

Genetic diversity and population structure of Indonesian foxtail millet (*Setaria italica* (L.) P. Beauv.) germplasm revealed by SSR markers

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ABSTRACT. Foxtail millet (*Setaria italica* (L.) P. Beauv.) is cultivated in arid and semi-arid regions of Indonesia, yet genetic information on Indonesian germplasm remains limited. This study assessed the genetic diversity and population structure of 21 foxtail millet accessions collected from five Indonesian provinces using 30 polymorphic SSR markers distributed across nine chromosomes. DNA was extracted from 12-14-day-old seedlings and amplified by PCR with annealing temperatures ranging from 50 to 57°C. The polymorphic information content (PIC) was calculated using PowerMarker v3.25, while cluster analysis, genetic distance matrices, and Principal Coordinate Analysis (PCA) were performed using NTSYS-pc 2.1. Cophenetic correlation analysis was conducted using WinBoot software. Results revealed moderate to high genetic diversity with PIC values ranging from 0.09 (b190) to 0.78 (p16, b247, b223), averaging 0.54. A total of 100 alleles were detected with 2-6 alleles per locus. UPGMA-based cluster analysis grouped the germplasm into two main clusters at a similarity coefficient of 0.28, with Cluster I containing 11 accessions and Cluster II containing 10 accessions. Genetic distance values ranged from 0.11 (Minna' and Lasse') to 0.87 (ICE-276-12B-1 and Balla Pulut/Wete), indicating substantial genetic variation. PCA analysis confirmed the clustering pattern, with a strong cophenetic correlation ($r = 0.81$; good fit).

Keywords: foxtail millet; genetic diversity; microsatellite markers; germplasm; Indonesia

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INTRODUCTION

Foxtail millet (*Setaria italica* (L.) P. Beauv.) ranks among the world's oldest domesticated cereals, with archaeological evidence tracing its cultivation to approximately 10,000 years ago in Northern China (Hariprasanna, 2017; Satomura, 2024). As a diploid species ($2n = 2x = 18$) with a relatively small genome (~490 Mb), foxtail millet has emerged as a promising model system for investigating C4 photosynthesis, abiotic stress tolerance, and orphan crop improvement (Bhinda *et al.*, 2022; Rana *et al.*, 2021). Globally, approximately 29,000 germplasm accessions are conserved, reflecting extraordinary genetic diversity shaped by millennia of farmer selection across diverse agroecological zones (Vetriventhan *et al.*, 2024; Hariprasanna *et al.*, 2025).

In Indonesia, foxtail millet occupies a unique position within traditional agricultural systems, particularly across West Sulawesi, South Sulawesi, East Nusa Tenggara, and Papua provinces. Local communities use foxtail millet as an alternative staple food, preparing traditional dishes such as pancakes, porridge, and puddings, due to its low allergenicity and superior digestibility (Ramlah *et al.*, 2020; Kalsi & Bhasin, 2023). The crop's exceptional nutritional profile, rich in iron, calcium, zinc, dietary fiber, and antioxidant compounds, combined with its gluten-free nature and low glycemic index, positions it as a strategic resource for addressing malnutrition and diet-related health challenges (Amadou, 2022; Abedin *et al.*, 2022; Yang *et al.*, 2022; Arora *et al.*, 2023). Furthermore, its remarkable adaptation to marginal lands, drought tolerance, and short growth cycle make foxtail

millet crucial for climate-resilient agricultural diversification in Indonesia's semi-arid regions (Ardie *et al.*, 2025; Arinarayanasamy *et al.*, 2025; Hidayati & Syarif, 2023).

Despite these advantages, Indonesian foxtail millet germplasm remains critically undercharacterized at the molecular level, hindering the implementation of systematic conservation strategies and genetic improvement programs. Comprehensive genetic diversity assessment is fundamental for sustainable germplasm management, enabling identification of unique allelic variants, understanding population structure, and facilitating strategic parental selection for breeding initiatives (Jadhav *et al.*, 2024; Padhiyar *et al.*, 2024). Recent advances in molecular marker technology have revolutionized crop genetic resource characterization, with Simple Sequence Repeat (SSR) markers remaining the preferred choice for diversity studies due to their codominant inheritance, high polymorphism, excellent reproducibility, and cost-effectiveness compared to SNP arrays or whole-genome sequencing (Arya *et al.*, 2022; Mishra *et al.*, 2022; Seth *et al.*, 2025; Sinha *et al.*, 2025; Rahul *et al.*, 2025). SSR markers have successfully elucidated genetic architecture in various millet species, including foxtail millet (Rahul *et al.*, 2025), finger millet (Lakhani *et al.*, 2024), proso millet (Zargar *et al.*, 2023), and pearl millet (Gunguniya *et al.*, 2023), demonstrating their robust utility across Poaceae phylogeny (Kumar *et al.*, 2024).

