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Genetic diversity and rhizome flesh color variation among Indonesian turmeric (*Curcuma longa* L.) accession revealed by SSR markers

Abstract. Turmeric (*Curcuma longa* L.) is a rhizomatous plant widely cultivated in Indonesia. Although recent studies have demonstrated that simple sequence repeat (SSR) markers are highly effective for assessing genetic diversity in *Curcuma longa* germplasm, most investigations have focused either on molecular diversity or breeding-oriented germplasm characterization traits of agronomic without integrating phenotypic commercial importance, particularly rhizome flesh color. The study was conducted from February to May 2026. This study aimed to evaluate the genetic diversity, phylogenetic relationships, and rhizome flesh color variation among Indonesian turmeric accessions collected by Plant Breeding Laboratory at the Faculty of Agriculture, Universitas Padjadjaran, and was grown at the Ciparanje Experimental Farm. Eighteen turmeric accessions from various locations in Indonesia were analyzed using six SSR markers to assess genetic variation and phylogenetic relationships. The results showed that 12 polymorphic alleles were identified, with the highest polymorphism information content (PIC) value of 0.58 observed for the CuMiSat-35 primer. Principal coordinate analysis and cluster analysis divided the accessions into two main clusters, with Jaccard genetic distances ranging from 0.00 to 0.80, indicating genetic diversity among the 18 turmeric accessions tested. The accession pairs with the longest kinship distances were found between accession CL-93 (South Sulawesi) and several other accessions, such as CL-01, CL-20, CL-25, CL-34, CL-36, CL-38, CL-47, CL-61, and CL-84, with a Jaccard coefficient of 0.80 and a genetic similarity of 20%, indicating the presence of genetic variation. Eighteen turmeric accessions exhibited varying rhizome flesh colors, which illustrates the differences in curcumin content.

Keywords: *Curcuma longa* · Curcumin · Simple sequence repeat (SSR) · Principal coordinate analysis · Flesh color expression

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Introduction

Turmeric (*Curcuma longa* L.) is a rhizome plant commonly found throughout Asia, including Indonesia. This plant is widely distributed across nearly all of Indonesia's islands, including Java, Sumatra, Kalimantan, Bali, Sulawesi, Maluku, and Nusa Tenggara, but the center of turmeric cultivation is on Java (Nihayati, 2023). Turmeric can thrive and grow wild near forests under environmental conditions with temperatures of 20–35°C and annual rainfall of 1,500 mm (Ahmad et al., 2020). Generally, Indonesians use turmeric as a spice that provides a natural yellow color, distinctive aroma, and flavor. Turmeric serves not only as a food coloring but also as a pharmacological ingredient.

Turmeric is included on the World Health Organization's (WHO) priority list as one of the most widely used medicinal plants across various countries and is frequently mentioned in pharmaceutical compendia (Listyana, 2018). Turmeric contains various bioactive compounds, including curcumin (the active component responsible for its yellow color and medicinal properties), demethoxycurcumin, oleoresin, bisdemethoxycurcumin, essential oils, proteins, and others (Shan & Iskandar, 2018). These compounds make turmeric beneficial as an anti-inflammatory, antioxidant, antimutagenic, antidiabetic, antibacterial, antiviral, antifungal, and hepatoprotective agent, and for treating kidney disease, arthritis, inflammation, and even Alzheimer's disease (Ayer et al., 2020; Zhang & Tang, 2023). The diverse range of bioactive compounds and pharmacological benefits in turmeric not only gives it high health value but also makes it an important commodity with a continuously growing demand.

According to the Badan Pusat Statistik (BPS), turmeric demand in Indonesia has fluctuated over the past five years. In 2025, total turmeric production is projected to decrease by 16.94% from 2024, to 147,505,182 kg (BPS, 2025). This decline in national turmeric production is due to farmers not planting high-quality turmeric seeds, which makes the crops more susceptible to disease. The development of turmeric cultivation for the food and pharmaceutical industries should be supported by the creation of superior varieties that meet market needs. Efforts to develop superior varieties based on genetic diversity are necessary to optimize the utilization of turmeric.

Information on turmeric's genetic diversity serves as the basis for parental selection in genetic improvement and the development of turmeric plants (Afidani et al., 2025). To date, genetic information has largely been analyzed using morphological markers, which rely on visual observations of plant phenotypes. However, morphological markers are plastic in response to environmental factors; some traits depend on the plant's growth stage, and the number of observed morphological traits is limited because many traits are controlled by multiple genes (polygenic), making it difficult to distinguish genotypes based solely on morphology (Sahoo et al., 2017). Genetic diversity analysis can also use isozyme (biochemical) markers, which are relatively fast, easy to use, and cost-effective; however, their polymorphism is limited and influenced by the plant's developmental stage (Qosim, 2020). Therefore, molecular markers are necessary to complement genetic information because they operate at the DNA level and are not influenced by environmental conditions or the plant's developmental stage.

Molecular markers are a method of analyzing plant genetic diversity that uses short chromosomal DNA sequences to measure genetic variation. Molecular markers act directly on chromosomal sequences, enabling the detection of hidden genetic variation, the quantification of population diversity, and the mapping of phylogenetic relationships and population structure (Ozbek, 2024). Simple sequence repeats (SSRs) are molecular markers widely used for identifying genetic diversity in plants. They consist of short sequence repeats (1–6 bases) distributed throughout the genome and are based on polymerase chain reaction (PCR) of genomic sequences that vary in number at specific loci, and they possess several advantages, including codominance, high levels of polymorphism, good reproducibility, and transferability between populations or related species (Bidyananda et al., 2024).

