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Genetic diversity among 24 clones of robusta coffee in Lampung based on RAPD markers

SRI RAMADIANA, DWI HAPSORO♥, RUSDI EVIZAL, KUKUH SETIAWAN, AGUS KARYANTO, YUSNITA YUSNITA

Department of Agronomy and Horticulture, Faculty of Agriculture, Universitas Lampung. Jl. Sumantri Brojonegoro No. 1, Bandar Lampung 35145, Lampung, Indonesia. Tel. +62 813-7915-5175, ♥email: dwi.hapsoro@fp.unila.ac.id

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Abstract. *Ramadiana S, Hapsoro D, Evizal R, Setiawan K, Karyanto A, Yusnita Y. 2021. Genetic diversity among 24 clones of robusta coffee in Lampung based on RAPD markers. Biodiversitas 22: 3122-3129.* This study aimed to estimate the genetic diversity among 24 clones of Robusta coffee from Lampung, Indonesia, by use of RAPD markers. The clones consisted of 18 local and 6 BP clones. These BP clones were developed from a breeding program of The Indonesian Coffee and Cocoa Research Institute. Genomic DNAs extracted from these clones were subjected to polymerase chain reaction and the amplified products were run using gel electrophoresis. Eleven random primers produced clear, reproduceable, scorable bands. Fifty four of 86 bands showed polymorphism and were used to construct a dendrogram based on UPGMA Jaccard's Similarity Coefficients. The genetic base of the population was narrow (average genetic similarity 68.4%), ranging from 26-93%. The genetic similarity of the local clones was higher than that of BP clones. The clones were clusterred into five groups. Group 1 contained one clone (BP 534), while each of Group II-V contained more than one clone. The average genetic similarity of BP 534 to each clone of Group II-V was 41%. The genetic similarity of clones in Group II, III, IV, and V were 55.5%, 43.0%, 81.1%, and 80.1%, respectively. This research should be very useful for selecting parents in a breeding program to produce better clones of Robusta coffee.

Keywords: *Coffea canephora*, clones, dendrograms, Indonesia, PCR, similarity, UPGMA

INTRODUCTION

Coffee belongs to the genus *Coffea*, sub-genus *Coffea*, family Rubiaceae and grows mostly in tropical and subtropical regions (Ferreiraa et al. 2019). The Arabica coffee (C. *arabica* L.) and Robusta coffee (C. *canephora* Pierre ex A. Froehner) are the two most cultivated coffee species world-wide which account for 60% and 40% of total world coffee production, respectively (Davis et al. 2019). Compared to the Arabica coffee, the Robusta coffee has higher caffeine content, wider geographical distribution, and more resistant to nematodes (Hendre and Aggarwal 2014) and leaf rust disease (Ulubelu Cofco Abadi. 2012). Indonesia is the fourth largest coffee producer in the world after Brazil, Vietnam, and Columbia (Eleven Coffees 2021; Walton 2020) and the third largest producer of Robusta coffee (Walton 2020). Lampung Province is one of the coffee production centers in Indonesia. However, the data presented by the Directorate General of Estate Crop (2018) revealed that coffee productivity in Lampung is only 722 kg ha^{-1}, much lower than that in Brazil (1,800 kg ha^{-1}) (USDA 2018), the leading producer. Not only is this low productivity caused by suboptimal cultural practices but also the use of low-yielding clones. However, the introduction of high-yielding clones sometimes resulted in variable successes because of their low adaptability to Lampung agroclimatic condition. Therefore, breeding programs should take into account gene pools of local origin and their genetic diversity. Knowing this genetic diversity is very important in order to be able to determine effective strategies to produce better clones. Since Robusta coffee is a cross-pollinating plant, crosses among genetically-distant genotypes would expectedly lead to maximum heterosis.

A pioneer study on genetic diversity of Robusta coffee was undertaken by Berthaud (1986) who identified two main diversity groups, i.e. Congolese group (from the Democratic Republic of Congo, the Central African Republic, and Cameroon) and Guinean group (from Guinea and the Ivory Coast). Montagnon et al. (1992) proposed that the Congolese group be divided into two subgroups, i.e. SG1 and SG2. Gomez et al. (2009), using 107 genotypes of Robusta coffee selected from those used by Dussert et al. (1999), identified five diversity groups across West and Central Africa, i.e. groups A (Congo and Cameroon), B (eastern-central Africa), C (western-central Africa, Cameroon, and northeastern Congo), D (Guinea and Ivory Coast), and E (Congo and Southern Cameroon).

