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GENETIC DIVERSITY BASED ON SIMPLE SEQUENCE REPEAT (SSR) MARKERS IN INDIAN JUJUBE (*ZIZIPHUS MAURITIANA* LAM.) FROM SUMBAWA ISLAND, INDONESIA

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SUMMARY

Jujube (*Ziziphus mauritiana* Lam.) is a culturally, ecologically, and economically significant species in arid and semi-arid regions worldwide, including Sumbawa Island, Indonesia. Its wide morphological and pomological variability necessitates assessment of the genetic diversity for utilization and future conservation. The 13 jujube accessions (10 wild and three partially domesticated from West Sumbawa [SB], Sumbawa Regency [KS], and Dompu [DP]) underwent analysis for genetic diversity using 27 SSR markers. Genetic diversity revealed variations in allelic richness and polymorphism levels, and the DP population had the highest allelic richness, followed by SB and KS. The principal component analysis (PCA) showed that KS accessions formed a genetically cohesive group, while DP accessions displayed more dispersion, with considerable intra-population variation and partial overlap with SB accessions. The SSR-based clustering divided the accessions into two major groups: cluster I (KS3L1, DP1L2, DP1L3, KS1L1, KS2L2, SB1L2, and SB2L1) and cluster II (SB2L2, DP2L1, SB1LI, DP2L2, DP3L1, and SB2L3), consistent with PCA results. The results confirmed distinct genotypic profiles among ecotypes and the effectiveness of SSR markers in capturing the population structure of *Z. mauritiana* across Sumbawa Island. The two distinct genotype groups reflect complex local adaptation and domestication processes, and SB accessions showed intermediate genetic signals likely influenced by ecological overlap and historical exchange of planting materials.

Keywords: Jujube (*Z. mauritiana*), genetic diversity, SSR polymorphism, UPGMA clustering, principal component analysis (PCA), ecotype, domestication

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Key findings: The SSR markers considerably revealed genetic diversity and structure among the jujube (*Z. mauritiana*) accessions from Sumbawa Island, identifying two distinct genotypic clusters. It indicates the presence of at least two distinct genotypes, reflecting complex patterns of domestication and local adaptation within the island's populations.

INTRODUCTION

Jujube (*Z. mauritiana* Lam.), commonly known as Indian jujube or ber, is a multipurpose tree species of significant ecological and economic value in arid and semi-arid regions across Asia, Australia, Africa, China, and the Middle East. This evergreen species thrives in diverse ecological conditions, including coastal saline soils and drylands. Alongside the Chinese jujube (*Z. jujuba* Mill.), *Z. mauritiana* has become one of the most economically important species in the genus *Ziziphus* (Muhammad *et al*., 2022).

In its native and domesticated ranges, *Z. mauritiana* provides critical ecosystem services, such as fruits, shelter for animals and humans, water conservation, and erosion control, due to its heavy and deep root system. However, the same traits contribute to its classification as an invasive species in Australia, where it rapidly regenerates after fire and repeated cutting. In contrast, in countries like India and Pakistan, where consumption of fruits is widely prevalent (Sareen *et al.,* 2023), these fruit-related traits further enhance its value as a resilient fruit crop capable of thriving under harsh climatic conditions. The ber plant is also crucial for its medicinal, ethnopharmacological, and ethnobotanical properties (Maaiden *et al*., 2020; Imran *et al*., 2023; Kaboli *et al*., 2023).

Reports have stated the highest morphological and phytochemical diversity emerged among the *Z. mauritiana* populations across different regions in previous studies (Mirheidari *et al*., 2022; Khadivi, 2023). The genus *Ziziphus* is a predominantly cross-pollinated tree and exhibits protandry (Muhammad *et al*., 2023). In China, over 45 different species have reached documentation, while more than 60 accessions have also had reports in Pakistan and 48 in India (Liang *et al*., 2019; Sharif *et al*., 2019; Sareen *et al*., 2023). In Indonesia, the species prevails in Sumatra (Askur and Ganning, 2021), Java (Kurniawan *et al*., 2019), Bali (Soraya *et al*., 2022), West Nusa Tenggara (Nairfana *et al*., 2022; Nikmatullah *et al*., 2023), and East Nusa Tenggara (Kurniawan and Pujiono, 2018). For West Nusa Tenggara, *Z. mauritiana* grows naturally on Sumbawa Island from lowland areas up to approximately 700 meters above sea level (masl) across all five regencies. Some ecotypes incurred semi-domestication when maintained in home gardens and on paddy field margins due to their desirable fruit traits (Nikmatullah *et al*., 2023). During the peak fruiting season, selling ber fruits in traditional markets persists, while selection for its domestication often relied on fruit characteristics such as taste, size, and color.