Information on the genetic diversity of turmeric in Indonesia based on SSR markers remains very limited. A study on the genetic diversity of turmeric was conducted by Afidani et al. (2025), though it utilized only six turmeric accessions. Several local studies have employed SSR markers, but only for the plant *Curcuma aeruginosa* (Fauzi, 2020; Purwoko et al., 2021; Purwoko et al., 2023), whereas for local *Curcuma longa* L., the literature and SSR data collections

remain incomplete, leaving gaps in information regarding allele distribution and superior genetic resources across various regions of Indonesia. Although recent studies have demonstrated that simple sequence repeat (SSR) markers are highly effective for assessing genetic diversity in *Curcuma longa* germplasm, most investigations have focused either on molecular diversity or breeding-oriented germplasm characterization without integrating phenotypic traits of agronomic and commercial importance, particularly rhizome flesh color (Ravindran et al., 2024). Furthermore, the limited number of Indonesian turmeric accessions evaluated with SSR markers has restricted understanding of the country's rich germplasm diversity, despite Indonesia being one of the centers of cultivation and utilization of turmeric (Afidani et al., 2025) Recent studies have emphasized the usefulness of SSR markers for detecting polymorphism, estimating genetic relationships, and identifying potential parental materials for breeding; however, the association between SSR-based genetic variation and visible rhizome flesh color remains largely unexplored (Ravindran et al., 2024). Since rhizome flesh color is closely associated with curcuminoid composition, carotenoid accumulation, antioxidant properties, and consumer preference, understanding its relationship with molecular diversity would provide valuable information for germplasm conservation and marker-assisted breeding (Mondal & Bandyopadhyay, 2024). Therefore, a comprehensive evaluation integrating SSR marker analysis with rhizome flesh color variation across diverse Indonesian turmeric accessions is needed to identify genetically distinct germplasm, clarify genotype–phenotype relationships, and establish a scientific basis for the development of superior turmeric cultivars with desirable quality traits and broad genetic backgrounds. Therefore, further research is needed to address these gaps. This study provides information on the informativeness of SSR markers and on genetic relationships among 18 accessions of local Indonesian turmeric, which serves as the basis for turmeric plant breeding programs and the development of varieties that are genetically and functionally superior in curcumin content.

Materials and Methods

Genetic Material and SSR Markers. The genetic material used in this study consisted of young leaves and rhizomes from 18 accessions of local Indonesian turmeric (Table 1). The local varieties used were sourced from the Faculty of Agriculture's collection, grown at the Ciparanje Experimental Garden, and collected during exploratory surveys across several provinces in Indonesia. Analysis was conducted using 6 CuMiSat SSR markers with high PIC values, as determined by previous studies (Gogoi et al., 2020; Senan et al., 2013). Annealing Temperature between 52℃ and 57℃ and consisted of 18 to 24 nucleotides.

Marker specifications are detailed in Table 2. This study was conducted from January to May 2026 at the Plant Breeding Laboratory, Department of Crop Science, Faculty of Agriculture, Universitas Padjadjaran University, Jatinangor, Sumedang, West Java, Indonesia.

Table 1. List of turmeric (*Curcuma longa* L.) accessions

Sample code	Accession code	Region of origin
1	CL-01	West Java
2	CL-20	East Java
3	CL-25	Malang, East Java
4	CL-34	Sidikalang Prefecture, Dairi, North Sumatera
5	CL-35	Pontianak, West Kalimantan
6	CL-36	Samarinda, East Kalimantan
7	CL-38	Kendari, Southeast Sulawesi
8	CL-47	Kerom, Papua
9	CL-61	Misol Island, Raja Ampat, West Papua
10	CL-66	Gorontalo
11	CL-70	Kisar Island, Southwest Maluku
12	CL-80	Mataram, West Nusa Tenggara
13	CL-84	Banten
14	CL-85	Lampung
15	CL-89	North Sulawesi
16	CEK 4	Cisaat, Sukabumi, West Java
17	CL-90	Central Sulawesi
18	CL-93	South Sulawesi

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Table 2. Specifications of the SSR markers

Marker		Sequence	*Repeat motif*	T_a	References
CuMiSat-19	*Forward*	CAT GCA AAT GGA AAT TGA CAC	$(AC)_{16}(AT)_6$	57°C	Senan *et al*., 2013
	Reverse	TGA TAA ATT GAC ACA TGG CAG TC			
CuMiSat-21	*Forward*	TCA TTC AAA AGT CCG ATG GAA	$(AAG)_9$	49°C	Gogoi *et al*., 2020
	Reverse	TTC GAG TGC AGA AGG AAT TA			
CuMiSat-22	*Forward*	AAT TTA TTA GCC CGG ACC AC	$(CTT)_{10}$	57°C	Senan *et al*., 2013
	Reverse	AAG AAA GTG AGT AGA AAC CAA AGC			
CuMiSat-28	*Forward*	TTC AAC TTC TCC TCG CTC AG	$(AAG)_7(GAT)_5$	56°C	Gogoi *et al*., 2020
	Reverse	GCA AGG TCT GCA TCT ATT TCT C			
CuMiSat-31	*Forward*	GGA GGA GAA GAA GCA GAA G	$(AGC)_6(AAG)_4$	49°C	Gogoi *et al*., 2020
	Reverse	GAC AGG CGA AGG AGG AAA C			
CuMiSat-35	*Forward*	GGT TCG TCG TGG AAA GTA AT	$(CTT)_{10}$	52°C	Gogoi *et al*., 2020
	Reverse	GCA TCT CAA CAG GGG CTG			

Note: T_a = Annealing temperature.