Recent international studies have established comprehensive frameworks for analyzing foxtail millet diversity. Ahmad *et al.* (2023) characterized 18 foxtail millet genotypes and landraces using 33 polymorphic SSR markers, revealing distinct geographical clustering patterns and identifying drought-tolerance-associated loci. Similarly, Sharma *et al.* (2025) integrated phenomic and genomic approaches to evaluate a global set of accessions, demonstrating the power of multi-trait assessment in core collection development. However, investigations on Southeast Asian germplasm, particularly Indonesian collections representing distinct agro-ecological adaptations, remain conspicuously absent from global diversity databases. This knowledge gap critically limits our understanding of foxtail millet's full genetic landscape and may overlook valuable adaptive alleles selected under tropical conditions.

Therefore, the present study aimed to comprehensively assess the genetic diversity and population structure of 21 Indonesian foxtail millet accessions collected from eight provinces using 30 polymorphic SSR markers distributed across all nine chromosomes. Specific objectives were to: (1) quantify genetic diversity parameters including polymorphic information content (PIC), allelic richness, and heterozygosity; (2) construct phylogenetic relationships through UPGMA clustering and Principal Coordinate Analysis; (3) evaluate genetic distance matrices to identify potential parental combinations for breeding programs; and (4) establish baseline molecular data for conservation prioritization and germplasm management strategies. The findings will provide essential genetic information to support Indonesian foxtail millet conservation efforts and facilitate breeding programs to develop improved varieties adapted to local conditions.

MATERIALS AND METHODS

Plant genetic materials. Twenty-one foxtail millet (*Setaria italica* (L.) P. Beauv.) accessions representing diverse agro-ecological zones across Indonesia were utilized in this study. The germplasm collection comprised 11 accessions from local collections across eight provinces (West Sulawesi, South Sulawesi, Bangka Belitung, East Nusa Tenggara, and Maluku) and 10 accessions from the Indonesian Cereals Research Institute (ICERI) germplasm bank in Maros, South Sulawesi, Indonesia (Table 1). The seeds were planted in the ICERI experimental fields, following standard agronomic practices and adequate irrigation and fertilization to ensure uniform growth conditions for sample collection.

DNA extraction and quality assessment. Fresh young leaf tissue was harvested from three individual plants per accession at 12-14 days after planting to ensure developmental uniformity and optimal DNA quality. Leaf samples were immediately frozen in liquid nitrogen and stored at -20°C until processing. Genomic DNA extraction was performed using a modified *cetyltrimethylammonium*

bromide (CTAB) protocol adapted from Doyle & Doyle (1990). Approximately 0.4 g of finely ground leaf tissue was mixed with 800 μ L extraction buffer containing 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% polyvinylpyrrolidone (PVP), and 0.2% β -mercaptoethanol. The mixture was incubated at 65°C for 60 minutes with gentle agitation every 15 minutes, followed by chloroform: isoamyl alcohol (24:1) extraction and centrifugation at 12,000 rpm for 10 minutes at 4°C. DNA was precipitated using 2/3 volume of ice-cold isopropanol at -20°C for 30 minutes, washed twice with 70% ethanol, air-dried, and resuspended in 50 μ L TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Table 1. Germplasm of foxtail millet in Indonesia

No	Germplasm Name	Number of accessions	Regions
1	Balla Biasa	1	Sumba Barat Daya District, East Nusa Tenggara Province
2	Balla Pulut	1	Sumba Barat Daya District, East Nusa Tenggara Province
3	Wete	1	Nagekeo District, East Nusa Tenggara Province
4	Wete Kune/Biasa	1	Nagekeo District, East Nusa Tenggara Province
5	Mani-Mani	1	Bangka District, Bangka Belitung Island
6	Banne	1	Selayar Island, South Sulawesi Province
7	Minna'	1	Polewali Mandar District, West Sulawesi Province
8	Lasse'	1	Polewali Mandar District, West Sulawesi Province
9	Bulawang	1	Polewali Mandar District, West Sulawesi Province
10	Lokal Buru-B-1	1	Buru Island, Maluku Province
11	ICE-276-12D	1	ICERI, Maros District, South Sulawesi Province
12	4/1	1	ICERI, Maros District, South Sulawesi Province
13	L. Hitam C	1	ICERI, Maros District, South Sulawesi Province
14	20076-376-2-7	1	ICERI, Maros District, South Sulawesi Province
15	ICE-276-1	1	ICERI, Maros District, South Sulawesi Province
16	ICE-376-1	1	ICERI, Maros District, South Sulawesi Province
17	20043	1	ICERI, Maros District, South Sulawesi Province
18	ICE-276-12B-1	1	ICERI, Maros District, South Sulawesi Province
19	2007-1135-2 (BC)	1	ICERI, Maros District, South Sulawesi Province
20	M 3/1 A	1	ICERI, Maros District, South Sulawesi Province
21	ICE-276-12A	1	ICERI, Maros District, South Sulawesi Province

DNA integrity was verified by 1% (w/v) agarose gel electrophoresis in 1 $\times$ TAE buffer at 100V for 30 minutes, visualized under UV transilluminator. DNA concentration and purity were quantified using a NanoDrop spectrophotometer (Thermo Scientific). Samples with A260/A280 ratios between 1.8 and 2.0 and A260/A230 ratios >1.8 were considered suitable for PCR amplification. Working DNA solutions were diluted to 10 ng/ μ L using 0.1 $\times$ TE buffer and stored at -20°C until use.