Genetic diversity within Robusta coffee cultivated in Lampung is needed for developing a breeding strategy to produce better clones. Genetic diversity of plant genotypes could be studied on the basis of morphological (Ngugi et al. 2019), biochemical (Mahmoud and Abd EL-Fatah 2020), and molecular (Motta et al. 2014; Omingo et al. 2017; Sousa et al. 2017) markers. One of the drawbacks of using morphological and biochemical markers is that they are influenced by the environment, while molecular markers are not influenced by the environment (Bekele and

Bekele 2014; Nadeem et al. 2018). One of such molecular markers used for the study of genetic diversity in crop plants is Random Amplified Polymorphic DNA (RAPDs). For quite many researchers, this marker is still a marker of choice because it is simple, applicable to any genomes, does not need sequence information and needs relatively small DNA quantities (Dhakshanamoorthy et al. 2015). RAPD markers have been reported to be an effective tool to detect genetic variation at DNA level in various plants such as date palm (Marsafari and Mehrabi 2013; Srivashtav et al. 2013), teak (Chaudhari et al. 2018; Chhajer et al. 2018), sugarcane (Hapsoro et al. 2015; Singth et al. 2017), tea (Islam et al. 2013; Martono and Syafarudin 2018), rice (Alam et al. 2014; Karande et al. 2017), and soybean (Al-Saghir and Salam 2011; Malik et al. 2017).

Studies on genetic diversity of Robusta coffee based on RAPD markers have also been reported (Awati et al. 2018; Achar et al. 2015: Tshilenge et al. 2009; Diniz et al. 2005; Cabral et al. 2002). Still, such a study is needed on Robusta coffee grown in a particular agroclimatic condition, such as that of Lampung. Results of this study could be used as the basis of selecting parental clones for hybridization to produce better clones of Robusta coffee adaptable to Lampung agroclimatic condition. This research was conducted with aim of investigating genetic diversity of 24 clones of Robusta coffee from Lampung, Indonesia, using RAPD markers.

MATERIALS AND METHODS

Plant materials

Twenty four coffee clones were used in this study (Table 1). The tested clones consisted of 18 local and 6 BP clones. The local clones were those developed by local farmers through selection and considered to be clones of choice. The BP clones were developed from a breeding program of The Indonesian Coffee and Cocoa Research Institute (ICCRI) and have been officially released by the Indonesian Department of Agriculture. BP actually stands for Besoekisch Proefstation, the former name of the ICCRI. The BP clones were cordially provided by The Office of Industrial Crop Research (Balittri) of North Lampung, Indonesia. All the tested clones were currently cultivated by local farmers (Table 1, Figure 1). Single node cuttings of each clone were planted in a mixture of soil and compost (1:1 v/v) contained in polybags, (one cutting per polybag), and maintained for 6-10 months in a greenhouse of the Faculty of Agriculture, the University of Lampung.

Genomic DNA extraction and PCR condition

Genomic DNA was isolated from healthy, disease-free leaves from second and third nodes from the growing tips of the coffee branches. Genomic DNA was extracted according to Diniz et al. (2005) with minor modifications using mixed alkyltrimethylammonium bromide (MATAB). The isolated genomic DNA was of high quality as indicated by the quality test where genomic DNA appeared as one band of high molecular weight in a gel electrophoresis and the value of absorbance of the DNA solution at 260 nm divided by that of 280 nm (A260/A280) was 1.8-2.0.

The genomic DNA was subjected to polymerase chain reaction (PCR) using Eurogene thermocycler. The PCR reaction was carried out at a volume of 25 µl in a 200 µl-microtube, 12.5 µl of PCR kit (QIAGEN), 50 ng/µl of template DNA, and 2 µl of a random primer. The PCR condition was set up as follows: one cycle of initial denaturation at 94°C for 3 minute followed by 30 cycles of denaturation at 94°C for 1 second, annealing at 37°C for 1 minute, and extension at 72°C for 2 minutes. Then the final extension was done at 72°C for 10 minutes. The amplified products were electrophoresed at 60 volt, 400 watt, for 2 hours in 1.2% (w/w) agarose gel, stained in ethidium bromide solution, and then visualized in a UV trans-illuminator. Initially, a set of 40 primers selected from the studies of Achar et al. (2015), Gimase et al. (2014), and Kathurima et al. (2012) were prescreened for PCR optimization of genomic DNA of 24 clones of Robusta coffee (*Coffea canephora* Pierre ex A. Froehner). Eleven out of the 40 primers produced sharp, clear, and reproduceable bands and were used in this study (Table 2).

