AGTCCCATAGGTTCCAAT	CGAGGTTATCCCCAAA	6	218
mTcCIR209	(TG)6 TAT(GA)9	TACGGGCTAATGGTGA	AGGTATGCTGTATTTATGGT	6	259
mTcCIR190	(TG)12	AAGAAACTGAAGCACAAT	CACAAAGAGCATAAACTG	7	166
mTcCIR218	(CT)11	TGACCAAGGAAGCTCTC	GGTGGGAAAGGTGGTA	8	187
mTcCIR211	(TC)9	TGGTGCTAACTCAAATC	CAAACAAGAAGGCTAAA	8	182
mTcCIR251	(CT)7 (CA)12	TCTATGGGATTTGATGAG	AGATACAGCAGGAACACA	9	188
mTcCIR155	(TC)12	CTTGGACTATTGGAAAAC	AAGGATACAATAAGGTAAATAC	10	274

Source: Pugh *et al.* (2004).

0.2 µM each primer and 1 PCR mix. Amplification of PCR products was performed with the following profile: pre-denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 15 seconds, 53°C for 15 seconds, 72°C for 15 seconds, and a final extension at 72°C for 10 minutes. The amplified SSR loci were separated on 6% non-denaturing polyacrylamide gel electrophoresis using 1 TBE buffer. The gels were stained with ethidium bromide for 20 minutes and DNA bands were visualized under UV light using the gel documentation system.

Data Analysis

The polymorphic fragments were scored visually in binary format (allele presence = 1 and allele absence = 0). A dendrogram was constructed based on the similarity matrix using NTSYS version 2.1 (Rohlf 2000). A similarity matrix was calculated using Dice coefficient followed by cluster analysis with the SAHN subprogram using the UPGMA clustering method as implemented in NTSYS. Principal component analysis (PCA) based on the genetic similarity matrix was carried out using DCENTER and EIGEN algorithm of the NTSYS-PC software package. Genetic distances between 12 cacao genotypes were calculated as 1-similarity.

Moreover, in present study we also calculated number of alleles (N_A), specific alleles (S_A), major allele frequency (M_{AF}), gene diversity (GD), expected heterozygosity (H_e) and polymorphic information content (PIC) values by using PowerMarker version 3.25 (Liu and Muse 2005). Specific allele refered to alleles with frequencies of less than 5% among the 12 cacao genotypes and major allele frequency (M_{AF}) was defined as allele with the highest frequency.

RESULTS AND DISCUSSION

Diversity of SSR Markers

Result of polymorphic survey showed that 14 markers exhibited polymorphic, whereas one marker showed monomorphic and another one showed no substantial amplification product. Genotyping analysis of 14 polymorphic SSR markers demonstrated that all cacao genotypes had clear and distinguish-able bands (Fig. 1). Therefore, all of polymorphic SSR loci resulted in this study can be scored and subsequently used for statistical analysis.

The polymorphic loci generated 70 alleles across 12 cacao genotypes. The number of alleles per locus ranged from 2 alleles (mTcCIR162) to 9 alleles (mTcCIR255) with an average of 5 alleles per locus. Total number of alleles as well as an average number of alleles obtained here were significantly higher than those of previous study done by Silva *et al.* (2011), which resulted 26 alleles (average 3.25) throughout eight SSR loci examined in cacao population derived from Brazilian Amazon. However, an average number of alleles produced in this study was lower than those obtained by Zhang *et al.* (2009) that was 14.2 alleles per locus and the one resulted by Kurniasih *et al.* (2011) that was 5.50 alleles per locus. The difference in these results might be due to differences in cacao accessions used and different numbers of SSR markers investigated. On the other hand, PIC values were also calculated, which were ranging from 0.14 (mTcCIR162) to 0.82 (mTcCIR255) with an average of 0.59. Eleven of 14 polymorphic SSR loci produced PIC values of 0.5 or above. The average value of gene diversity and expected heterozygosity were 0.64 and 0.53, respectively (Table 3). Marker mTcCIR255 presented the highest number of gene diversity,

Fig. 1. Genotyping analysis of two polymorphic SSR markers (primer CIR190 and CIR155) separated using 6% non-denaturing polyacrylamide gel electrophoresis. Lanes: K1, Sulawesi 1; K2, IAARD 12; K3, ICCRI 03; K4, ICCRI 04; K5, IAARD 2; K6, IAARD 9; K7, IAARD 11; K8, Sulawesi 2; K9, IAARD 4; K10, IAARD 1; K11, IAARD 6; K12, IAARD 7. Sulawesi 1, Sulawesi 2, ICCRI 03 and ICCRI 04 are released cacao varieties; IAARD 1, IAARD 12, IAARD 4, IAARD 2, IAARD 11, IAARD 6, IAARD 9 and IAARD 7 are local cacao genotypes collected from Kolaka, Southeast Sulawesi.