SSR marker selection and PCR amplification. Thirty genomic SSR primer pairs were selected based on genome-wide distribution across all nine chromosomes of *S. italica*, previously reported polymorphism in foxtail millet diversity studies, and high-quality amplification in related *Setaria* species. Primers were commercially synthesized by Genetika Science Indonesia (<https://ptgenetika.com/>) (Table 2). Multiplex PCR amplifications were conducted in 10 μ L reaction volumes containing 1 μ L template DNA (10 ng/ μ L), 6.25 μ L MyTaq HS Red Mix 2 $\times$ (Bioline) containing 0.2 mM each dNTP, 1.5 mM MgCl₂, MyTaq HS DNA Polymerase with red dye and stabilizers, 0.5 μ L each of forward and reverse primers (10 μ M), and 2.25 μ L nuclease-free water. Amplification was performed using the following thermal cycling program: initial denaturation at 94°C for 4 minutes; 35 cycles of denaturation at 94°C for 40 seconds, primer-specific annealing at 50-57°C for 40 seconds, and extension at 72°C for 1 minute; final extension at 72°C for 5 minutes; and holding at 4°C.

Table 2. The sequence 30 primer SSR of foxtail millet

No	Name of SSR primer	Chromosome location	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Number of bases	Annealing temperature
1	p16	1	TTTCTCCCTCTCTCGATT CC	GAAGAGAGAAGCAGCGTT C	40	54
2	p92	1	TGGAATTGGAACCCTTT CG	GCCATGCAAACAGTACCA TC	39	54
3	b260	1	GAAGAGAGAAGCAGCG TTC	AAACCACACTTGCCTGA	37	54
4	p56	2	GATGTGTACGGGTGCA TTG	TGGGTTTCAGGGCTCTCTC	39	55
5	p80	2	GCCGTTGGATTGATTA TGG	TGTGGTTAGTTTATGTGGC TTG	42	53
6	b115	2	GGTAGCGACGGATCTAC AGC	GCTAGCAAATGCTGTCATG G	40	56
7	b151	2	AATTTCTCCAAGAGGAC AACAA	TCCTCGACATATAACCCCTC ACC	44	54
8	p98	3	ATTCATCAGTAGCACAG C	TGGAACCTAAGAACAGGAA AC	38	50
9	b186	3	CCCGTATAAATGTCATC ATCCC	GCACCTGGCTTCCCTT	38	54
10	b226	3	TACCTCCCGTTCCGTTTT GT	CGCATTGATGGCTTACAGT T	40	55
11	b236	4	TCTGGACCAGCATTCTG TCTT	GGTAACTCTGCTTGGACGA G	41	56
12	b247	4	GATTGCTCTCTCACACA CACG	GCCCCGATGGCTGCTAGT	38	57
13	b255	4	GAGGACAGCGGCCATT	CCTCCCTCCATTTACTTTG G	36	54
14	p17x	5	CGGACACCTGAAAGAC GAA	GTCACTTGTTGTTGTTGCG	38	54
15	p75	5	ATGCCATGGGAATTTGA ACC	GTTTGATGCAGGACGAGA GG	40	55
16	b223	5	GGCATTAACTACATTGA CAGTGG	AAAACCAACAGTCCCTC GT	43	54
17	b190	6	GAAATTTTACAAGTGTT GGTG	TGATCGGAGCAGAGTGTT GA	41	53
18	SICAAS6035	6	AATACCACACAAGCATC AGGAG	GGCGATGGAGTGCATTTTA TTA	44	54
19	SICAAS6101	6	CATGGTGCCTTGCAATT AGA	TGCAGTTCAGTGAGACAT ACAAAAC	45	54
20	p59	7	TAATTTTGTGGCGTGGG ATG	GCACTGGTTTTGTTGAATG G	40	53
21	b107	7	AGAACGAGGTGGTGTGT GG	GGGTCTCACGCTCTCATCA	38	57
22	b142	7	TGGTAAAACTCCCATAT TGAGC	GCCCCATCCTTGATAACAG A	42	54
23	p6	8	AAGGATGGAATTTGCCA CTG	TTTCGACGATTTGCTTCAA C	40	52
24	b185	8	GCACGTGTGACTTTCCA CAT	GTGAATGGCACACGAAAC TG	40	55
25	b258	8	GGGCCAATAATGGTTGC ATA	TTGCACATCCAAATCTTTC C	40	52
26	p38	9	GTCGTCCCACGTATGAA ACC	TGATTTACCTACCGATT GC	41	54
27	b241	9	CACGCACGTAGTATTGC TAT	GTTCTGGGCTTCTGGCTG	38	54
28	m2	9	ATCGGAAAGCAGCGCA C	TCGTTCTCCACCACTGC	35	57
29	b265	9	AATAATGGAGAGGCAG CATCC	CGAATCAAGGTGTGCGTG	39	55
30	b269	9	GTGCGTGCCTCCCTTTA	CCAGATGCTTCCACGGT	34	55

