RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
58 (1) 66-75, 2026
http://doi.org/10.54910/sabrao2026.58.1.7
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

GENETIC DIVERSITY ANALYSIS IN LOCALLY ADAPTED COFFEE (*COFFEA* SPP.) GENOTYPES IN CENTRAL SULAWESI, INDONESIA

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SUMMARY

Robusta (*Coffea arabica* L.) and Arabica (*Coffea canephora* A. Froehner) are the major agricultural commodities in Central Sulawesi, Indonesia, known for their unique flavor mostly influenced by local microclimates. The following study aimed to analyze the genetic diversity of 12 coffee genotypes procured from the Central Sulawesi Region using RAPD markers. Ten RAPD primers (OPA01, OPA02, OPA07, OPA16, OPD08, OPA13, OPG03, OPI07, OPY10, and OPX20) generated 103 DNA bands, with 102 as polymorphic. The DNA profiles' binary data underwent analysis using Jaccard similarity coefficients and the unweighted pair group method with arithmetic mean (UPGMA) clustering. Genetic similarity ranged from 7% to 72%, with an average of 31%. Cluster analysis grouped the coffee genotypes into two main clusters unrelated to their taxonomic classifications, implying extensive genetic divergence potentially driven by local environmental conditions. Four non-conventional primers (OPA02, OPA13, OPA16, and OPD08) successfully amplified the DNA in several local genotypes. However, the local genotype Robusta Dombu did not amplify with any primer, pointing to possible mutation, highlighting its distinct genetic makeup. The results demonstrated a significant genetic diversity among the local coffee genotypes. Robusta Dombu, being a local coffee genotype, emerges as a potential candidate for developing climate-resilient and high-quality cultivars.

Keywords: Arabica, biodiversity, *Coffea arabica*, *Coffea canephora*, local adaptation, RAPD, Robusta

Key findings: The study showed the high genetic diversity in *Coffea* spp. genotypes by using RAPD markers. The phylogenetic clusters have not shown their belonging to two main ancestors, Robusta and Arabica, suggesting their highest variability during adaptation to local conditions.

Communicating Editor: Dr. Tabynbaeva Laila

Manuscript received: May 15, 2025; Accepted: August 04, 2025.

Citation: Rifka, Nurfadilah, Magfirah H, Suwitra I K, Suwastika I N (2026). Genetic diversity analysis in locally adapted coffee (*Coffea* spp.) genotypes in central Sulawesi, Indonesia. *SABRAO J. Breed. Genet. 58 (1) 66-75. http://doi.org/10.54910/sabrao2026.58.1.7*.

INTRODUCTION

Arab traders introduced *Coffea arabica* beans to the world in the 17th century in India, and since then, it has spread beyond Africa (Ukers, 1922). *Coffea* sp. originated from Africa, specifically, Ethiopia (Wellman, 1961). In Europe, the coffee arrived through trade routes controlled by European colonials, including the Dutch. In the 18th century, the Dutch introduced coffee plants to their colonies in Indonesia, which later became a major hub for global coffee production (Clarence-Smith and Topik, 2003).

Coffee is a highly valuable crop with significant economic importance that represents a promising export commodity for Indonesia. The Indonesian Government has developed around 35 superior coffee cultivars, including 12 cultivars of the Arabica line, 20 of the Robusta type, and also three cultivars from Liberica (Randriany and Dani, 2018). In Indonesia, coffee production is prominent in several regions, notably in the Central Sulawesi province. In this province, coffee cultivation covers an area of 10.3 hectares, with an annual production of approximately 2700 tons (Nurman *et al*., 2021). The key coffee-producing areas include Sigi Regency and Poso Regency. Each district cultivates coffee with distinct traits shaped by microclimates and traditional practices. Coffee features are varied; Sigi Regency produces the bean-type coffee called Kulawi, Kamanuru, Gawalise, Ratu, Matantimali, and Palolo coffees. The Poso Regency is popular for producing Kalemago and Koji coffees, and Donggala Regency offers Sojol, Rano Balaesang Tanjung, and Napeto Nupabomba coffees (Ikbal *et al*., 2013).