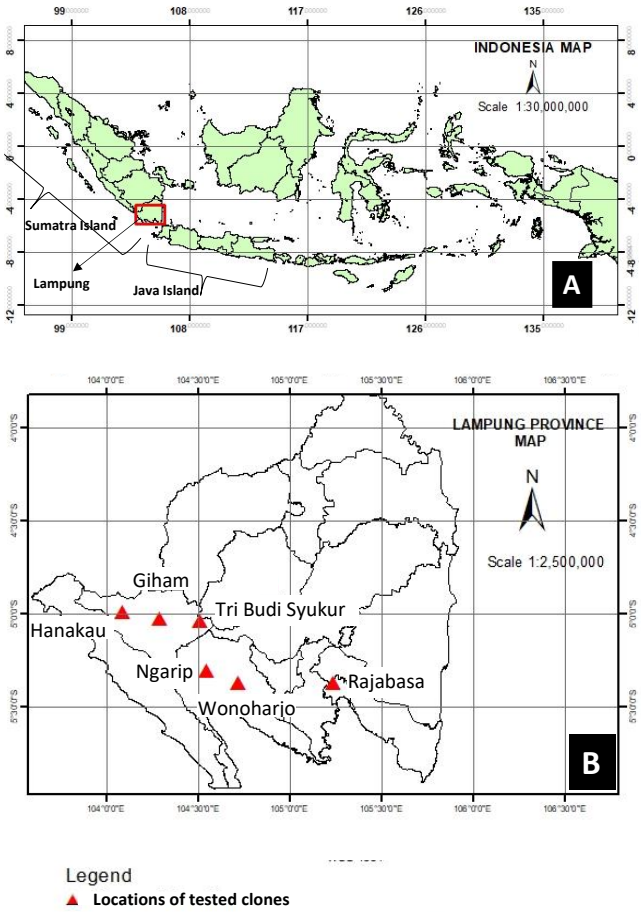

Figure 1. Locations of tested Robusta coffee clones used in this study. A. A map of Indonesia showing where Lampung Province, Sumatra Island, and Java Island are. B. A map of Lampung Province and locations of tested clones as indicated by triangles

Table 1. The Coffea canephora clones used in this study and their geographical locations

Clones	Village, sub district, district, province	Coordinate points	Elevation (m asl.)
Bakir 1	Rajabasa, Rajabasa, Bandar Lampung, Lampung	N 5º,22',19"; E 105º,14',15"	135
Bakir 2	Rajabasa, Rajabasa, Bandar Lampung, Lampung	N 5º,22',19"; E 105º,14',15"	135
Bakir 3	Rajabasa, Rajabasa, Bandar Lampung, Lampung	N 5º,22',19"; E 105º,14',15"	135
Bakir 4	Rajabasa, Rajabasa, Bandar Lampung, Lampung	N 5º,22',19"; E 105º,14',15"	135
Komari	Ngarip, Ulu Belu, Tanggamus, Lampung	N 5º,18',28"; E 104º,32',56"	874
Tugino	Ngarip, Ulu Belu, Tanggamus, Lampung	N 5º,18',28"; E 104º,32',56"	874
Wanto	Ngarip, Ulu Belu, Tanggamus, Lampung	N 5º,18',28"; E 104º,32',56"	976
Wardi	Ngarip, Ulu Belu, Tanggamus, Lampung	N 5º,18',28"; E 104º,32',56"	976
Lengkong	Tribudi Syukur, Kebun Tebu, West Lampung. Lampung	N 5º,02',15"; E 104º,30',45"	860
Tugu Biru	Tribudi Syukur, Kebun Tebu, West Lampung, Lampung	N 5º,02',15"; E 104º,30',45"	860
Rope Dale	Tribudi Syukur, Kebun Tebu, West Lampung, Lampung	N 5º,02',15"; E 104º,30',45"	860
Srintil	Tribudi Syukur, Kebun Tebu, West Lampung, Lampung	N 5º,02',15"; E 104º,30',45"	860
Rona	Tribudi Syukur, Kebun Tebu, West Lampung, Lampung	N 5º,02',15"; E 104º,30',45"	860
Imam 1	Giham, Sekincau, West Lampung, Lampung	N 5º,01',31"; E 104º,17',21"	1,034
Garudak	Tribudi Syukur, Kebun Tebu West Lampung, Lampung	N 5º,02',15"; E 104º,30',45"	860
Aegawa	Hanakau, Sukau, West Lampung, Lampung	N 4º,59',27"; E 104º,05',12"	865
Bodong	Wonoharjo, Sumber Rejo, Tanggamus, Lampung	N 5º,22',07"; E 104º,43',00"	461
Blirik	Wonoharjo, Sumber Rejo, Tanggamus, Lampung	N 5º,22',07"; E 104º,43',00"	461
BP 538	These BP clones were developed from a breeding program by		
BP 203	The Indonesian Coffee and Cocoa Research Institute and		
BP 534	have been officially released by The Indonesian Department		
BP 42	of Agriculture.		
BP 913			
BP 308			