Source: Primer No. 1-17; 20-30 (Kim *et al.*, 2012); Primer No. 18-19 (Trivedi *et al.*, 2018)

Gel electrophoresis and allele scoring. PCR products were separated on 8% polyacrylamide gels (29:1 acrylamide: bisacrylamide) prepared in 1× TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.3) with 0.05% (v/v) TEMED and 0.1% (w/v) ammonium persulfate as polymerization catalysts. Gels (20 × 20 cm) were cast using Dual Mini-Vertical Complete System MGV-202-33 (C.B.S. Scientific, Del Mar, CA, USA) and polymerized for 45 minutes at room temperature. Electrophoresis was conducted at 100V for a 10-minute pre-run, followed by sample separation at 150V for

approximately 2-3 hours until the bromophenol blue dye reached the bottom of the gel. Gels were visualized using a modified silver staining protocol involving fixation in 10% acetic acid (20 minutes), silver impregnation in 0.2% AgNO₃ solution (20 minutes), and development in 3% Na₂CO₃ with 0.05% formaldehyde (3-5 minutes) until bands were clearly visible. Fragment sizes were estimated by comparison with PhiX174 DNA/HinfI molecular weight markers (72-1.353 bp) run on each gel. The marker, consisting of 11 fragments ranging from 72 to 1.353 bp, was loaded in the first and last lanes of each gel to account for potential variation in migration across the gel. Fragment sizes were determined by plotting the log of the known marker fragment sizes against their migration distances to generate a standard curve for each gel. SSR alleles were scored as present (1) or absent (0) based on visual data.

Data analysis. A binary data matrix (21 accessions) was constructed in Microsoft Excel and converted to appropriate formats for subsequent analyses. Prior to binary conversion, SSR alleles were scored based on the presence of specific fragment sizes as resolved on agarose gels, with each unique fragment size treated as a distinct allele. The multi-allelic genotype data were used for Polymorphic Information Content (PIC) calculation to ensure accurate estimation of allelic diversity at each locus. Polymorphic information content (PIC) values were calculated for each SSR locus using PowerMarker version 3.25 (Liu & Muse, 2005) according to the formula: $PIC = 1 - \sum (P_i^2)$, where P_i represents the frequency of the i th allele. The binary data matrix was used exclusively for similarity-based analyses (Jaccard's coefficient and UPGMA clustering), as binary scoring is appropriate for presence/absence data in these analyses. PIC values were classified as highly informative ($PIC > 0.75$), moderately informative ($0.50 < PIC \leq 0.75$), or low informative ($PIC \leq 0.50$) according to Botstein *et al.* (1980). Jaccard's similarity coefficients were calculated between all pairs of accessions using NTSYS-pc version 2.11 (Exeter Software, Setauket, NY, USA), and genetic distance matrices were calculated as the complement of similarity coefficients ($GD = 1 - \text{Similarity}$). Hierarchical cluster analysis was performed using the *Unweighted Pair Group Method with Arithmetic Mean* (UPGMA) algorithm based on *Jaccard's similarity coefficients*, with bootstrap support assessed through 1,000 replicates using WinBoot software. The goodness-of-fit between the original similarity matrix and cluster-derived cophenetic matrix was evaluated by calculating the cophenetic correlation coefficient (r) using NTSYS-pc, with significance tested via the Mantel test with 1,000 permutations. Cophenetic correlation values were interpreted as a very good fit ($r > 0.9$), a good fit ($0.8 < r \leq 0.9$), a poor fit ($0.7 < r \leq 0.8$), or a very poor fit ($r \leq 0.7$) following Rohlf (2000). *Principal Coordinate Analysis* (PCA) was conducted using NTSYS-pc 2.11 to visualize genetic relationships among accessions in multidimensional space, providing complementary confirmation of clustering patterns derived from UPGMA analysis.

RESULTS AND DISCUSSION

SSR primer amplification. The transferability assessment of 30 foxtail millet SSR primers on twenty-one millet genotypes (Table 3) revealed variable amplification success rates. All 30 primers (100%) amplified successfully. Among these, 4 primers (13.3%) produced 6 alleles each, while the remaining 26 primers (86.7%) produced 2–4 alleles per locus. The amplified products ranged in size from 82 bp to 362 bp across the tested germplasm, demonstrating considerable size polymorphism (Fig. 1).