In coffee germplasm, genetic diversity is a crucial element of plant breeding, serving as a source of genetic information for improving cultivars. The genetic diversity is vital for developing effective strategies in producing superior clones (Pangestika *et al*., 2021). The assessment of genetic diversity can use morphological and molecular markers. However, due to environmental influences, the morphological markers are often less reliable. In contrast, molecular markers seemed more precise for evaluating genetic variability, as the DNA incurs no effects from environmental factors (Budi and Mawardi, 2021).

Unfortunately, in Central Sulawesi province, Indonesia, the local coffee germplasm lacks detailed descriptions and basic information regarding its genetic and physiological properties. Addressing this gap necessitates genetic analysis, such as the random amplified polymorphic DNA, or RAPD scrutiny. The RAPD is a PCR-based molecular marker, useful for DNA fingerprinting (Pangestika *et al*., 2021). The RAPD markers are advantageous due to their simplicity in preparation, ease of use, and rapid results. Unlike other molecular markers, RAPD also requires a small amount of DNA as a template and does not need prior knowledge of a target genome (Pancaningtyas and Susilo, 2022).

Despite Central Sulawesi's importance as a coffee-producing region, its local germplasm has not reached genetic characterization using non-conventional molecular markers, making this study the first of its kind. Although RAPD has been widely operational in coffee research, previous studies in Indonesia have primarily relied on conventional primers. In contrast, our study introduces the use of non-conventional RAPD primers that have no previous application to coffee. This study compiles fundamental information on the genetic diversity of local coffee genotypes, shaped by local environmental conditions through adaptation, which could contribute to future efforts in improving coffee production and quality.

MATERIALS AND METHODS

Plant material

Twelve local coffee genotypes came from five different locations, i.e., District Donggala (Rano and Alindau villages), Sigi (Dombu village), Tolitoli (Bangkir village), and Poso (Alitupu village). Those locations are famous for coffee farms with different ecological conditions, including cultivars with diverse properties, varied microclimates, and altitudes. The name of each genotype pertains to the

Table 1. Sequences of 10 RAPD primers used in this study.

Primers	Sequences (5'-3')	References
OPA01	CAGGCCCTTC	(Budi and Mawardi, 2021)
OPA02	TGCCGAGCTG	This study*)
OPA07	GAAACGGGTG	(Masumbuko *et al*., 2003)
OPA13	CAGCACCCAC	This study*)
OPA16	AGCCAGCGAA	This study*)
OPD08	GTGTGCCCCA	This study*)
OPG03	GAGCCCTCCA	(Kathurima *et al*., 2012; Ramadiana *et al*., 2021)
OPI07	CAGCGACAAG	(Kathurima *et al*., 2012; Gimase *et al*., 2014; Omingo *et al*., 2017; Ramadiana *et al*., 2021; Pangestika *et al*., 2021)
OPX20	CCCAGCTAGA	(Kathurima *et al*., 2012; Gimase *et al*., 2014; Omingo *et al*., 2017; Pangestika *et al*., 2021)
OPY10	CAAACGTGGG	(Kathurima *et al*., 2012; Gimase *et al*., 2014; Omingo *et al*., 2017; Pangestika *et al*., 2021)

*Non-conventional Coffee-RAPD primers, which has never been used for coffee analysis.

local farming community with its common use. For the RAPD analysis, the study used 10 RAPD primers (OPA01, OPA02, OPA07, OPA13, OPA16, OPD08, OPG03, OPI07, OPX20, and OPY10) (Table 1).

DNA isolation and RAPD analysis

Performing total DNA extraction utilized the DNA Purification Kit NucleoSpin Plant II (Machery-Nagel). The procedure followed the kit instructions and started with 100 mg of fresh leaf weight. DNA purity assessment continued by the absorbance at 260 and 280 nm using a NanoDrop spectrophotometer.

The PCR process ran in a Bio-Rad T100 Thermal Cycler. The DNA amplification succeeded in using MyTaq HS Red Mix Bioline-DNA Polymerase enzyme, with 10 different RAPD primers (Table 1), 50 ng purified DNA as a template, and dH_2O. The total PCR reaction was 20 μl. The PCR protocol was as follows: pre-denaturation at 95 °C for 3 min, followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 35 °C for 30 s, and elongation at 72 °C for 2 min, with a final extension at 72 °C for 10 min. The PCR products entailed separation in 1% agarose (Nacalai Tesque) in electrophoresis gel, and visualization used ethidium bromide (Promega) under a UV transilluminator. The band size calculation depended on the 1 kb DNA Ladder (Maestrogen).