The bands were scored for presence (1) and absence (0) for each clone. The data were organized into a matrix and subjected to cluster analysis using R statistical software (Venables et al. 2020). A dendrogram was constructed using similarity matrix calculation function and unweighted pair-group method using arithmetic averages (UPGMA) (Venables et al. 2020). The similarity data were also subjected to principal coordinate analysis (PCA) to generate groupings of the tested clones using GenAlEx 6.41 software (Peakall and Smouse 2012)

RESULTS AND DISCUSSION

The eleven selected primers amplified 86 DNA fragments, of which 54 were polymorphic (62.8%) (Table 2). The number of bands per primer varied from 6-11 with an average of 7.8. The amplified products ranged in size from 100-2000 bp. OPE 18 generated the highest number of bands (11) ranging from 250-2000 bp, while OPN 18, OPR 1, and OPO 12 produced the lowest number of bands (6) ranging from 300-1500 bp. The polymorphism level ranged from 36-100% per primer, with an average of 64%. The highest polymorphism level (100%) was obtained by OPN 18, while the lowest (29%) by OPL 18. Figure 2 showed an example of a banding pattern of amplified products of PCR using the tested clones'DNA as templates and primer OPO 5.

Table 3 showed the level of genetic similarities among the 24 Robusta clones as indicated by UPGMA Jaccard's similarity coefficients. The data revealed that genetic similarity was in the range of 25.6% (BP 534 and Blirik) to 93.0% (Bakir 1 and Lengkong), with an average of 68.4%. This high percentage indicates that the clones were genetically similar to each other. In Lampung, coffee plants, including Robusta coffee, are introduced plants, which originated from Africa (Herrera and Lambot 2017). Around the year 1900, non-selected genetic materials of Robusta coffee were directly brought from Uganda and Democratic Republic of the Congo to Java and indirectly brought to Java through Europe, and then subjected to selection from 1900-1930 (Montagnon et al. 1998). Java became a primary selection center of Robusta coffee, and some of the selected genetic materials were then sent to Brazil around 1912 and sent back to Uganda and Democratic Republic of the Congo around 1916 (Montagnon et al. 1998).

Cramer (1957), based on literature on coffee researches in Indonesia, stated that Robusta coffee plants were imported from the House of Horticulture of L. Linden (Brussels) to Java in the year 1900 and distributed to several plantations in East Java. The germplasms that were coming from the center of genetic diversity of Robusta coffee were used by the ICCRI (formerly Besoekisch Proefstation) to produce superior clones. Kristian (2019) stated that Robusta coffee was introduced to Lampung from Java around 1910 by the Government of Dutch East Indies.

Table 2. Random primers used in PCR reaction using genomic DNA of 24 clones of *Coffea canephora* (var. *robusta*) as templates

Name of primers	Reference	Primer sequences (5'– 3')	Number of bands	Number of polymorphic bands	Polymorphic bands (%)	Sizes of the amplified products (bp)
OPR 1	Achar et al. (2015)	TGCGGGTCCT	6	3	50	500-1500
OPN 16	Achar et al. (2015)	AAGCGACCTG	8	6	75	250-1500
OPO 12	Achar et al. (2015)	CAGTGCTGTG	6	4	67	400-1500
OPM 13	Achar et al. (2015)	GGTGGTCAAG	7	4	57	375-1500
OPO 5	Achar et al. (2015)	GTGTCTCAGG	10	8	80	100-1750
OPO 15	Achar et al. (2015)	CCAAGCTGCC	9	4	44	300-2000
OPL 18	Achar et al. (2015); Gimase et al. (2014)	ACCACCCACC	7	2	29	350-1500
OPN 18	Achar et al. (2015); Gimase et al. (2014); Kathurima et al. (2012)	GGTGAGGTCA	6	6	100	300-1000
OPG 03	Kathurima et al. (2012)	GAGCCCTCCA	8	7	87.5	375-1500
OPE 18	Kathurima et al. (2012)	GGACTGCAGA	11	4	36	250-2000
OPI 07	Kathurima et al. (2012); Gimase et al. (2014)	CAGCGACAAG	8	6	75	300-1250
Total			86	54		
Average			7.8	4.9	62.8	