Sampling. Sampling was conducted using a simple method: the tips of the leaves were cut off, placed in ziplock bags, and labeled with the accession number. Leaf samples were collected from young leaves of plants older than 12 weeks after planting (WAP), while rhizome samples were collected at 16 WAP, when the plants had entered the rhizome development stage and curcumin accumulation had already begun (Ayer et al., 2020).

DNA Extraction. In this study, DNA extraction was performed using the cetyl trimethyl ammonium bromide (CTAB) method, modified by Murray & Thompson (1980) with the addition of 2% polyvinylpyrrolidone (PVP), sodium dodecyl sulfate (SDS), and β-mercaptoethanol (BME). The addition of these components was useful for obtaining pure DNA by removing polyphenols with BME, binding phenolic components, and suppressing oxidation with PVP (Sari & Restanto, 2022). A total of 0.1 grams of young turmeric leaf pieces were placed in a mortar and ground to a fine powder with a pestle, followed by the addition of 900 µL of 2% CTAB+PVP and 100 µL of SDS. The sample was then transferred to a 2-mL tube, and 0.1% BME (0.9 µL) was added; the sample was then incubated in a heating block at 65°C for 15 minutes. Purification was performed by adding 1000 µL of chloroform, followed by precipitation using 600 µL of cold isopropanol in a 1:1 ratio relative to the collected supernatant. The DNA pellet was washed with 70% ethanol and dried on silica gel for 2 hours. The DNA pellet was dissolved in 100 µL of TE buffer.

DNA Quantity and Quality Assay. The DNA quantity assay is performed using a UV-vis spectrophotometer, which measures DNA concentration based on the principle of UV absorption and utilizes specific wavelengths that can be absorbed by DNA molecules. DNA is measured at 260 nm, while proteins are measured at 280 nm. Meanwhile, DNA quality testing is performed via agarose gel electrophoresis, with 1.5% agarose, to assess the likelihood of amplification during PCR.

PCR Amplification and Visualization of PCR Results. Turmeric DNA amplification was performed using 6 SSR primers (Table 2). DNA from each turmeric accession was amplified in a 10 µL reaction volume containing 2 µL of 20 ng template DNA, 7.5 µL of GoTaq® Green Master Mix (Promega), 0.5 µL each of forward and reverse primers at a concentration of 10 µM, and

4.5 µL of NFW. The PCR reaction was performed on a SelectCycler II Gradient Thermal Cycler with the following PCR program: Initial denaturation was performed at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the appropriate temperature (Table 2) for 30 seconds, and extension at 72°C for 30 seconds. The PCR reaction was concluded with a final extension cycle at 72°C for 5 minutes, followed by incubation at 4°C for 10 minutes. The PCR results were then visualized using electrophoresis on a 2% agarose gel for further analysis.

Observation of Rhizome Flesh Color. The color of the rhizome flesh was visually assessed using the RHS Color Chart (sixth edition, 2019). The color of the rhizome flesh of each turmeric accession was observed directly after the main rhizome was cut into 3 cm sections and then cut diagonally 1 cm from a representative section. The observation results were matched to the closest color code to ensure consistent, objective color determination.

Data Analysis. Analysis of the research data was conducted in stages in accordance with the research objectives. The results of rhizome flesh color measurements were classified into color categories. Photographs of the electrophoresis gels from the SSR markers showed DNA band patterns of specific sizes, based on molecular weight, using GelAnalyzer. The data were analyzed based on the presence of polymorphic bands, which were scored using a binary scoring process. The scored data were analyzed for similarity using the Jaccard coefficient in RStudio with vegan package 2.7.5 version, followed by principal coordinate analysis (PCoA) to determine genetic variation and contribution to diversity using GelAlEx, and cluster analysis using the Unweighted Pair Group Method with Arithmetic (UPGMA) to construct a phylogenetic tree linked to the results of rhizome flesh color analysis using RStudio software 4.6.0 with ape package 5.8.1 version (Bhandari et al., 2017).

Results and Discussion

Analysis of SSR Marker Performance. The performance of the SSR markers can be assessed by visually examining the DNA bands resulting from PCR amplification. The results of agarose gel electrophoresis using 2% agarose (Figure 1) show that all primers amplified well, as indicated by the presence of DNA bands for all primers. To detect SSR marker polymorphisms, DNA molecular weight was measured using GelAnalyzer software with a 100-bp DNA ladder standard. Visualization on an agarose gel allows for the separation of fragments over a wide range up to 20,000 bp, although the resolving power of agarose gels is lower than that of polyacrylamide gels. Therefore, fragment sizes were calculated based on 50-bp resolution, in accordance with previous research (Green & Sambrook, 2019). Differences in the sizes of the fragments used to identify SSR marker polymorphisms. The results of this study identified 12 polymorphic alleles, with an average of 2 alleles per locus, based on the analysis using six SSR markers. A summary of the statistical analysis of polymorphisms generated by the SSR markers is presented in Table 3.