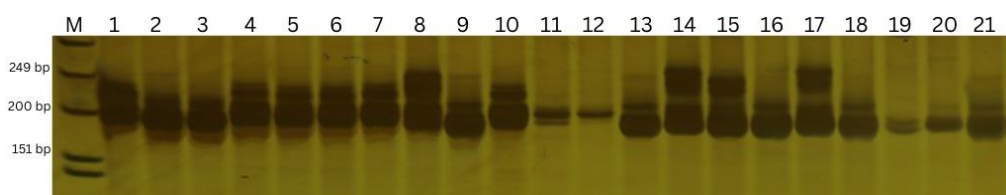


Fig. 1. Amplification with P16 primers. Note. **M:** Marker, **1:** ICE-276-1, **2:** ICE-276-12B-1, **3:** ICE-376-1, **4:** 2007-1135-2 (BC), **5:** 20043, **6:** 20076-376-2-7, **7:** 4/1, **8:** M 3/1 A, **9:** L. hitam C, **10:** Lokal Buru-B-1, **11:** Mani-mani, **12:** Balla Pulut, **13:** Balla Biasa, **14:** Wete kune/biasa, **15:** Wete, **16:** Banne, **17:** Minna', **18:** Lasse', **19:** Bulawang, **20:** ICE-276-12A, **21:** ICE-276-12D

Genetic diversity parameters

Analysis of 30 polymorphic SSR markers across 21 foxtail millet accessions generated a total of 100 alleles, with the number of alleles per locus ranging from 2 to 6 (mean 3). The polymorphic information content (PIC) values ranged from 0.09 to 0.78 with an average value of 0.54, indicating moderate to high genetic diversity. Gene diversity ranged with primer p16, b247, and b223, exhibiting the highest diversity (0.78). According to established classification criteria, these primers displayed high polymorphism ($PIC > 0.75$), while the majority (17/30 loci) displayed moderate polymorphism ($0.50 < PIC \leq 0.75$), three loci showed high polymorphism ($PIC > 0.75$), and ten loci showed low polymorphism ($PIC \leq 0.50$).

Table 3. Data Pprofile of 30 SSR markers used in foxtail millet germplasm in Indonesia

No	Primer	Location of chromosomes	Number of Alleles	Allele Size (bp)	(Polymorphism) PIC	Melting Temperatur (Tm) ⁰ C
1	p16	1	6	189 – 254	0,78	54
2	p92	1	2	147 – 157	0,50	54
3	b260	1	4	125 – 169	0,71	54
4	p56	2	2	166 – 185	0,17	55
5	p80	2	4	191 – 216	0,64	53
6	b115	2	3	167 – 184	0,54	56
7	b151	2	3	182 – 194	0,54	54
8	p98	3	2	143 – 151	0,50	50
9	b186	3	3	136 – 145	0,63	54
10	b226	3	2	239 – 264	0,49	55
11	b236	4	3	118 - 134	0,59	56
12	b247	4	6	82 - 111	0,78	57
13	b255	4	2	161 – 175	0,31	54
14	p17x	5	3	200 – 244	0,40	54
15	p75	5	5	190 – 288	0,68	55
16	b223	5	6	118 – 220	0,78	54
17	b190	6	2	311 – 362	0,09	53
18	SICAAS6035	6	3	143 – 171	0,53	54
19	SICAAS6101	6	6	155 – 249	0,66	54
20	p59	7	3	175 - 191	0,59	53
21	b107	7	3	210 - 255	0,51	57
22	b142	7	4	184 – 239	0,64	54
23	p6	8	2	175 – 185	0,31	52
24	b185	8	4	148 – 175	0,59	55
25	b258	8	2	200 – 249	0,34	52
26	p38	9	3	157 – 169	0,54	54
27	b241	9	4	109 – 137	0,66	54
28	m2	9	2	143 – 151	0,49	57
29	b265	9	2	224 – 239	0,49	55
30	b269	9	4	126 - 167	0,68	55
Total			100	Size range:	16,18	
Average			3	82 - 362	0,54	50 - 57

Population structure and genetic relationships. UPGMA cluster analysis based on Jaccard's similarity coefficient grouped the 21 foxtail millet accessions into two main clusters at a similarity coefficient of 0.28 (**Fig. 2**). Cluster I contained 11 accessions, including five ICERI collection accessions (ICE-276-1, ICE-276-12B-1, ICE-376-1, ICE-276-12A, ICE-276-12D) in sub-cluster IA, and six accessions from various regions including four from East Nusa Tenggara (Wete kune/biasa, Balla Pulut, Wete, Balla Biasa), one from Bangka Belitung (Mani-mani), and one ICERI collection (L.Hitam C) in sub-cluster IB. Cluster II comprised 10 accessions, with sub-cluster IIA containing nine diverse accessions from West Sulawesi (Bulawang, Minna', Lasse'), South Sulawesi (Banne), Maluku (Lokal Buru-B-1), and four ICERI collections, while sub-cluster IIB contained only one accession (M3/1A).