Table 3. Allelic diversity of 14 polymorphic SSR markers across 12 cacao genotypes.

Marker name	Allele number	Specific alleles[1)]	Major allele frequencies[2)] (%)	Genetic diversity	H_o[3)]	PIC[4)]
mTcCIR255	9	3	0.29	0.84	0.67	0.82
mTcCIR76	6	2	0.63	0.58	0.33	0.55
mTcCIR190	5	1	0.33	0.75	0.92	0.71
mTcCIR82	4	1	0.75	0.41	0.33	0.39
mTcCIR251	6	2	0.38	0.74	0.83	0.69
mTcCIR144	3	-	0.54	0.59	0.25	0.52
mTcCIR155	3	-	0.46	0.64	0.58	0.57
mTcCIR184	8	1	0.29	0.82	0.42	0.79
mTcCIR69	5	1	0.42	0.70	0.67	0.66
mTcCIR162	2	-	0.92	0.15	0.17	0.14
mTcCIR211	3	-	0.58	0.56	0.25	0.49
mTcCIR291	5	1	0.38	0.74	0.42	0.69
mTcCIR109	5	-	0.42	0.71	0.83	0.67
mTcCIR209	6	1	0.54	0.66	0.75	0.63
Mean	5	0.93	0.49	0.64	0.53	0.59

[1)]Specific alleles are referred to alleles with a frequency of less than 5%.
[2)]Major allele is referred to the allele with the highest frequency.
[3)]Expected heterozygosity.
[4)]Polymorphic information content.

while marker mTcCIR190 revealed the highest number of expected heterozygosity. This finding indicates that SSR markers used in present study have a high degree of diversity and would be potential to discriminate cacao genotypes. Furthermore, SSR markers which have high values of PIC and gene diversity can be chosen as prospective markers to fingerprint cacao germplasms in order to improve cacao breeding programs in the future.

Major allele frequencies were also determined at each locus, ranging from 29% (mTcCIR255 and mTcCIR184) to 92% (mTcCIR162) with an average of 49%. About 49% of the 12 cacao genotypes showed common major alleles in all of 14 polymorphic SSR markers observed in present study. In addition, we also found 13 specific alleles at nine SSR loci as shown in Table 3. Specific allele was defined as alleles with a frequency of less than 5%. Marker mTcCIR255 had the highest number of specific alleles compare to the other eight markers. The specific alleles displayed unique fingerprinting that can distinguish certain genotypes from the others. As mentioned by Izzah *et al.* (2013), specific alleles offered an effective means for cultivar identification, cultivar protection and DUS testing.

Genetic Diversity and Phylogenetic Relationships of 12 Cacao Genotypes

Genetic similarity values between cacao genotypes were used to generate a dendrogram as shown in Figure 2, which clearly explained the relationships among the 12 cacao genotypes. A similarity coefficient of 36% was used as the threshold level for UPGMA clustering, of which all cacao genotypes were successfully classified into three main groups (Fig. 2). The first group (group I) represented five genotypes that consisted of three local cacao genotypes and two released cacao varieties, whereas the second group (group II) comprised four cacao genotypes. The third group (group III) held three cacao genotypes. Furthermore, a similarity coefficient was also used to produce a two-dimensional scale diagram (PCA analysis, Fig. 3). The groupings obtained by PCA were also similar to those identified by the UPGMA cluster analysis.

In detail, group I contained five cacao genotypes including Sulawesi 1, IAARD 1, ICCRI 04, IAARD 12 and IAARD 4. Sulawesi 1 and ICCRI 04 are released cacao varieties. Meanwhile, group II comprised four cacao genotypes including IAARD 2, IAARD 11 and IAARD 6, which clustered together with a cacao varieties that have been released, i.e. Sulawesi 2. These four cacao genotypes were clustered in group II at a similarity coefficient of 39%. This clustering result demonstrated that six local cacao genotypes obtained from Kolaka shared at about 36-39% similarity with three released cacao varieties. On the other hand, group III which consisted of three cacao genotypes including ICCRI 03, IAARD 7 and IAARD 9 was separated at a genetic similarity of 42%. Interestingly two local genotypes IAARD 7 and IAARD 9 showed