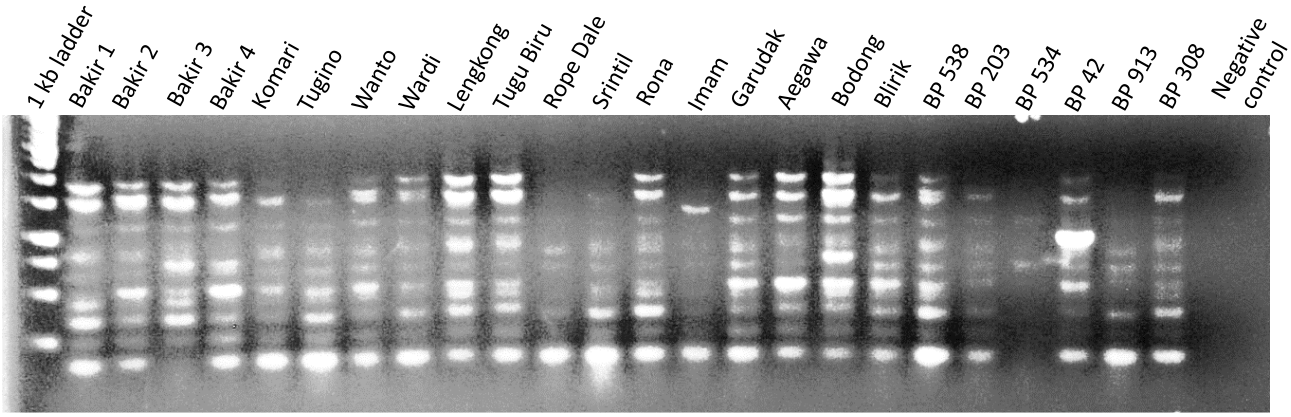

Figure 2. Banding pattern of amplified PCR products of genomic DNA from 24 Robusta coffee clones amplified with primer OPO 5

Since Java became a primary selection center of Robusta coffee from 1900-1930 (Montagnon et al. 1998), it is probable that the introduced clones in Lampung were the results of selection, so they were probably genetically narrow. The local farmers then grew them from seeds and vegetative parts and did continuous plant selection. They selected better coffee plants, took some branches (as scions) and grafted them on branches of mature plants (as rootstocks). These practices led to identification of some local superior clones of Robusta coffee. Since these selected local clones were derived from genetically limited germplasms, they understandably displayed high genetic similarity as shown in our study. Syafaruddin et al. (2014) also reported high genetic similarity (60%) of farmer-selected Robusta coffee in Bengkulu Province (also in Sumatra island). They did not mention where the Robusta coffee plants in Bengkulu came from, but Kristian (2019) stated that Robusta coffee in Bengkulu was also introduced from Java. As what happened in Lampung, the local farmers in Bengkulu did selection against seed-derived plants, then vegetatively propagated the selected ones, resulting in clones of farmer choice. This might be the reason for the high similarity.

Our study showed that BP clones showed lower genetic similarity (58.7%) than the local ones (73.5%). The reason might be as follows. The BP clones were developed from a breeding program using germplasm from Africa, which had wide genetic base. The local clones, on the other hand, as described before, were developed by local farmers from selected germplasm introduced from Java (Kristian 2019), which had narrow genetic base.

Some studies on genetic diversity of Robusta coffees in some regions in Africa showed lower genetic similarity, for example in Uganda 40.0% (Ngugi and Aluka 2019), Tanzania 28.8% (Ng'homaa et al. 2017), and Congo 39.0% (Tshilenge et al. 2009). The disparity of results between these studies and our study might be mostly due to the origin of the tested population. While these studies used populations from accessions conserved in ex-situ collections (Tshilenge et al. 2009), cultivated landraces and

gene bank accessions (Ngugi and Aluka 2019), and cultivated and wild Robusta coffee (Ng'homaa et al. 2017), our study used population developed from selection. Because of their high diversity, some of the accessions used by these authors could be good candidates for improving Robusta coffee in Lampung.

The dendrogram constructed on the basis of UPGMA Jaccard's similarity coefficients showed that the tested clones were divided into two main clusters (Figure 3). The first cluster consisted of only one clone (BP 534), and the second cluster consisted of all the local clones (18 clones) and 5 BP clones. Based on PCA analysis, the two clusters were divided into five groups (Figure 4). Table 4 showed the average values of genetic similarity of each group, with Group IV showing the highest (81.1%), followed by Group V (80.1%), Group II (55.3%), and Group III (43.0%) (Table 4).