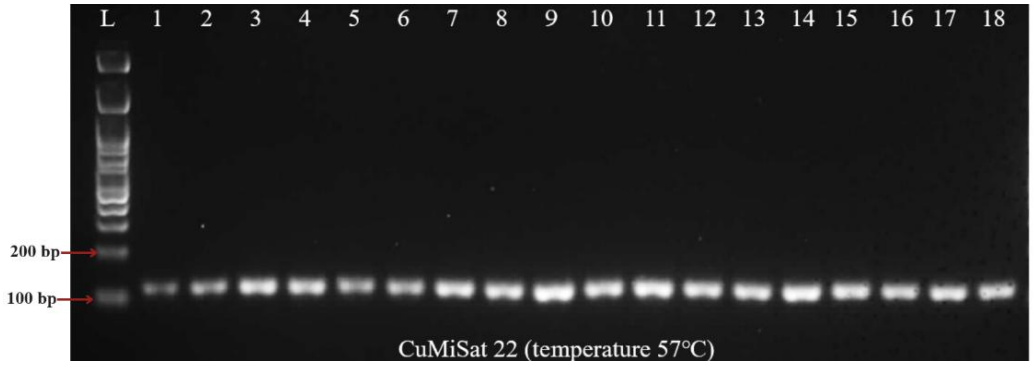

Figure 1. Example of DNA visualization for 18 accessions at the CuMiSat 22 marker

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Table 3. Characteristics of 6 SSR markers from the analysis of 18 local turmeric accessions

Marker	Allele size (bp)	Number of alleles per locus (Na)	Heterozygosity (uHe)	Polymorphism percentage (%)	PIC
CuMiSat-19	154-131	1	0.000	5.55	0.00
CuMiSat-21	171-126	2	0.108	11.11	0.10
CuMiSat-22	128-99	2	0.457	11.11	0.44
CuMiSat-28	105-73	1	0.000	5.55	0.00
CuMiSat-31	174-114	3	0.375	16.67	0.36
CuMiSat-35	334-242	3	0.603	16.67	0.58
Total		12			1.48
Average		2			0.24

Note: PIC = polymorphism information content

The number of alleles detected in this study was lower than that reported in the study by Gogoi et al. (2023), which detected 26 alleles based on the same six SSR markers in 152 turmeric genotypes. Differences in allele numbers are thought to be related to differences in the genetic material used (genetic background or accession origin) and interactions between the primer and the DNA template, resulting in variations in target amplification because each individual has a different number of base repeats, which also affects polymorphism frequency (Terryana et al., 2020; Ramlah & Astina, 2025).

The highest unbiased heterozygosity value of 0.603 was obtained for the CuMiSat-35 primer, and the lowest value of 0.000 was obtained for the CuMiSat-19 and 28 primers. The unbiased heterozygosity value indicates the likelihood that a marker can distinguish between two alleles within a population. The higher the unbiased heterozygosity value, the greater a marker's ability to indicate genetic diversity (Serrote et al., 2020). The heterozygosity value is related to the PIC, which indicates the level of informativeness of the primer in distinguishing individuals (accessions).

Polymorphism information content (PIC) indicates the degree to which a primer can distinguish between individuals based on the number of alleles and their frequency distribution, thereby allowing for an estimation of the primer's strength and serving as the basis for selecting efficient molecular markers (Aswathi et al., 2022). There are three categories of PIC values: low informativeness (PIC < 0.25), moderate informativeness (0.25 < PIC < 0.5), and high informativeness (PIC > 0.5) (Serrote et al., 2020). The results of this study show that the average PIC value for the six primers was 0.24, with the highest PIC value observed for the CuMiSat-35 primer at 0.58. The average PIC value in this study was lower than that reported in previous studies, namely Gogoi et al. (2023), Afidani et al. (2025), Singh et al. (2015), and Aswathi et al. (2022), which reported average PIC values for the same primers of 0.76, 0.88, 0.48, and 0.42, respectively. The higher the PIC value, the more informative the primer is in indicating genetic diversity.

The differences in PIC values obtained using these CuMiSat primers are likely due to differences in the genetic material used. The degree of polymorphism is also attributed to intra-species variation, which reflects differences in the number of loci among turmeric cultivars (Singh et al., 2015). Intra-species variation can occur due to mutations such as point mutations, insertions, or deletions of SSR units/motifs, thereby altering the size of the molecular markers. Mutations can also be caused by DNA polymerase strand slippage, resulting in the template sequence and the new sequence being non-complementary, thus leading to sequence contraction and expansion of the repeat motifs (Tsykun et al., 2017). Thus, of the six SSR primers used in this study, only CuMiSat-35, with a PIC value of 0.58, was classified as highly informative in distinguishing the 18 turmeric accessions from Indonesia. This indicates that the CuMiSat-35 primer is capable of revealing different genetic expressions in each accession, as evidenced by the presence of codominant alleles.