Data analysis

Based on the appearance of each DNA band, the data scoring as binary data states 1 (for presence) and 0 (for absence) of the DNA band. Genetic relationships underwent analysis based on the binary data using the program NTSYS (Numerical Taxonomy and Multivariate Analysis) ver. 2.11a (Rohlf, 2000). The dendrogram construction employed Jaccard's coefficient similarity and the sequential, agglomerative, hierarchical, and nested (SAHN) - unweighted pair group method with arithmetic mean(UPGMA) clustering method (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

The timely investigations aimed to analyze the genetic variation of 12 local Sulawesi coffee genotypes using the RAPD analysis, employing 10 RAPD primers. They comprised six conventional primers (OPA01, OPA07, OPG03, OPI07, OPY10, and OPX20), often used for analyzing coffee, and four non-conventional primers (OPA02, OPA13, OPA16, and OPD08). Their use is the first time in analyzing coffee genotypes (Table 1). Twelve coffee genotypes collected from five regions bore scrutiny, consisting of six local cultivars from Dombu, Rano, and Bangkir villages (Table 2). Moreover, the evaluation included six cultivars from Alindau and Alitupu villages, produced

Table 2. Coffee genotypes and their geographical locations.

Genotypes	Locations	Coordinate points	Elevations (masl)	Date of collection
Robusta Dombu	Dombu village, Sigi	0°57′29.5″S 119°47′03.5″E	1,800	September, 2023
Liberika Dombu	Dombu village, Sigi	0°57′29.5″S 119°47′03.5″E	1,800	September, 2023
Tipika Dombu	Dombu village, Sigi	0°57′29.5″S 119°47′03.5″E	1,800	September, 2023
Robusta Rano	Rano village, Donggala	0°04′07.2″S 119°42′06.2″E	120	September, 2023
Dara Rano	Rano village, Donggala	0°04′29.3″S 119°42′02.3″E	126	September, 2023
Bangkir	Bangkir village, Tolitoli	0°47′52.64″N 120°15′5.829″E	69	September, 2023
Arabica Alindau-1	Alindau village, Donggala	0°20′42.306″S 119°45′56.982″E	25	August, 2023
Arabica Alindau-2	Alindau village, Donggala	0°20′41.958″S 119°45′57.354″E	20	August, 2023
Robusta Alindau	Alindau village, Donggala	0°20′43.62″S 119°45′58.428″E	17	August, 2023
Lampung Alindau	Alindau village, Donggala	0°20′42.18″S 119°45′57.228″E	19	August, 2023
Robusta Alitupu	Alitupu village, Poso	1°25′19.030″S 120°20′27.260″E	1,226	August, 2023
Lampung Alitupu	Alitupu village, Poso	1°26′6.604″S 120°22′4.666″E	1,224	August, 2023