In Sumbawa, Indonesia, considerable morphological variations have succeeded in the documentation of *Z. mauritiana* ecotypes, including differences in leaf size, shape, and color; thorn morphology; fruit size, shape, and color; flesh thickness; and taste (plain, sour, and sweet) (Nikmatullah *et al.*, 2023). These jujube ecotypes also vary for their secondary metabolite profiles based on biochemical analysis (Nairfana *et al*., 2022). Given this diversity among these jujube ecotypes, further exploration of genetic divergence is vital to support domestication, breeding, and the development of value-added products. With greater species' potential in food, medicinal, and cultural applications, it was essential to assess its genetic diversity to support future conservation and utilization strategies. The SSR markers have proven effective in distinguishing the closely related *Ziziphus* species, such as *Z. mauritiana*, *Z. jujuba*, and *Z. nummularia*, as well as detecting intraspecific variations (Uddin *et al*., 2021; Sareen *et al*., 2023). Accordingly, the employment of SSR markers sought to investigate the genetic diversity among the inter-island *Z. mauritiana* ecotypes.

Molecular markers appeared to be powerful tools for evaluating genetic variation and elucidating relationships within and among the crop plant species. Several marker systems have had successful employment in previous studies on *Ziziphus* genetic relationships. These are amplified fragment length polymorphisms (AFLP) (Shahhoseini *et al*., 2023), inter-simple sequence repeats (ISSR) (Abdel-Sattar *et al*., 2024), *matK* (Uddin *et al*., 2024a, 2024b), and random amplified polymorphic DNA (RAPD) (Singh *et al*., 2009; Daghighi *et al*., 2020). Others included start codon-targeted (SCoT) polymorphisms (Kaboli *et al*., 2023), sequence-related amplified polymorphisms (SRAP), simple sequence repeats (SSR) (Sareen *et al*., 2023), internal transcribed spacer (ITS) (Uddin *et al*., 2024a), and single nucleotide polymorphisms (SNPs) (Uddin *et al*., 2024b).

Among these markers, SSR markers proved particularly more productive due to their codominant inheritance, high allelic diversity, and precise genome localization (Ramesh *et al*., 2020; Li *et al*., 2025). The SSRs have reached effective utilization for molecular fingerprinting and genetic diversity analyses across various wild and domesticated *Ziziphus* species, as well as among ecotypes of the same species from distinct geographical regions, including China, Pakistan, and India (Liang *et al*., 2019; Uddin *et al*., 2021; Sareen *et al*., 2023; Uddin *et al*., 2024a, 2024b). The SSR markers have demonstrated the capacity to differentiate the genetic variation within *Ziziphus* populations, suggesting their potential utility in detecting intra-island genetic diversity in *Z. mauritiana*. Accordingly, the following study aimed to apply SSR markers for evaluating genetic diversity and relationships among the *Z. mauritiana* accessions collected from Sumbawa Island, Indonesia.

MATERIALS AND METHODS

Study area

This research on jujube (*Z. mauritiana*) took place in three districts on Sumbawa Island, Indonesia: West Sumbawa (SB), Sumbawa Regency (KS), and Dompu (DP) (Figure 1). The selection of these districts depended on previous studies confirming the presence of well-established *Z. mauritiana* populations in these areas (Nairfana *et al*., 2022; Nikmatullah *et al*., 2023). All three districts shared characteristics of semi-arid regions with predominantly non-technical irrigation systems. Their climatic conditions, particularly annual rainfall and temperature, reflect a subtle gradient of aridity. West Sumbawa (SB) receives the highest annual rainfall, ranging from 1250 to 1500 mm, followed by Sumbawa Regency (KS) with 1000 to 1250 mm, and Dompu (DP) with the lowest rainfall (between 750 and 1000 mm).

Figure 1. Location of sampling site of *Z. mauritiana* L. reported in this study.

Table 1. *Z. mauritiana* accessions with origin, habitus, and domestication status.