Genetic Diversity and the Phylogeny of Turmeric. Genetic diversity is a key aspect of molecular analysis that reflects variations in genetic material, serving as the basis for distinguishing between individuals. Genetic diversity analysis is conducted to assess the level of diversity among turmeric varieties distributed across Indonesia. Diversity analysis can be assessed based on genetic distance, which aims to

identify a group of individuals sharing distinctive characteristics, thereby distinguishing them from other groups. The Jaccard coefficient is a commonly used statistical measure to estimate genetic distance between individuals based on binary data regarding allele presence (Bag et al., 2019).

The results of genetic distance calculations using the Jaccard coefficient (Table 4) show that the genetic distance values for the 18 local turmeric accessions range from 0.00 to 0.80. This relatively wide range of genetic distance values indicates the presence of genetic variation among the 18 local turmeric accessions. The genetic variation observed in this study is consistent with previous research, such as that by Putri et al. (2020) and Afidani et al. (2025), which reported extensive genetic diversity in Indonesian turmeric germplasm, with similarity coefficient ranges of 0.01–0.83 and 0.11–0.33, respectively. These variations in genetic distance among accessions indicate different levels of genetic relatedness, thereby allowing for the formation of specific clustering patterns.

Genetic relationships were visualized more clearly using Principal Coordinate Analysis (PCoA) based on a matrix of genetic distances among accessions. Through PCoA analysis, it is possible to observe the clustering of accessions and identify the most distinct accessions, those located furthest apart on the plot which have the potential to be selected for crossbreeding. The PCoA results based on the Jaccard coefficient (Figure 2) show that principal coordinate 1 (Coord. 1) and principal coordinate 2 (Coord. 2) explain 54.32% and 23.86% of the total variation, respectively. Thus, these two principal coordinates account for 78.19% of the genetic diversity of all turmeric accessions. This high percentage indicates that the majority of the information related to genetic differences is represented by the two principal axes, and therefore the pattern of accession distribution on the PCoA plot can representatively describe the distribution of genetic diversity (Wang et al., 2022; Zebua, 2025)

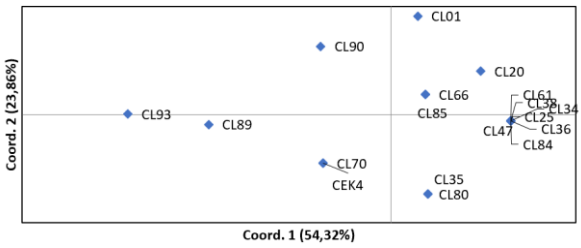

Figure 2. Principal coordinate analysis (PCoA) of 18 turmeric accessions based on the Jaccard coefficient

The PCoA plot shows that several samples cluster at the same point—specifically, at the coordinates (0.065, -0.006), where there are 7 accessions is CL-25, CL-34, CL-36, CL-38, CL-47, CL-61, and CL-84. The fact that these seven accessions are located at the same point indicates that they are genetically identical to one another. The PCoA analysis also shows that accessions CL-93 (-0.143, 0.000) and CL-01 (0.014, 0.091) are far apart from the other accessions. These geographically distant accessions indicate distinct genetic diversity and a high degree of dissimilarity from other accessions (Zebua, 2025).

Table 4. Jaccard coefficient for 18 local turmeric accessions

CL01	CL20	CL25	CL34	CL35	CL36	CL38	CL47	CL61	CL66	CL70	CL80	CL84	CL85	CL89	CEK4	CL90	CL93	
0.000																		**CL01**
0.285	0.000																	**CL20**
0.500	0.285	0.000																**CL25**
0.500	0.285	0.000	0.000															**CL34**
0.667	0.500	0.285	0.285	0.000														**CL35**
0.500	0.285	0.000	0.000	0.285	0.000													**CL36**
0.500	0.285	0.000	0.000	0.285	0.000	0.000												**CL38**
0.500	0.285	0.000	0.000	0.285	0.000	0.000	0.000											**CL47**
0.500	0.285	0.000	0.000	0.285	0.000	0.000	0.000	0.000										**CL61**
0.500	0.285	0.285	0.285	0.500	0.285	0.285	0.285	0.285	0.000									**CL66**
0.667	0.500	0.500	0.500	0.285	0.500	0.500	0.500	0.500	0.285	0.000								**CL70**
0.667	0.500	0.285	0.285	0.000	0.285	0.285	0.285	0.285	0.500	0.285	0.000							**CL80**
0.500	0.285	0.000	0.000	0.285	0.000	0.000	0.000	0.000	0.285	0.500	0.285	0.000						**CL84**
0.500	0.285	0.285	0.285	0.500	0.285	0.285	0.285	0.285	0.000	0.285	0.500	0.285	0.000					**CL85**
0.667	0.667	0.667	0.667	0.500	0.667	0.667	0.667	0.667	0.500	0.285	0.500	0.667	0.500	0.000				**CL89**
0.667	0.500	0.500	0.500	0.285	0.500	0.500	0.500	0.500	0.285	0.000	0.285	0.500	0.285	0.285	0.000			**CEK4**
0.500	0.500	0.500	0.500	0.667	0.500	0.500	0.500	0.500	0.285	0.500	0.667	0.500	0.285	0.285	0.500	0.000		**CL90**
0.800	0.800	0.800	0.800	0.667	0.800	0.800	0.800	0.800	0.667	0.500	0.667	0.800	0.667	0.285	0.500	0.500	0.000	**CL93**