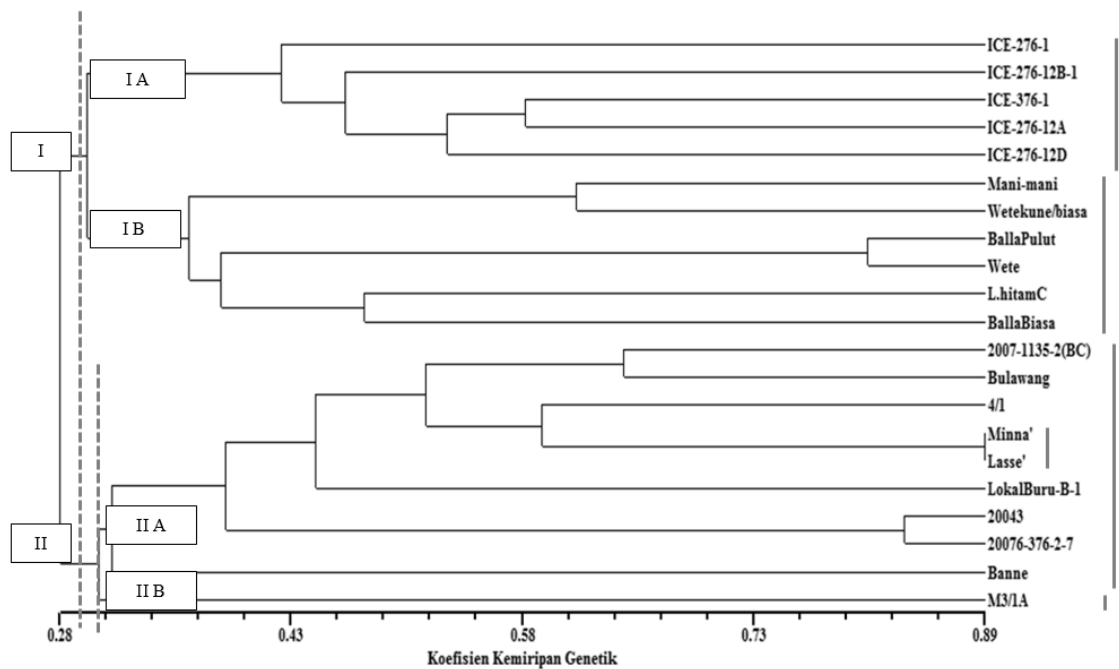


Fig. 2. Dendrogram of foxtail millet germplasm in Indonesia based on information on 30 SSR primers using the NTSYS-pc 2.1 program

Genetic distance (Table 4) (values ranged from 0.11 to 0.87, with the smallest distance observed between Minna' and Lasse' (0.11), both originating from West Sulawesi, indicating a close genetic relationship. Conversely, the largest genetic distance (0.87) was detected between ICE-276-12B-1 and Balla Pulut/Wete, suggesting substantial genetic divergence. The close genetic relationship between Minna' and Lasse', confirmed by their clustering at a similarity coefficient of 0.89, indicates limited genetic variability between these two local accessions and suggests they would not be suitable as parental combinations for breeding programs.

Principal Coordinate Analysis (PCA) (Fig. 3) corroborated the UPGMA clustering pattern, with the first three principal components explaining a substantial portion of the genetic variation. The cophenetic correlation coefficient (r 0.81) indicated a good fit between the original similarity matrix and the dendrogram, confirming the reliability of the genetic relationships depicted in the cluster analysis. Population structure analysis revealed genetic stratification consistent with both geographical origin and morphological characteristics, although some accessions showed unexpected clustering patterns potentially attributable to historical germplasm exchange or selection pressures.

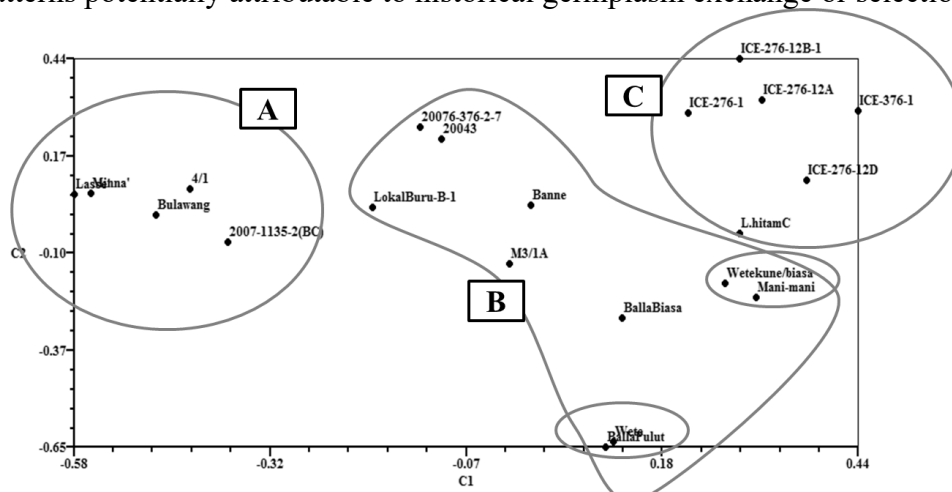


Fig. 3. The relative position of foxtail millet germplasm in Indonesia using 30 millet SSR primers based on the results of two-dimensional PCA analysis

Table 4. Genetic Distance of 30 SSR markers used in foxtail millet germplasm in Indonesia