Acession	Location	Habitus	Altitude (m asl)	Latitude/ Longitude (GPS)	Plant status
SB1L1	Poto Tano-1	Coastal	5	8°31'58.0"S 116°50'08.0"E	Wild
SB1L2	Kertasari	Coastal	5	8°41'26.9"S 116°46'35.7"E	Wild
SB2L1	Taliwang -1	Garden/forest edge	72	8°44'15.9"S 116°49'39.1"E	Wild
SB2L2	Taliwang-2	Garden/forest edge	84	8°44'35.0"S 116°49'15.6"E	Wild
SB2L3	Jereweh	Garden/forest edge	93	8°51'58.3"S 116°49'41.2"E	Wild
KS1L1	Labuan Badas	Coastal	5	8°27'14.4"S 117°21'01.3"E	Wild
KS2L1	Sebasang, Moyo Hulu	Rice field	320	8°39'36.2"S 117°27'01.8"E	Wild
K32L1	Lantung, Moyo Hulu	Garden	520	8°45'16.2"S 117°31'21.9"E	Wild
DP1L2	Doro Nchanga	Savanna	32	8°26'24.7"S 117°58'33.9"E	Wild
DP1L3	Persiapan Sori Tonga Pekat	Savanna	52	8°27'15.9"S 117°59'47.4"E	Wild
DP2L1	Doromelo, Manggalewa, Dompu Village	Backyard	120	8°30'59.3"S 118°18'17.6"	Grew naturally and maintained (domesticated)
DP2L2	Dompu Village	Backyard	110	8°32'22.3"S 118°26'57.2"E	Grew naturally and maintained (domesticated)
DP2L3	Dompu Village	Backyard	115	8°31'54.3"S 118°28'39.7"E	Grew naturally and maintained (domesticated)

Plant material and DNA extraction

The collected jujube (*Z. mauritiana* Lam.) accessions used in this study came from Sumbawa Island, Indonesia. It shows considerable morphological variation, especially in leaf size and color, the number and size of thorns, and the shape, size, color, and flavor of the fruit, as well as the biochemical composition of secondary metabolites of leaves (Nairfana *et al*., 2022; Nikmatullah e*t al.*, 2023). These phenotypic differences among the jujube accessions suggested diverse ecotypes, with some of which being selectively maintained and cultivated in home gardens by local communities, primarily for better fruit quality and adaptability.

For genetic diversity analysis, the collection of jujube tree leaf samples occurred during the period of September to October 2024. In each district, researchers took leaf samples from lowland areas of previously characterized *Z. mauritiana*, with the detailed characteristics provided in Table 1 (Nairfana *et al*., 2022; Nikmatullah *et al*., 2023). In the presented study, 13 *Ziziphus* accessions, comprising 10 wild and three partially domesticated, entailed collection from different geographical locations of Sumbawa Island, West Nusa Tenggara, Indonesia. The leaf samples of jujube accessions received analysis using SSR markers.

Young and unopened selected apical leaves of *Z. mauritiana* were chosen samples after confirming them as healthy and undamaged. Samples, as placed in plastic bags containing silica gel to prevent moisture and degradation, proceeded in transporting to the laboratory and storing at room temperature (26 °C–27 °C) until used for DNA extraction (approximately one month).

The molecular analysis comprised grinding 1 g of young leaf tissues in liquid nitrogen and extracting genomic DNA using a modified cetyltrimethylammonium bromide (CTAB) method (Aboul-Maaty and Oraby,

2019; Kurnianingsih *et al*., 2025) with 2% CTAB, 2% β-mercaptoethanol, and 1% polyvinylpyrrolidone (PVP-40). The DNA concentration and purity assessment used a NanoDrop spectrophotometer (Thermo Scientific) before storing the DNA samples at −20 °C.

PCR reaction to amplify the SSR markers

Twenty-seven SSR primers (13 primers from Sareen *et al*. [2023] and 14 primers from Liang *et al*. [2019]) served the assessment of genetic diversity in 13 Z. *mauritiana* accessions from Sumbawa Island. The PCR performed consisted of a 10 µl reaction containing 4.8 µl of sterilized distilled water, 2.0 µl of genomic DNA (13 ng/µl), 0.5 µl of forward and 0.5 µl of reverse primer (5 µM), and 0.5 µl of $MgCl_2$ (25 mM). It also contained 1.0 µl of 10 × PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 0.5 µl of dNTP mix (0.2 mM each of dATP, dGTP, dCTP, and dTTP), and 0.2 µl of Taq polymerase (5 U/µl). The PCR conditions included initial denaturation (5 min at 94 °C), 35 cycles of 1 min denaturation at 94 °C, 1 min annealing at respective temperature for each primer (Table 2), 1 min extension at 72 °C, and a final extension for 7 min at 72 °C. Amplification products entailed separation on 1% agarose gel in 1 × TBE buffer, with each fragment size estimated using a 50 bp DNA ladder (MBI Fermentas, Lithuania). The fragments incur visualization by using ethidium bromide, with permanent photographs of gels taken in a gel documentation system (Bio-Rad laboratories-segrate, Milan, Italy).