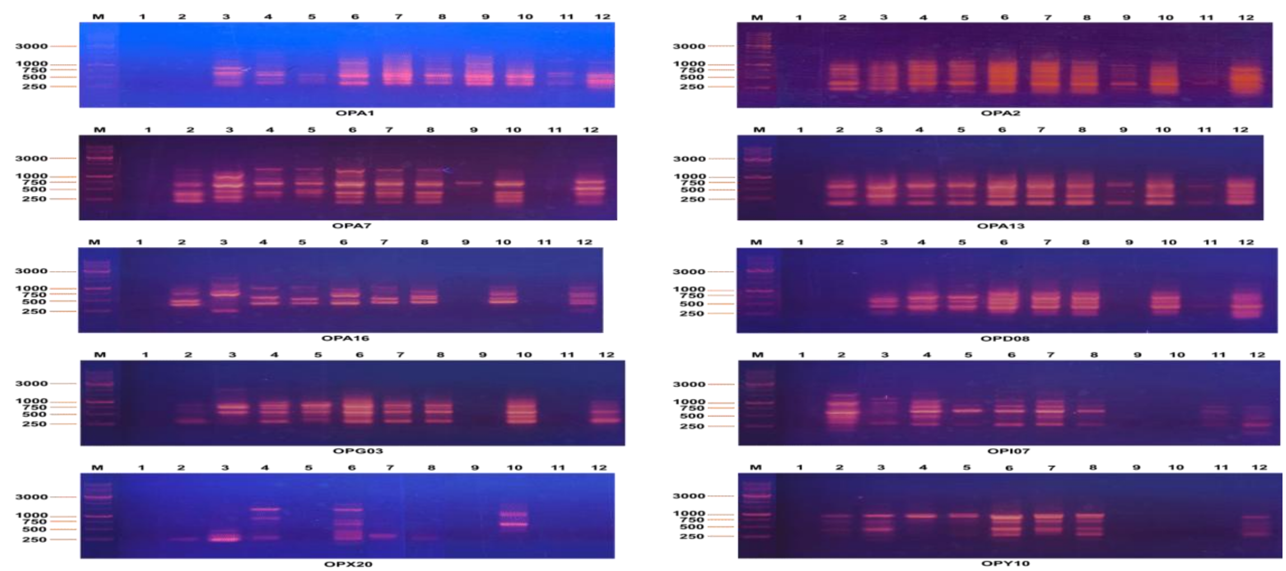

Figure 1. DNA fragment amplification using 10 different primers (as listed under each image) for 12 different coffee genotypes—1: Robusta Dombu, 2: Tipika Dombu, 3: Liberika Dombu, 4: Robusta Rano, 5: Dara Rano, 6: Bangkir, 7: Arabica Alindau-1, 8: Arabica Alindau-2, 9: Robusta Alindau, 10: Lampung Alindau, 11: Robusta Alitupu, 12: Lampung Alitupu, and M: DNA ladder. Genotype of Robusta Dombu (No. 1) cannot be amplified by using all the tested primers.

through different breeding programs. The local coffee genotypes were the cultivars from traditional cultivation, as selected by the farming community over generations. The breeding genotypes reached their development through professional breeding programs before being introduced to the local farmers. The ability of RAPD markers to classify the crop cultivars and determine their genetic diversity has garnered documentation in past research involving different plant species (Shehab, 2023; Nama and Altameme, 2023; Marasabessy *et al.,* 2024; Karuwal *et al.,* 2024).

The electrophoresis yielded distinct and consistent DNA bands, confirming the reliability of the amplification across primers. However, the effectiveness of the primers varied in their ability to generate the DNA bands (Figure 1). Interestingly, four non-conventional primers

(OPA02, OPA13, OPA16, and OPD08) successfully amplified the DNA in several genotypes, except for Robusta Dombu, Robusta Alindau, and Robusta Alitupu, indicating potential genomic divergence. This could be because of a high genetic divergence, primer mismatch, or technical limitations, highlighting the need for a population-specific primer design. Past studies reported the tested RAPD primers produced scorable and polymorphic bands in coffee, emphasizing the importance of careful primer selection and empirical validation, especially when working with genetically diverse coffee genotypes (Kathurima *et al.*, 2012; Awati *et al.*, 2018). These results highlight the adaptability and effectiveness of the non-conventional primers in detecting genetic variation, particularly in under-explored local coffee germplasm. These primers could serve as valuable tools in future studies based on genetic diversity, especially in populations with unique genetic backgrounds.

Notably, none of the primers used were able to amplify the DNA from the local Robusta Dombu genotype. The results were contrary to previous research, where primers OPX20 and OPY10 successfully amplified and produced polymorphic bands for *Coffea canephora* (Ramadiana *et al.,* 2021; Pangestika *et al.,* 2021). This may indicate the presence of mutation and sequence divergence at the primer binding sites, suboptimal DNA quality, and also some other technical limitations affecting amplification. Gimase *et al.* (2014) reported several RAPD primers failed to amplify DNA from specific Robusta accessions, underscoring that primer efficiency was not universal across all coffee genotypes. Additionally, Awati *et al.* (2018) mentioned particular *Coffea canephora* genotypes exhibited the highest genetic dissimilarity and likely required genotype-specific primers to achieve effective amplification. Overall, the presented results emphasized that not a single set of RAPD primers can be universally effective for amplification in all the coffee genotypes. The highest genetic variability among the coffee genotypes, particularly from regions that have not attained extensive studies, necessitates careful selection and validation of primers for specific target populations. Hence, the promising study utilized only 11 coffee samples for the analysis based on genetic relationship.