Note: Numbers highlighted in yellow = farthest genetic distance; numbers highlighted in green = closest genetic distance

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Kinship analysis was conducted to determine the degree of genetic similarity among 18 accessions of local Indonesian turmeric based on SSR marker data that had been processed into Jaccard coefficients. A low Jaccard coefficient value, close to 0, indicates a small genetic distance and means that the individuals share a high degree of genetic similarity; the opposite is also true. Based on a cluster analysis that generated a dendrogram using the UPGMA method, the 18 turmeric accessions were divided into two main clusters, with the separation of the two clusters occurring at a Jaccard coefficient of 0.667 (Figure 3). Cluster 1 consists of 3 accessions originating from the island of Sulawesi, with the first subcluster consisting solely of CL-93, while the second subcluster consists of CL-89 and CL-90. Cluster 2 consists of 15 other accessions originating from various regions and forms a subcluster that separates accession CL-01 from the other 14 accessions in the second subcluster. Accessions CL-93 and CL-01 show fairly distinct genetic differences from the other accessions in the same cluster. However, genetic background from the accession play an important rules phenotype expression.

Accessions CL-93 (South Sulawesi) and CL-01 (West Java) appear to have low genetic similarity to other accessions, with similarity ranging from 0.20 to 0.71 (Table 5). Based on their phylogenetic relationships, accession CL-93 is most distantly related to several accessions, such as CL-01, CL-20, CL-25, CL-34, CL-36, CL-38, CL-47, CL-61, and CL-84, with a Jaccard coefficient of 0.80 and a genetic similarity of 0.20, or 20%. Meanwhile, the closest phylogenetic relationship was observed between accession CL-25 (Malang, East Java) and several other accessions, such as CL-34, CL-36, CL-38, CL-47, CL-61, and CL-84, with a Jaccard coefficient of 0.0, indicating a genetic similarity of 1.0 or 100%. Based on this dendrogram, the grouping of accessions and the phylogenetic relationships of turmeric are not based on the region of origin of the samples.

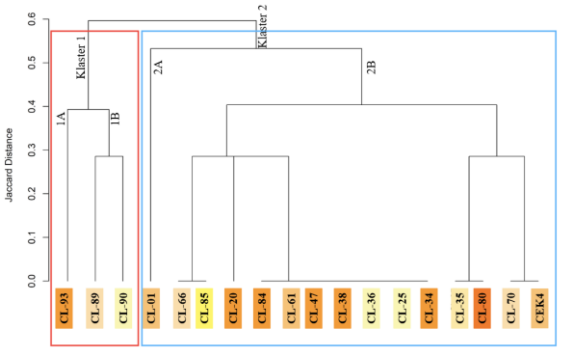

Figure 3. Dendrogram of 18 local turmeric accessions based on SSR markers using the Jaccard coefficient

Note: The color notation indicates the color of the rhizome flesh for each turmeric accession, based on color analysis using the RHS Color Chart, Sixth Edition, 2019

The results of the cluster analysis are consistent with those of the principal coordinate analysis, which did not group turmeric accessions based on their regional origin. The dendrogram, which illustrates the degree of genetic similarity among turmeric accessions, has provided an opportunity to refine the selection stage in the breeding program. Crossbreeding between parental candidates that are genetically distant can maximize the chances of obtaining segregants of superior varieties (Terryana et al., 2020).

Tabel 5. Jaccard similar\ity coefficient for 18 local turmeric accessions

CL01	CL20	CL25	CL34	CL35	CL36	CL38	CL47	CL61	CL66	CL70	CL80	CL84	CL85	CL89	CEK4	CL90	CL93	
1.000																		**CL01**
0.714	1.000																	**CL20**
0.500	0.714	1.000																**CL25**
0.500	0.714	1.000	1.000															**CL34**
0.334	0.500	0.714	0.714	1.000														**CL35**
0.500	0.714	1.000	1.000	0.714	1.000													**CL36**
0.500	0.714	1.000	1.000	0.714	1.000	1.000												**CL38**
0.500	0.714	1.000	1.000	0.714	1.000	1.000	1.000											**CL47**
0.500	0.714	1.000	1.000	0.714	1.000	1.000	1.000	1.000										**CL61**
0.500	0.714	0.714	0.714	0.500	0.714	0.714	0.714	0.714	1.000									**CL66**
0.334	0.500	0.500	0.500	0.714	0.500	0.500	0.500	0.500	0.714	1.000								**CL70**
0.334	0.500	0.714	0.714	1.000	0.714	0.714	0.714	0.714	0.500	0.714	1.000							**CL80**
0.500	0.714	1.000	1.000	0.714	1.000	1.000	1.000	1.000	0.714	0.500	0.714	1.000						**CL84**
0.500	0.714	0.714	0.714	0.500	0.714	0.714	0.714	0.714	1.000	0.714	0.500	0.714	1.000					**CL85**
0.334	0.334	0.334	0.334	0.500	0.334	0.334	0.334	0.334	0.500	0.714	0.500	0.334	0.500	1.000				**CL89**
0.334	0.500	0.500	0.500	0.714	0.500	0.500	0.500	0.500	0.714	1.000	0.714	0.500	0.714	0.714	1.000			**CEK4**
0.500	0.500	0.500	0.500	0.334	0.500	0.500	0.500	0.500	0.714	0.500	0.334	0.500	0.714	0.714	0.500	1.000		**CL90**
0.200	0.200	0.200	0.200	0.334	0.200	0.200	0.200	0.200	0.334	0.500	0.334	0.200	0.334	0.714	0.500	0.500	1.000	**CL93**