	ICE-276-1	ICE-276-12B-1	ICE-376-1	2007-1135-2(BC)	20043	20076-376-2-7	4/1	M3/1A	L.hitamC	LokalBuru-B-1	Mani-mani	BallaPulut	BallaBiasa	Wetekune/biasa	Wete	Banne	Minna'	Lasse'	Bulawang	ICE-276-12A	ICE-276-12D
ICE-276-1	0.00																				
ICE-276-12B-1	0.58	0.00																			
ICE-376-1	0.50	0.45	0.00																		
2007-1135-2(BC)	0.67	0.80	0.78	0.00																	
20043	0.67	0.70	0.60	0.63	0.00																
20076-376-2-7	0.67	0.67	0.65	0.65	0.17	0.00															
4/1	0.68	0.73	0.74	0.44	0.57	0.48	0.00														
M3/1A	0.78	0.72	0.75	0.65	0.65	0.73	0.69	0.00													
L.hitamC	0.69	0.64	0.53	0.65	0.65	0.67	0.75	0.72	0.00												
LokalBuru-B-1	0.72	0.73	0.68	0.43	0.60	0.63	0.57	0.63	0.75	0.00											
Mani-mani	0.69	0.74	0.58	0.69	0.72	0.76	0.72	0.64	0.58	0.61	0.00										
BallaPulut	0.78	0.87	0.73	0.65	0.77	0.77	0.69	0.65	0.65	0.73	0.62	0.00									
BallaBiasa	0.72	0.79	0.63	0.69	0.63	0.66	0.71	0.77	0.52	0.71	0.62	0.58	0.00								
Wetekune/biasa	0.75	0.75	0.60	0.71	0.65	0.71	0.74	0.68	0.65	0.65	0.38	0.65	0.63	0.00							
Wete	0.79	0.87	0.76	0.71	0.74	0.74	0.70	0.71	0.70	0.76	0.65	0.19	0.52	0.67	0.00						
Banne	0.63	0.69	0.61	0.72	0.67	0.67	0.67	0.76	0.69	0.81	0.75	0.67	0.72	0.64	0.72	0.00					
Minna'	0.79	0.75	0.78	0.57	0.65	0.68	0.44	0.73	0.81	0.63	0.81	0.77	0.71	0.78	0.74	0.64	0.00				
Lasse'	0.77	0.77	0.75	0.50	0.60	0.63	0.37	0.73	0.79	0.60	0.79	0.75	0.69	0.75	0.71	0.67	0.11	0.00			
Bulawang	0.72	0.80	0.78	0.35	0.63	0.60	0.48	0.68	0.67	0.53	0.76	0.70	0.66	0.78	0.78	0.64	0.47	0.43	0.00		
ICE-276-12A	0.55	0.51	0.41	0.75	0.70	0.67	0.65	0.75	0.64	0.70	0.69	0.77	0.75	0.70	0.75	0.74	0.75	0.73	0.77	0.00	
ICE-276-12D	0.66	0.63	0.45	0.74	0.67	0.67	0.77	0.76	0.61	0.67	0.63	0.74	0.60	0.61	0.65	0.77	0.79	0.76	0.79	0.48	0.00

Genetic diversity and population differentiation. The moderate to high genetic diversity detected in Indonesian foxtail millet germplasm (average PIC 0.54) indicates substantial genetic variation within the studied collection. In comparison, Rahul *et al.* (2025) reported the PIC value of 27 accessions of browntop millet using SSR primers varied from 0 to 0.68. Lv *et al.* (2025) reported an average PIC value of 0.38 for Chinese proso millet germplasm using 15 SSR markers, while Xue *et al.* (2025) documented Shannon-Wiener indices ranging from 1.86 to 2.08 for quantitative traits in 1,558 foxtail millet accessions. The relatively higher PIC values in our study may reflect either genuine genetic diversity within Indonesian germplasm or differences in marker selection and primer informativeness.

The detection of 100 alleles across 30 SSR primer pairs (average 3 alleles per locus) demonstrates considerable allelic richness in Indonesian foxtail millet germplasm. This allelic diversity is higher than the 76 alleles from 25 markers reported by Rahul *et al.* (2025) in browntop millet, suggesting that foxtail millet germplasm harbors substantial genetic variation suitable for breeding programs. The identification of both common (high major allele frequency) and rare alleles (low major allele frequency) indicates the presence of unique genetic variants that may carry valuable agronomic traits or adaptive characteristics.

Geographical patterns and genetic structure. The clustering pattern observed in our study revealed a partial association between genetic grouping and geographical origin, with accessions from the same region often clustering together. The extremely close genetic relationship between Minna' and Lasse' from West Sulawesi (genetic distance 0.11) exemplifies geographical clustering and suggests recent common ancestry or ongoing gene flow within localized populations. This pattern parallels findings from Lv *et al.* (2025), who reported that Chinese proso millet varieties from the

Northwest and Loess Plateau regions showed greater genetic diversity and distinct clustering patterns based on geographical origin.