Figure 4 showed that the BP clones were positioned in different groups, i.e. one in Group I, one in Group II, one in Group III, and three in Group V. As for the local clones, most of them (12 clones) were positioned in Group V, two in Group II, one in Group III, and three in Group IV (Figure 4). Some BP clones were positioned in the same groups with the local clones. One possible reason was that some BP clones were introduced to local farmers in some regions of Lampung, then hybridization occurred with the local clones, leading to new local ones. Being alone in Group I, clone BP 534 shared small portion of its genetic composition with each member of other groups as indicated by the relatively small genetic similarity (41%) with clones in the other groups (Table 3, Figure 3).

The PCA analysis also revealed no location-specific groupings of the tested clones. Some clones cultivated in the same locations were clustered in different groups. For example, clones Bakir 1, Bakir 2, Bakir 3 and Bakir 4 were cultivated in the same location (Rajabasa), but they were positioned in different groups. One group could also consist of clones of different locations. For example, each of Group II and Group III contained clones cultivated in different locations.

Table 4. Clustering of the tested Robusta coffee clones from Lampung into groups presented on the basis of PCA analysis. The average genetic similarity among clones in each group is presented

Group I	Group II	Group III	Group IV	Group V	
BP 534	BP 913	Bakir 3	Wanto	BP 308	Tugu Biru
	Blirik	BP 203	Bakir 4	BP 42	Komari
	Imam 1		Bakir 2	Srintil	Wardi
				Rope Dale	BP 538
				Tugino	Bodong
				Garudak	Lengkong
				Rona	Bakir 1
				Aegawa	
-	55.3%	43.0%	81.1%	80.1%	
			Genetic similarity		

Table 3. UPGMA Jaccard's Similarity Coefficients generated from RAPD data of 24 clones of *Coffea canephora* from Lampung, Indonesian

	Bakir 1	**Bakir 2**	**Bakir 3**	**Bakir 4**	**Komari**	**Tugino**	**Wanto**	**Wardi**	**Lengkong**	**Tugu Biru**	**Rope Dale**	**Srintil**	**Rona**	**Imam 1**	**Garudak**	**Aegawa**	**Bodong**	**Blirik**	**BP 538**	**BP 203**	**BP 534**	**BP 42**	**BP 913**	**BP 308**
Bakir 1	1																							
Bakir 2	0.818	1																						
Bakir 3	0.683	0.595	1																					
Bakir 4	0.733	0.767	0.63	1																				
Komari	0.8	0.756	0.62	0.674	1																			
Tugino	0.739	0.696	0.6	0.689	0.881	1																		
Wanto	0.778	0.814	0.6	0.854	0.717	0.696	1																	
Wardi	0.841	0.837	0.66	0.711	0.818	0.756	0.756	1																
Lengkong	0.93	0.8	0.67	0.717	0.864	0.8	0.761	0.86	1															
Tugu Biru	0.886	0.8	0.67	0.717	0.907	0.8	0.761	0.82	0.909	1														
Rope Dale	0.818	0.733	0.56	0.652	0.837	0.773	0.696	0.8	0.841	0.841	1													
Srintil	0.773	0.727	0.59	0.682	0.791	0.81	0.767	0.75	0.837	0.795	0.854	1												
Rona	0.818	0.773	0.68	0.767	0.881	0.814	0.773	0.8	0.884	0.884	0.773	0.81	1											
Imam 1	0.581	0.5	0.38	0.455	0.595	0.571	0.5	0.6	0.643	0.605	0.692	0.68	0.61	1										
Garudak	0.795	0.791	0.65	0.705	0.814	0.791	0.711	0.81	0.86	0.818	0.75	0.79	0.833	0.585	1									
Aegawa	0.787	0.783	0.61	0.702	0.886	0.822	0.745	0.89	0.848	0.889	0.783	0.78	0.822	0.556	0.8	1								
Bodong	0.867	0.783	0.65	0.739	0.804	0.783	0.783	0.84	0.889	0.889	0.745	0.78	0.822	0.591	0.761	0.87	1							
Blirik	0.614	0.533	0.45	0.489	0.628	0.605	0.568	0.67	0.674	0.636	0.605	0.63	0.643	0.629	0.659	0.622	0.622	1						
BP 538	0.907	0.818	0.64	0.733	0.8	0.739	0.778	0.88	0.886	0.844	0.818	0.77	0.778	0.619	0.756	0.787	0.909	0.651	1					
BP 203	0.622	0.732	0.43	0.643	0.636	0.651	0.614	0.64	0.609	0.644	0.651	0.64	0.614	0.475	0.591	0.667	0.667	0.378	0.659	1				
BP 534	0.395	0.415	0.34	0.4	0.405	0.415	0.415	0.44	0.419	0.419	0.45	0.47	0.381	0.394	0.425	0.409	0.442	0.256	0.463	0.457	1			
BP 42	0.721	0.674	0.61	0.667	0.78	0.756	0.674	0.7	0.744	0.786	0.714	0.75	0.846	0.622	0.69	0.689	0.767	0.575	0.762	0.585	0.41	1		
BP 913	0.548	0.537	0.49	0.564	0.6	0.615	0.5	0.56	0.571	0.571	0.575	0.61	0.615	0.594	0.59	0.523	0.558	0.5	0.585	0.556	0.43	0.68	1	
BP 308	0.756	0.667	0.69	0.659	0.732	0.667	0.667	0.69	0.738	0.738	0.667	0.7	0.75	0.568	0.769	0.644	0.721	0.564	0.756	0.575	0.43	0.78	0.67	1