Note: Numbers highlighted in yellow = farthest genetic distance; numbers highlighted in green = closest genetic distance

Based on the phylogenetic analysis depicted in the dendrogram, the results are consistent with those of Putri et al. (2020), who analyzed turmeric diversity using PBA markers, indicating that genetic diversity in turmeric in Indonesia is extensive and not based on the region of origin. Genetic variation in turmeric accessions is thought to result from geographical dispersal patterns that lead to natural mutations and alter genomic composition, thereby causing allelic variation at specific loci (Putri et al., 2020; Nandakumar et al., 2023). Meanwhile, the mismatch between cluster grouping and the regional origin of the accessions indicates that turmeric genotypes are geographically and genetically independent (Afidani et al., 2025). This inter-accession mixing is commonly found in plants propagated vegetatively due to factors such as gene flow, the exchange of planting material between regions, domestication and selection, and mutation (Li et al., 2021; Gogoi et al., 2023). Thus, the 18 Indonesian turmeric accessions in this study exhibit extensive genetic diversity and varied phylogenetic relationships due to domestication that triggers natural mutations, thereby influencing genetic variation, particularly allele frequencies.

Genetic Relatedness and Turmeric Rhizome Flesh Color. An analysis of the relationship between genetic relatedness and the color of turmeric rhizome flesh is one of the initial steps in determining how genetic relationships influence curcumin content as indicated by the color of the rhizome flesh. The results of this study indicate that the formed genetic relationships do not group turmeric accessions with the same rhizome flesh color (Figure 3). Similarly, regarding rhizome core diameter, the dendrogram was unable to distinguish based on core diameter size. Cluster one, CL-89, has a rhizome core diameter of 5.03, while in cluster two, the rhizome core diameter ranges from 4.30 to 10.42. Thus, the dendrogram of genetic relationships based on SSR markers does not show significant grouping regarding rhizome flesh color in relation to curcumin content.

Curcumin content is influenced by many factors, such as growing environment, seasonal variations, farming techniques, and rhizome maturity. Environmental conditions such as low temperatures and water shortages can reduce curcumin content in turmeric because they can inhibit growth and photosynthesis, which affect the formation of curcuminoids (Chintakovid et al., 2022; Farrar et al., 2021). Additionally, curcumin content is influenced by phenolic content, which is directly proportional to curcumin levels (Malahayati et al., 2021). Thus, molecular information is crucial as preliminary data for identifying genetic variations that influence phenotypic traits. In plant breeding, this information serves as a basis for selecting potential parental lines for crossing and provides an initial indication of differences in curcumin content, thereby potentially yielding segregants with broad diversity and facilitating the development of superior varieties.

Analysis of Rhizome Flesh Color. Analysis of rhizome flesh color is one of the initial analyses used to determine the curcumin content in turmeric. The intensity of the rhizome flesh color reflects the curcumin content. According to the findings of Pal et al. (2020), the content of curcuminoids, antioxidants, and iron can be determined by the color of the rhizome flesh. This is further supported by the findings of Malahayati et al. (2021), which show that the curcuminoid content in yellow turmeric is higher than in white turmeric, as curcuminoids are the compounds responsible for the yellow color. In this study, 18 turmeric accessions exhibited varying rhizome flesh colors ranging from light yellow to reddish-orange (Figure 4).

Figure 4. Flesh color of the rhizomes of 18 local turmeric accessions.
Note: Sample codes 1 (CL-01), 2 (CL-20), 3 (CL-25), 4 (CL-34), 5 (CL-35), 6 (CL-36), 7 (CL-38), 8 (CL-47), 9 (CL-61), 10 (CL-66), 11 (CL-70), 12 (CL-80), 13 (CL-84), 14 (CL-85), 15 (CL-89), 16 (CEK 4), 17 (CL-90), 18 (CL-93).

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Table 6. Variations in rhizome flesh color

No.	Color category	Rhizome flesh color	Accession
1.	Light yellow	10A	CL-85
2.	Orange	23A	CL-25, CL-36, CL-90
		N25C	CL-35, CL-34
		24A	CL-66, CL-70, CL-89
		25B	CL-01, CL-61, CEK 4
		N25B	CL-20, CL-38, CL-47, CL-84, CL-93
3.	Reddish orange	25A	CL-80

Based on an analysis of rhizome color using a standardized color chart (RHS Color Chart, sixth edition, 2019), the 18 turmeric accessions can be categorized into several groups, as shown in Table 6 below. the flesh color of turmeric rhizomes in the 18 accessions ranged from light yellow to reddish orange. The most dominant rhizome flesh color was the orange category (code N25B), found in 5 accessions (CL-20, CL-38, CL-47, CL-84, CL-93), while the least common color was light yellow (code 10A) in CL-85. This variation in rhizome flesh color indicates genetic diversity among accessions, making it a potential distinguishing characteristic. Additionally, the color of the rhizome flesh can explicitly indicate the curcumin content in turmeric. However, curcumin content is not solely represented by the color of the rhizome flesh but is also positively correlated with the diameter of the primary rhizome core and the length of the secondary rhizome (Dudekula et al., 2022).