Overall, the primers generated a total of 103 DNA bands, ranging from 172 to 2165 bp, wherein 102 bands were polymorphic (Table 3). The primers OPA01 and OPI07 yielded the highest number of polymorphic bands (15 each), while the primers OPA13 and OPY10 produced the fewest, with seven each. On average, each primer generated 10.3 bands, including 10.2 polymorphic bands, resulting in a polymorphic band percentage of 99%. The results authenticated that the present set of primers was reliable and accurate in analyzing genetic variability of the coffee genotypes grown locally. Similarly, past studies reported a 95% polymorphism rate among the *Coffea canephora* accessions in Temanggung, supporting the high sensitivity of RAPD markers in Indonesian coffee populations (Pangestika *et al.,* 2021).

For 11 coffee genotype samples, the genetic similarity matrix ranges from 0.07 to 0.72, with an average of 0.31 (Table 4). It indicates that, on average, a 31% genetic similarity existed between the coffee genotypes based on RAPD markers. The highest similarity coefficient (0.72) was evident between the coffee genotypes Arabica Alindau-1 and Arabica Alindau-2, suggesting these two genotypes were considerably genetically similar. Both genotypes originated from the same location; however, they differed in fruit shape: Arabica Alindau-1 has an oval fruit, while Arabica Alindau-2 has a round fruit. Conversely, the lowest similarity value (0.07) was noticeable between the coffee genotypes Robusta Alitupu and Local Tipika Dombu, indicating substantial genetic divergence between these two accessions. The said broad range of similarity was consistent with the findings of Omingo *et al.* (2017), who reported the RAPD-based genetic similarity values ranging from 0.06 to 1.00 across the various coffee accessions, indicating substantial genetic variability in the cultivated coffee.

The study results revealed local coffee genotypes have the topmost genetic similarity (0.35) compared with the coffee genotypes developed through breeding programs (0.29).

Table 3. RAPD primers and the number of DNA fragments produced in different coffee genotypes.

Primers	Sequences (5′-3′)	DNA size (bp)	DNA bands			Polymorphic (%)
			Total	Monomorphic	Polymorphic	
OPA01	CAGGCCCTTC	336-1966	15	0	15	100
OPA02	TGCCGAGCTG	214-1197	10	1	9	90
OPA07	GAAACGGGTG	218-1553	13	0	13	100
OPA13	CAGCACCCAC	172-728	7	0	7	100
OPA16	AGCCAGCGAA	252-1136	11	0	11	100
OPD08	GTGTGCCCCA	230-847	8	0	8	100
OPG03	GAGCCCTCCA	306-2165	9	0	9	100
OPI07	CAGCGACAAG	178-1565	15	0	15	100
OPX20	CCCAGCTAGA	241-1520	8	0	8	100
OPY10	CAAACGTGGG	291-973	7	0	7	100
Total			103	1	102	
Average			10.3	0.1	10.2	99%

Table 4. Similarity coefficient of 11 coffee genotypes based on 10 RAPD markers.

Coffee genotypes	Tipika Dombu	Liberika Dombu	Robusta Rano	Dara Rano	Bangkir	Arabica Alindau-1	Arabica Alindau-2	Robusta Alindau	Lampung Alindau	Robusta Alitupu	Lampung Alitupu
Tipika Dombu	1.00										
Liberika Dombu	0.29	1.00									
Robusta Rano	0.29	0.29	1.00								
Dara Rano	0.24	0.29	0.63	1.00							
Bangkir	0.27	0.32	0.45	0.40	1.00						
Arabica Alindau-1	0.25	0.39	0.55	0.53	0.48	1.00					
Arabica Alindau-2	0.28	0.44	0.53	0.51	0.53	0.72	1.00				
Robusta Alindau	0.09	0.13	0.15	0.16	0.12	0.16	0.15	1.00			
Lampung Alindau	0.17	0.25	0.37	0.44	0.42	0.46	0.46	0.17	1.00		
Robusta Alitupu	0.07	0.14	0.18	0.20	0.17	0.19	0.20	0.25	0.13	1.00	
Lampung Alitupu	0.25	0.31	0.28	0.38	0.44	0.38	0.41	0.17	0.33	0.18	1.00