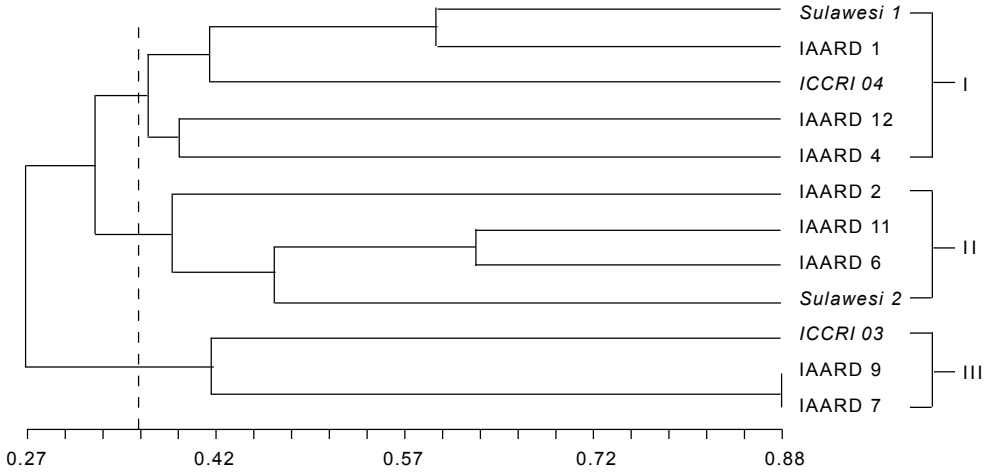

Fig. 2. Cluster analysis of 12 cacao genotypes based on the similarity matrix. Four released cacao cultivars are identified by italic letter; IAARD 1, IAARD 12, IAARD 4, IAARD 2, IAARD 11, IAARD 6, IAARD 9 and IAARD 7 are local cacao genotypes obtained from Kolaka, Southeast Sulawesi.

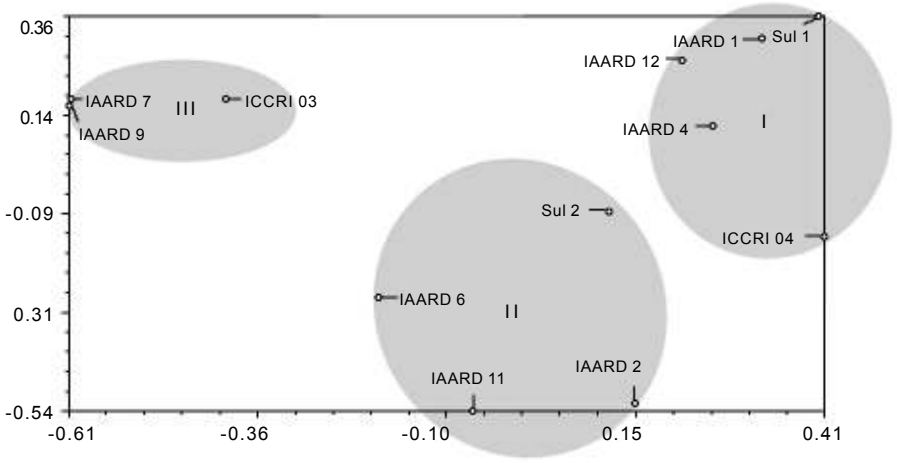

Fig. 3. Diagram showing the relationships among 12 cacao genotypes based on principal component analysis (PCA) using 14 SSR markers. Three clusters (I, II and III) are shaded in grey. Sulawesi 1, Sulawesi 2, ICCRI 03 and ICCRI 04 are released cacao varieties; IAARD 1, IAARD 12, IAARD 4, IAARD 2, IAARD 11, IAARD 6, IAARD 9 and IAARD 7 are local cacao genotypes collected from Kolaka, Southeast Sulawesi.

genetically identical at similarity coefficient of 88% even though they have different names. Possible reason is the two cacao genotypes in fact are same genotypes but adopted by diffèrent farmers and then given by different names. This phenomenon often found in germplasm exploration activities. Therefore, genetic diversity analysis using molecular markers is really helpful to differentiate a genotype from another. Nevertheless, one of these two genotypes that clustered separately from the other local genotypes would be a good candidate for breeding material. In addition, both of these genotypes (IAARD 7 and IAARD 9) have several advantages including weight per dried bean of >1 g and fat content of dried beans about 55% (data not shown).