Besides color, rhizome diameter plays an important role in assessing gene expression related to curcumin content. Rhizome diameter and color are important phenotypic traits that are closely associated with the accumulation of bioactive compounds in turmeric (*Curcuma longa*) and other rhizomatous medicinal plants (Ravindran et al., 2023). Larger rhizomes generally contain a greater volume of storage parenchyma tissue, which supports higher accumulation of curcuminoids, essential oils, phenolics, and other secondary metabolites, while intense orange-yellow rhizome coloration is typically associated with elevated concentrations of curcumin, demethoxy-curcumin, and bisdemethoxycurcumin (Chintakovid et al., 2025; Kaur et al., 2023). Recent studies have demonstrated that genotypes with darker orange rhizomes exhibit significantly higher curcuminoid content than pale-colored genotypes, suggesting that rhizome color can serve as a practical indicator of phytochemical quality (Chintakovid et al., 2025). At the molecular level, these variations are regulated by differential expression of genes involved in the phenylpropanoid and curcuminoid biosynthetic pathways, including phenylalanine ammonia-lyase (PAL), 4-coumarate-CoA ligase (4CL), diketide-CoA synthase (DCS), and curcumin synthase genes (CURS1, CURS2, and CURS3) (Ayer et al., 2020; Ravindran et al., 2023). Expression analyses revealed that DCS expression is positively associated with curcumin, demethoxycurcumin, bisdemethoxy-curcumin, and total curcuminoid content, whereas CURS1, CURS2, and CURS3 contribute to the regulation of curcuminoid composition among cultivars, leading to differences in rhizome pigmentation and phytochemical profiles (Ayer et al., 2020). Furthermore, transcriptomic investigations have identified additional candidate genes involved in secondary-metabolite biosynthesis and rhizome development, supporting the hypothesis that morphological traits such as rhizome diameter and color are closely linked to gene-expression patterns governing bioactive compound accumulation (Fadhullah et al., 2024). Similar transcriptomic approaches in other rhizomatous species, such as ginger, have demonstrated the importance of gene regulatory networks in controlling rhizome-associated traits and metabolic pathways (Deng et al., 2023). Therefore, integrating rhizome phenotyping with molecular markers based on DCS, CURS, PAL, and related biosynthetic genes offers a promising strategy for breeding turmeric cultivars with enhanced nutraceutical and pharmaceutical value (Ayer et al., 2020; Fadhullah et al., 2024).

Supporting data on rhizome core diameter are presented in Table 7. The results of rhizome core diameter measurements in this study show a positive correlation with rhizome flesh color,

consistent with the findings of Dudekula et al. (2022). The largest rhizome core diameter was found in CL-84 at 10.42 mm with color code N25B, while CL-85, with the lighter color code 10A, had a rhizome core diameter of 4.66 mm. The larger the rhizome core diameter, the higher the curcumin content (Aarthi et al., 2018). Thus, information on rhizome flesh color and rhizome core diameter can serve as a basis for assessing curcumin content and genetic variation in turmeric.

Molecular information is important as an initial indicator for identifying genetic variation that influences phenotypic expression. In plant breeding, this information serves as a basis for selecting potential parent lines for crossing and provides an initial indication of differences in curcumin content, there by offering the potential to produce segregants with broad diversity and to develop new superior varieties. Accession CL-93 has the potential to be used as a superior parent candidate, as it exhibits genetic characteristics that differ from those of other accessions. Furthermore, based on the color of the rhizome flesh and the diameter of the rhizome core, accession CL-93 is also suspected to have a relatively high curcumin content.

Table 7. Rhizome core diameter data for 18 local turmeric accessions

Sample code	Accession code	Rhizome flesh color code	Rhizome core diameter (mm)
1	CL-01	25B	5.12
2	CL-20	N25B	5.18
3	CL-25	23A	5.32
4	CL-34	N25C	6.09
5	CL-35	N25C	4.49
6	CL-36	23A	4.85
7	CL-38	N25B	6.09
8	CL-47	N25B	5.45
9	CL-61	25B	6.27
10	CL-66	24A	4.30
11	CL-70	24A	4.85
12	CL-80	25A	4.88
13	CL-84	N25B	10.42
14	CL-85	10A	4.66
15	CL-89	24A	5.03
16	CEK 4	25B	6.61
17	CL-90	23A	4.98
18	CL-93	N25B	6.00

Conclusion

The level of genetic diversity among the 18 local turmeric accessions in this study was high, based on the results of cluster and principal coordinate analyses using six SSR markers capable of distinguishing local turmeric accessions. The genetic diversity in turmeric indicates the possibility of natural mutations resulting from domestication, thereby influencing genetic variation, particularly allele frequencies. The CuMiSat-35 marker has the highest PIC value of 0.58, which is capable of distinguishing each local turmeric accession and can be used for further turmeric research. Genetic diversity information based on SSR markers can serve as preliminary information in the selection of crossing parents regarding differences in curcumin content in turmeric breeding programs.

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