Data analysis

Recording allele presence continued numerically, with a score of 1 indicating the presence and 0 indicating the absence of a given allele. For each population and SSR locus, genetic parameters including the number of different alleles (Na), the effective number of alleles (Ne), observed heterozygosity (h), and Shannon's diversity index (I) reached calculations using GenAlEx version 6.51b2 (GenAlEx software: https://biology.anu.edu.au/research/software/ genalex (Peakall and Smouse, 2021). Clustering analysis, as performed, used PAST 4.0 software, employing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm based on the Euclidean similarity index to generate a dendrogram. Principal Component Analysis (PCA) also ensued using PAST 4.0 to visualize patterns of variation among the samples obtained from leaves of different jujube accessions.

RESULTS AND DISCUSSION

SSR marker polymorphism

Twenty-seven SSR primers generated 70 reproducible bands across 13 *Z. mauritiana* accessions, averaging 1.21 fragments per primer (ranging from 0 to 2 bands of 80 to 450 bp) (Figure 2). The 26 primers exhibited polymorphism at both population and individual levels, while the primer ZCMS14 failed to amplify any alleles. The primer BFU1157 amplified alleles only in the jujube populations obtained from KS and DP regions, and primer E.63 only amplified alleles in DP populations. The other 24 primers produced 1–2 alleles based on the jujube genotypes and their origin. The average number of alleles per SSR locus was lower than the same reported previously in *Z. mauritiana*, Z. *jujuba*, and other related species. Singh *et al.* (2009) obtained 9.28 bands per primer using the ISSR marker, while Uddin *et al.* (2024a) reported 8.92 alleles per locus in *Z. jujube* using SSR. In another study, Uddin *et al*. (2021) successfully employed SSR markers on the genotype of four *Ziziphus* species originating from Pakistan and China and obtained an average of 2.82–3.366 alleles per species. Similarly, Sareen *et al.* (2023) declared 2.47 alleles per primer by analyzing the genetic diversity of species *Z. mauritiana* and *Z. nummularia* across diverse geographical locations in China. However, these differences may be due to several biological factors, including local adaptation, evolution and domestication history, and the narrower scope of the jujube germplasm collection.

Table 2. The SSR primers used to assess the genetic diversity of *Z. mauritiana* germplasm in Sumbawa Island, Indonesia.