Genetic distance between 12 cacao genotypes used in this study was also calculated as shown in Table 4. Knowing the genetic distance value is very important in order to determine the best parental line for crossing combination. The best combination was chosen between accessions which have the highest genetic distance value. In this study, the highest genetic distance value was 0.88 that found between ICCRI 04 and ICCRI 03 (Table 4). That value indicates the best parental line combination which can be used for crossing to obtain superior hybrids. Conversely, the lowest genetic distance value (0.47) exhibited that the combination should not be used for crossing. In addition, we also found that seven combinations of cacao accessions had genetic distance value of

Table 4. Genetic distance matrix of 12 cacao genotypes.

Clone	Sulawesi 1	IAARD 12	ICCRI 03	ICCRI 04	IAARD 2	IAARD 9	IAARD 11	Sulawesi 2	IAARD 4	IAARD 1	IAARD 6	IAARD 7
Sulawesi 1	0											
IAARD 12	0.57	0										
ICCRI 03	0.76	0.70	0									
ICCRI 04	0.55	0.73	0.88	0								
IAARD 2	0.78	0.72	0.83	0.59	0							
IAARD 9	0.76	0.74	0.56	0.82	0.75	0						
IAARD11	0.74	0.75	0.74	0.69	0.56	0.66	0					
Sulawesi 2	0.55	0.68	0.74	0.62	0.62	0.63	0.59	0				
IAARD 4	0.65	0.61	0.83	0.68	0.71	0.77	0.70	0.63	0			
IAARD 1	0.40	0.64	0.80	0.62	0.73	0.71	0.71	0.53	0.53	0		
IAARD 6	0.69	0.67	0.58	0.72	0.65	0.53	0.77	0.47	0.61	0.64	0	
IAARD 7	0.78	0.80	0.59	0.81	0.77	0.13	0.69	0.66	0.72	0.69	0.56	0

Sulawesi 1, Sulawesi 2, ICCRI 03 and ICCRI 04 are released cacao varieties; IAARD 1, IAARD 12, IAARD 4, IAARD 2, IAARD 11, IAARD 6, IAARD 9 and IAARD 7 are local cacao genotypes collected from Kolaka, Southeast Sulawesi.

>80%, higher than the other combinations (Table 4). Therefore, these seven combinations can be selected as parental lines to generate superior cacao progenies that would be useful for cacao breeding program, particularly to release new varieties.

Information about genetic distance value will help breeders to select the best parental lines without any doubts. This result is in accordance with the statement of Rubiyo (2013) that in order to increase the possibility of obtaining new superior varieties, there should be crossing between two parental genotypes that have high genetic distance value. However, in other plants genetic similarity value among parents was poorly correlated with yield for instance in barley (Kim *et al.* 2010) and maize (Balestre *et al.* 2008). Nevertheless, in maize, there was a medium correlation between genetic distance and heterosis (r = 0.40) as well as genetic distance and specific combining ability (r = 0.38) (Balestre *et al.* 2008). Another study done by Hung *et al.* (2012) in maize exhibited that there was a positive correlation between phenotypic parental distances and within-family genetic variance estimates for about half of the traits measured. Thus, the choice of promising segregating populations can be based on selecting phenotypically diverse parents.

Overall, the results obtained in present study is known to be more complete than previous study done by Kurniasih *et al.* (2011) also Syafaruddin and Nasution (2012), which did not show about principal component analysis (PCA) and calculate genetic distance value. Nevertheless, some of cacao genotypes used in this study were same with the two previous studies. For instance, two local cacao genotypes, IAARD 9 and IAARD 12 were the same to those used by Syafaruddin and Nasution (2012). Meanwhile, the two released varieties ICCRI 03 and ICCRI 04 were also used by Kurniasih *et al.* (2011). However, in comparison with those two previous studies, we analyzed the data in detail which would give more information to the breeders on how to select the best cacao genotype as parental lines for artificial crossing.

CONCLUSION

The result obtained in present study could give a new insight regarding cacao genetic diversity collected from Kolaka and would be useful for future breeding programs. Based on UPGMA clustering, the local genotypes either IAARD 7 or IAARD 9 which clustered separately from the other genotypes, can be selected as clonal candidate for cacao crop improvement. The other considerations are these two genotypes (IAARD 7 and IAARD 9) have a good bean quality shown by weight per dried bean of >1 g and fat content of dried beans about 55%. Besides, we also found seven crossing combinations with high value of genetic distance that could be used to generate superior progenies for cacao breeding. Furthermore, we proved that SSR markers revealed as a powerful tool for assessing genetic diversity and phylogenetic relationships of cacao germplasms.

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