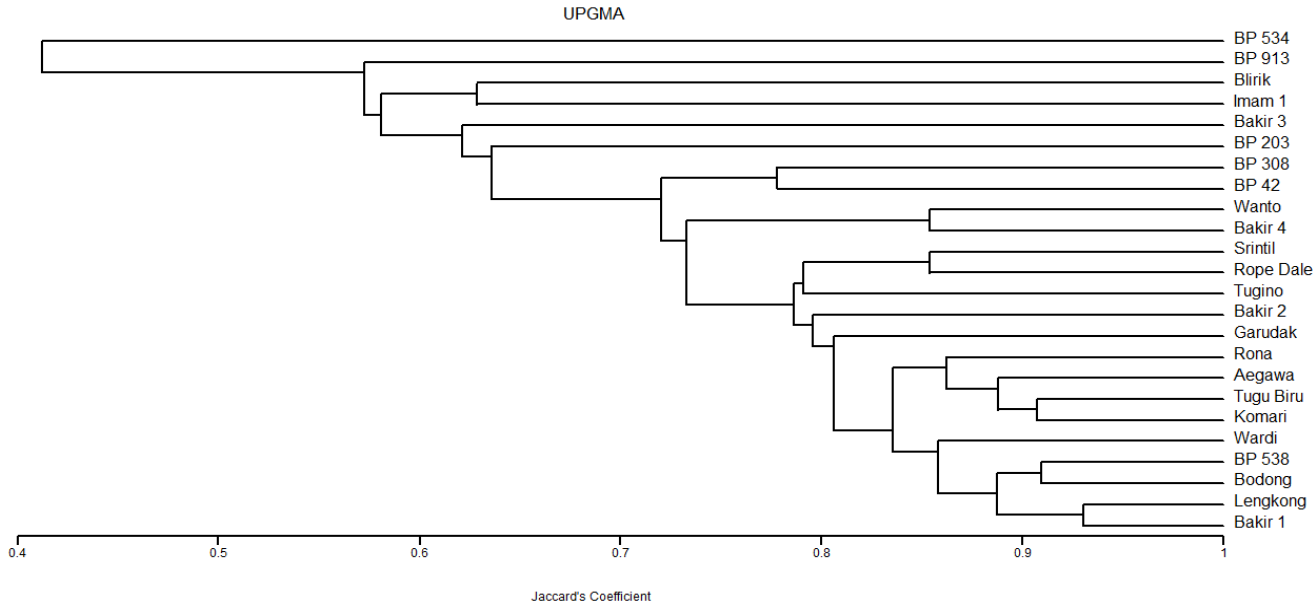

Figure 3. A dendrogram generated using UPGMA cluster analysis based on 11 RAPD markers on 24 clones of Robusta coffee grown in Lampung, Indonesia