However, the presence of accessions with unexpected clustering patterns, such as germplasm from geographically distant regions grouping together, suggests factors beyond simple geographical isolation influence genetic structure. Xue *et al.* (2025) similarly observed that hierarchical clustering of 1,558 foxtail millet accessions revealed complex genetic relationships not entirely explained by geographical origin, attributing this to historical germplasm exchange, human-mediated seed movement, and convergent selection for similar agronomic traits across different regions.

The division of Indonesian foxtail millet germplasm into two main clusters, with a low similarity coefficient (0.28), indicates substantial genetic differentiation within the collection. This genetic structure may reflect historical population isolation, different introduction events from diverse source populations, or differential selection pressures across Indonesia's varied agroecological zones. The relatively low genetic similarity between some cluster groups suggests the potential for heterosis when crossing accessions from different clusters, providing valuable information for breeding strategies.

Despite these findings, certain limitations must be considered. The small sample size of 21 accessions, combined with the disproportionate representation of ICERI Maros materials (47.6% of total accessions), limits the generalizability of the observed genetic patterns to the broader Indonesian foxtail millet germplasm. Accordingly, the results should be regarded as preliminary, and expanded studies employing larger, geographically diverse collections are warranted to elucidate a more complete picture of genetic structure.

Implications for germplasm conservation and breeding. The identification of genetically distinct groups within Indonesian foxtail millet germplasm has important implications for conservation strategies and breeding programs. Accessions with high genetic distance, such as ICE-276-12B-1 and Balla Pulut/Wete (genetic distance 0.87), represent valuable parental combinations for developing breeding populations with broad genetic base and high heterotic potential. This approach aligns with recommendations from Xue *et al.* (2025), who emphasized the importance of utilizing diverse germplasm from different genetic groups to broaden the genetic base of elite cultivars.

The moderate to high polymorphism exhibited by most SSR markers (average PIC 0.54) confirms their utility for various genetic applications, including germplasm characterization, genetic mapping, and marker-assisted selection in Indonesian foxtail millet. Rahul *et al.* (2025) similarly demonstrated that SSR markers with high PIC values (>0.5) were particularly effective for distinguishing genotypes and facilitating breeding decisions. The establishment of DNA fingerprints for Indonesian foxtail millet accessions using these polymorphic markers would facilitate germplasm management, variety protection, and quality control in seed production systems.

Comparative perspectives and future directions. Comparing our findings with recent studies on related millet species reveals both similarities and unique characteristics of Indonesian foxtail millet germplasm. While Xue *et al.* (2025) analyzed genetic diversity primarily through phenotypic traits across 1,558 foxtail millet accessions and identified five distinct groups through cluster analysis, our molecular marker-based approach detected two main genetic clusters among 21 accessions, demonstrating complementarity between phenotypic and molecular characterization approaches. Similarly, Lv *et al.* (2025) integrated both phenotypic and molecular data to evaluate 1,582 proso millet accessions, identifying four genetic groups through UPGMA analysis and demonstrating the value of multi-faceted germplasm assessment.

The cophenetic correlation coefficient ($r = 0.81$) confirms that the UPGMA dendrogram adequately represents the pairwise genetic relationships among accessions, consistent with values reported in comparable SSR-based studies such as Salem *et al.*, (2024) ($r = 0.71$) and Göçmen *et al.*, (2025) ($r = 0.80$). According to Rohlf (2000), cophenetic correlation values exceeding 0.80 are considered indicative of a good fit, supporting the reliability of the genetic structure observed in this

study. Nevertheless, future investigations should incorporate broader germplasm collections from underrepresented Indonesian regions and integrate complementary marker systems, such as SNPs, to further validate and refine these findings.

The limited genetic diversity observed between some closely related accessions (e.g., Minna' and Lasse') highlights the need for germplasm enrichment through the introduction of diverse materials from other regions and countries. Lv *et al.* (2025) emphasized the importance of collecting wild varieties and introducing foreign germplasm to enhance genetic diversity, a recommendation equally applicable to Indonesian foxtail millet conservation programs. Systematic collection efforts targeting underexplored regions and integration of wild relatives could substantially broaden the genetic base available for future breeding initiatives.

CONCLUSION

This study demonstrated moderate cross-species transferability of 30 foxtail millet SSR markers to Indonesian germplasm, with di-nucleotide repeats showing the highest amplification success. Analysis of 21 accessions revealed 100 alleles (2–6 per locus; average 3), with PIC values ranging from 0.09 to 0.78 (mean 0.54), indicating moderate to high genetic diversity. UPGMA grouped the accessions into 2 clusters at similarity coefficients of 0.28–0.89, with genetic distances ranging from 0.11 to 0.87 (Minna'–Lasse' very close; ICE-276-12B-1–Balla Pulut/Wete highly divergent). PCA confirmed the clustering pattern, and a cophenetic correlation of $r = 0.81$ validated dendrogram reliability. These findings provide essential molecular baseline data for conservation, germplasm management, and breeding design, where highly divergent pairs (0.87) represent promising parental combinations to maximize heterosis.

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