This suggested that local coffee genotypes exhibited less genetic diversity compared with coffee genotypes obtained through breeding. The reduced diversity might be due to the local coffee being developed by farmers from selected germplasm in their regions over generations, resulting in a narrower genetic base. These results align with the research by Kathurima *et al.* (2012), who observed a dissimilarity index of less than 5% among the arabica genotypes, confirming the narrow genetic base mostly observed in traditionally cultivated coffee cultivars. Ramadiana *et al*. (2021) reported the maximum genetic similarity in farmer-cultivated coffee (0.73) versus the breeding coffee (0.59), indicating that farmer selections often derive from a narrower germplasm pool.

Among the coffee genotypes developed through breeding, Robusta Alindau and Robusta Alitupu exhibited the lowest genetic similarity (0.25). However, these results were opposite from the past research where the robusta coffee had the highest average values of genetic similarity (0.65) (Pangestika *et al.*, 2021). Additionally, the coffee genotypes Lampung Alindau and Lampung Alitupu have a genetic similarity of 0.33. Contrastingly, the genotypes Arabica Alindau-1 and Arabica Alindau-2 showed the optimum genetic similarity (0.72). These findings suggested that even within breeding programs, plants do not always exhibit high genetic similarity. This variability can be ascribable to the differences in environmental conditions, breeding techniques, and genetic origins of genotypes.

Cluster analysis of the 11 coffee genotypes revealed the formation of two main clusters (Figure 2). The first cluster predominantly includes the local genotypes, along with Arabica and Lampung coffee. The second cluster comprises only the genotypes Robusta Alindau and Alitupu, which were also distinct from the other coffee genotypes. Within the first cluster, two subclusters emerged, i.e., the first subcluster only had the local coffee genotype Tipika Dombu. On the other hand, the second subcluster contains a diverse group of coffee genotypes. The second subcluster included local genotypes, such as Liberika Dombu, Robusta Rano, Dara Rano, Arabica Alindau-1, Arabica Alindau-2, Bangkir, Lampung Alindau, and Lampung Alitupu. The results showed clustering patterns gained more influence from genotype genetic background and type than geographical proximity. These results agreed with past findings, which revealed the two major clusters among coffee accessions based on RAPD analysis and clearly separated the species and varietal groups (Silvestrini *et al.*, 2008). Gimase *et al.* (2014) found that coffee arabica genotypes formed the distinct clusters from interspecific hybrids and Robusta accessions using both SSR (simple-sequence repeats) and RAPD markers.

The latest analysis also revealed location-specific groupings for some coffee genotypes. Local coffee genotypes obtained from the same location tend to cluster together, whereas breeding coffee genotypes, despite coming from different locations, often group based on type. Coffee genotypes Alindau and Alitupu Robusta do not cluster with Lampung coffee, despite Lampung coffees being of the robusta cultivar. This authenticated the significant genetic differences, with a similarity coefficient of only 16%, suggesting that Lampung coffees had closer genetic affinities with other cultivars than with other robusta coffees. This type of divergence supports the conclusions of past studies, where the robusta clones from Lampung did not cluster strictly by location but rather by genetic similarity (Ramadiana *et al.*, 2021). Similarly, Pangestika *et al.* (2021) reported coffee accessions from nearby regions formed distinct clusters, indicating that geographical origins do not always reflect genetic relatedness.