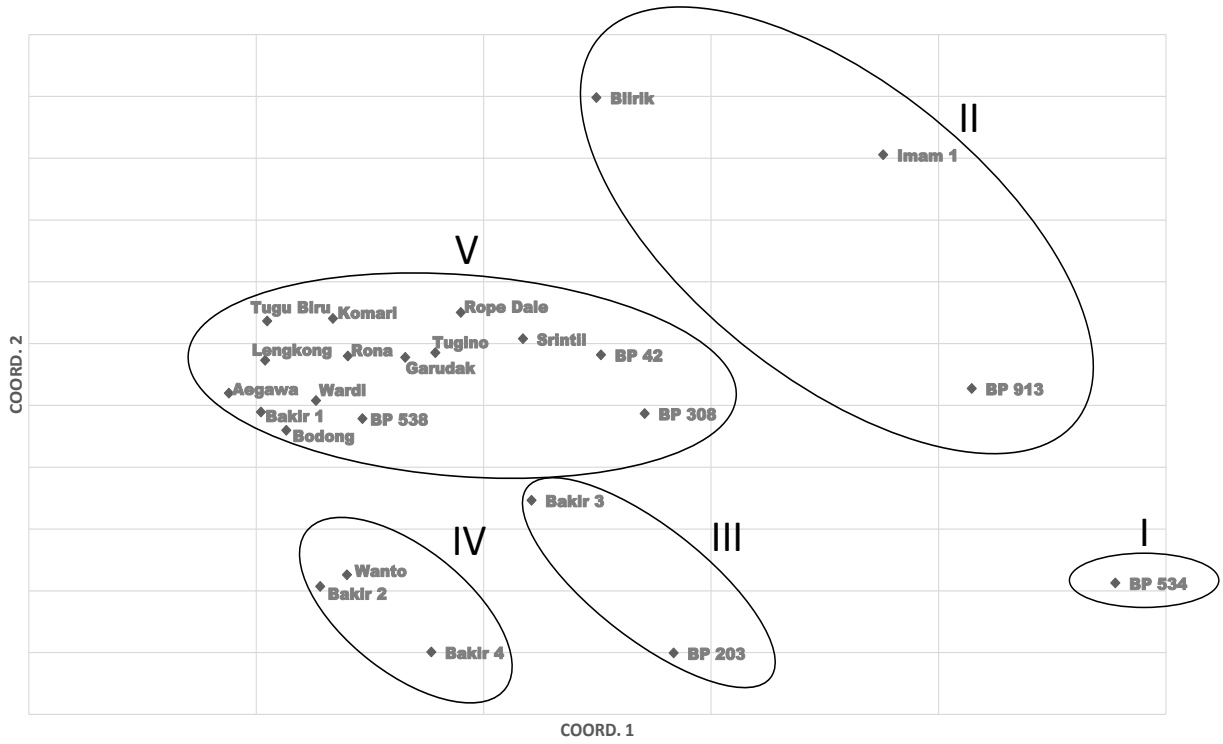

Figure 4. Estimated grouping based on principal coordinate analysis (PCA) of 24 Robusta coffee clones grown in Lampung genotyped with 11 RAPD markers.

This research is very important for designing a breeding program of Robusta coffee for adaptability to Lampung agroclimatic condition and other important traits. Firstly, the dendrogram showed that the genetic base of Robusta coffee in Lampung is narrow and could limit progress in variety development. Therefore, introduction of Robusta coffee genotypes from centers of genetic diversity is necessary to widen the genetic base. Secondly, both the tested local and BP clones were of farmers' choice. Tugino and Wanto, for example, were local clones won a coffee contest (2015) in Indonesia held by the ICCRI, coming as the best and the second-best, respectively. All of the tested

clones could potentially complement to each other through hybridization to result in new, better clones. From genetic standpoints, much better clones could result from hybridization of progenitors of low genetic similarity, by taking advantage of heterosis. Based on the dendrogram (Figure 3), PCA (Figure 4), and data on Table 4, BP 534 would be a good candidate as a parent to be crossed with each of the other tested clones because of its quite low genetic similarity with, or quite high genetic distance from other tested clones. BP 534 was reported to be high-yielding, have good taste, and show wide adaptability (Hulupi 2016). The clones which clustered in Group II and Group III would be potentially good parents for hybridization because they have relatively low genetic similarity to each other (43.0-55.3%)(Table 4, Figure 4). In general, the clones from different groups would potentially become good parents because they have quite low genetic similarity. Crossing such clones could lead to exploitation of heterosis that could possibly lead to the development of superior clones.

In conclusion, clones of Robusta coffee cultivated by Lampung local farmers had narrow genetic base with average genetic similarity of 68.4%. The local clones had higher similarity (73.5%) than BP clones (58.7%), which were officially released by the Government of Indonesia. The tested clones were clustered into two groups, one of which consisted of only one clone (BP 534). Therefore, BP 534 would be good as a parent to be crossed with the clones derived from the other groups because of their quite high genetic distance. However, because of the narrow genetic base of Robusta coffee from Lampung, it is necessary to introduce genetic materials from outside Indonesia to produce better clones. As far as we know, this is the first report on genetic diversity of Robusta coffee grown in Lampung, making it important for a breeding program for the development of better clones of Robusta coffee for the Lampung Province. As far as international coffee community is concerned, this report might be important in order to take advantage of the tested clones and to help increase genetic variability of Robusta coffee in Lampung by introducing genotypes from outside Indonesia.

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