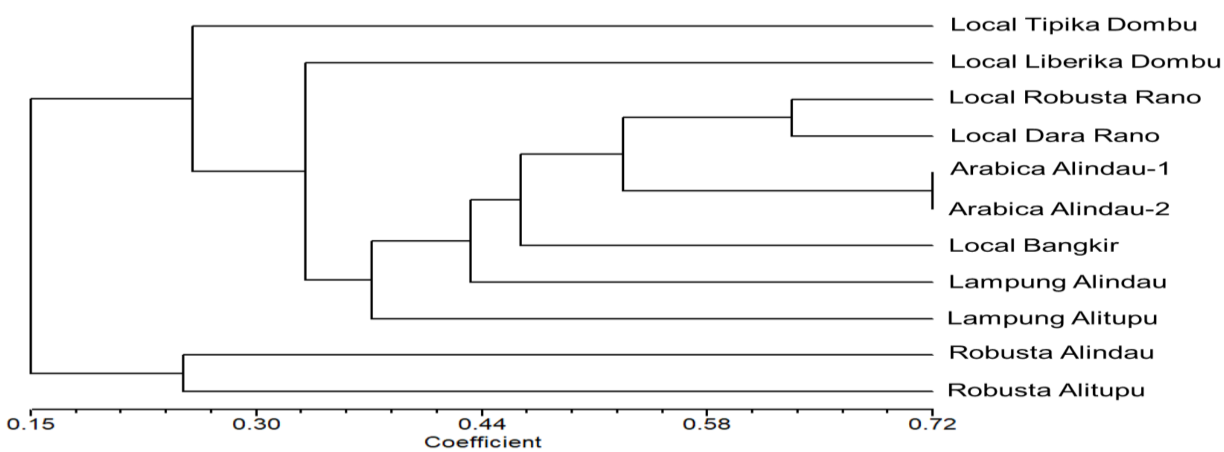

Figure 2. UPGMA dendrogram of 11 coffee genotypes based on the RAPD analysis. The clustering did not follow the taxonomic lines of Arabica and Robusta, suggesting high intra-population genetic variation.

In Indonesia, the initial planting of robusta coffee occurred in Sumatra, particularly in Lampung, at the end of the 19th century (Clarence-Smith and Topik, 2003). However, robusta coffee's later introduction to Sulawesi was in the early 20th century. The observed genetic variations were likely due to variations in the domestication processes and locations in growing these coffee genotypes. Previous studies revealed genetic variation within coffee species bore considerable effects from geographic and environmental factors, which significantly contributed to the genetic diversity among coffee populations (Kiwuka *et al*., 2021; Senra *et al*., 2023). Therefore, the presented results suggested that robusta coffee in Sulawesi may have undergone different natural and artificial selection processes compared with the Lampung coffee, leading to the genetic differences observed in this study.

This research provides valuable insights for designing coffee breeding programs that become tailored to the agro-climatic conditions and unique characteristics of Central Sulawesi, Indonesia. By incorporating both conventional and non-conventional primers, we were able to successfully assess the extent of genetic variations among the local coffee genotypes. The inability of all the primers to amplify the Local Robusta Dombu genotype highlights the limitations of relying solely on widely used molecular tools. The study results further underscore the need for localized molecular approaches that may consider the unique genetic backgrounds of indigenous coffee populations. However, understanding such types of variations is crucial for the development of resilient and high-performing coffee cultivars adapted to specific environments. Silvestrini *et al.* (2008) highlighted the usefulness of RAPD markers in assessing interspecific diversity and emphasized their application in conservation strategies for coffee germplasm. Similarly, Mishra *et al.* (2011) stressed the importance of species-specific primers for detecting unique genetic relationships in Indian coffee populations, supporting the need for tailored molecular approaches with diverse genetic backgrounds such as those found in Sulawesi, Indonesia.

The local coffee genotype Robusta Dombu, in particular, appears promising as a potential parent in hybridization efforts due to its low genetic similarity with other examined genotypes. The inability of the 10 primers to amplify its DNA suggested that the primer binding sites may differ substantially, pointing to significant genetic divergence. Consequently, the local coffee Robusta Dombu holds considerable potential for the development of superior coffee cultivars through hybridization, leveraging heterosis to improve the desirable traits.

CONCLUSIONS

The study confirmed a high level of genetic diversity among the evaluated coffee genotypes in Central Sulawesi, with the average genetic similarity as low as 31%. However, it is important to mention that the material analyzed represents only a small subset of the coffee germplasm and breeding materials available in Central Sulawesi, Indonesia. Future work should expand the germplasm scope and examine correlations between genetic diversity and agronomic traits to support local breeding strategies. Therefore, more comprehensive molecular analysis will be useful for characterizing these traits and ultimately selecting elite coffee cultivars for various breeding programs.

ACKNOWLEDGMENTS

The authors are grateful to the Faculty of Mathematics and Natural Sciences, Tadulako University, Indonesia, for funding the research through the DIPA Grant with the contract number 1197t/UN28.2/PL/2023. They also thank the coffee farming community that provided the samples.

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