No	Name of Primer	Sequence	Repeat Motif	TM	Reference
1.	BFU0263	F-GGTTTTTGTGGGTATGGAGGT R-AGGAAAACAAAGGGATGGAGA	$(CT)_{11}$	50	Sareen *et al*., 2023
2.	BFU0286	F-GATTGTTGCTGGTTTCCATGT R-CTGGACTCTCCGATGCAGTAG	$(AG)_{10}$	51	Sareen *et al*., 2023
3.	BFU0377	F-CCAGCTGGTATCCAATTGCT R-ACGACGATGCCATGAAAGAT	$(CT)_{10}$	50	Sareen *et al*., 2023
4.	BFU0473	F-GTCCTGATGTGGAGTGCATTT R-TCTACAAGGACGAATCGTTGC	$(AG)_{9}$	52	Sareen *et al*., 2023
5.	BFU0581	F-TGAGAAGGTTGAAGATGCTCTC R-CCTGACATCCATTTGAAGGAA	$(CA)_{7}$	50	Sareen *et al*., 2023
6.	BFU1157	F-TCC CTA AAT TAC CCT TCC CAAT R-AAAGCGACAGCGAAAACTGT	$(GA)_{9}$	50	Sareen *et al*., 2023
7.	BFU1205	F-TGTTGCTGGTTCAATTCCAG R-CTTATGGCTTTTTCATTTTGTGA	$(CA)_{8}$	48	Sareen *et al*., 2023
8.	BFU1409	F-CAAATGATGGATCGAGCAAA R-AATGGAGGACAAACCGTCAC	$(CA)_{6}$	48	Sareen *et al*., 2023
9.	BFU1178	F-CCTTGGTGGATTTTGGTTTG R-TATACTTTGGCAGCGGTGTG	$(TG)_{9}$	50	Sareen *et al*., 2023
10.	BFU0308	F-TTTCCACCCCAAAATACCAA R-AGACGCTGGATGAGGATGAT	$(TC)_{11}$	49	Sareen *et al*., 2023
11.	BFU0479	F-GAAAACCATTGTTGGAGACCA R-TGAACCAAGCAACAAAAATCA	$(TC)_{9}$	47	Sareen *et al*., 2023
12.	ZCMS14	F-GAAGCTCCAATAACACGTTACC R-ACAATTCCCCAAATCTAAACTG	$(AG)_{8}$	49	Sareen *et al*., 2023
13.	ZCMS1	F-CTCATCTTCTAAAACCAAAAACC R-CTCTGTCAACATATCTGGCTTG	$(AG)_{10}$	50	Sareen *et al*., 2023
14.	E25	TTCTTCAGTTCAGAGGCAGC GTTTGCCCGTTTCTTCGTCG	$(AG)_{13}$	56	Liang *et al*., 2019
15.	E29	CAGCGTCACTCATTCCACTTTC CCCTCCATTGCTTGAGCTTGT	$(AG)_{12}$	57	Liang *et al*., 2019
16.	E31	CAACGAAAGCCAGTGACAAGG GCAACGCCAGGGAGATAGAA	$(GA)_{10}$	56	Liang *et al*., 2019
17.	E45	TCTTTGCCGCACCCATTAG CCCACCTTACCGAAATACCC	$(AG)_{10}$	55	Liang *et al*., 2019
18.	E63	GATACGGATACGGACGGAGA CGCAAACAGGAACGGAGAAA	$(GA)_{7}$	55	Liang *et al*., 2019
19.	G16	ATGCGGATTCAACTACTCTTTTG TGGCTTGAGCAATGAAGAACTAT	(TC)16	52	Liang *et al*., 2019
20.	G48	TTGGATCGGTACAAAATCAGCTA AGCTCAGAATCCAATCTCTCCTT	$(CT)_{12}$	53	Liang *et al*., 2019
21.	G58	CTTCCTTGATTATGCATACTGGG CTGACTAGTGCTGCTGCTACCTT	$(AC)_{10}$	55	Liang *et al*., 2019
22.	G61	GACTAATCTCTGCAACATGCACC CTTCACATGTTGAAAGACGAGGT	$(AG)_{9}$	54	Liang *et al*., 2019
23.	G64	CAATCACTGTGGTGGGGATAC CGTACGACAAATTCTCCAAAAAC	$(TCAGGG)_{6}$	52	Liang *et al*., 2019
24.	JN714371	TCCTTCTCCCCAAGCGTAC ACCCCACAACAAAAAGGCCA	$(CT)_{20}$	58	Liang *et al*., 2019
25.	JN714381	AGGTGCTCAAAACCAGGACCCA GACTCGGCATTGAGGCCGATGA	$(AG)_{15}$	58	Liang *et al*., 2019
26.	JN714388	CCATTGTCCTCAATCCCTGAGT TGTCCGCATAGACACATGCAC	$(CT)_{29}$	58	Liang *et al*., 2019
27.	JN714362	TCGACAAAAGGAGATGGGTTGG GCTCTCCCTTACACACTCACT	$(GA)_{19}$	58	Liang *et al*., 2019

Figure 2. Amplification profiles of SSR primers BFU0283, BFU0377, and BFU1409 across 13 *Z. mauritiana* accessions from Sumbawa Island. The figure illustrates polymorphic allele patterns observed in each ecotype, highlighting variation in banding profiles that reflect underlying genetic diversity among populations.

Table 3. Polymorphism and diversity index of SSR primers used in the study.

No.	Primers	Na	Ne	h	uh
1	E.25	1.333	1.267	0.148	0.222
2	E.29	1.000	1.000	0.000	0.000
3	E.31	1.000	1.000	0.000	0.000
4	E.45	1.333	1.157	0.107	0.133
5	E.63	0.667	1.157	0.107	0.133
6	G.16	1.333	1.267	0.148	0.222
7	G.48	1.000	1.000	0.000	0.000
8	G.58	1.333	1.157	0.107	0.133
9	G.61	1.000	1.000	0.000	0.000
10	G.64	1.000	1.000	0.000	0.000
11	JN714371	1.333	1.157	0.107	0.133
12	JN714388	1.000	1.000	0.000	0.000
13	JN714381	1.000	1.000	0.000	0.000
14	JN14362	1.000	1.000	0.000	0.000
15	BFU 0263	2.000	1.731	0.415	0.556
16	BFU 0286	1.333	1.615	0.320	0.400
17	BFU 0377	2.000	1.731	0.415	0.556
18	BFU 0473	1.667	1.574	0.308	0.422
19	BFU 0581	1.333	1.615	0.320	0.400
20	BFU 1157	1.333	1.424	0.255	0.356
21	BFU 1205	1.667	1.465	0.267	0.333
22	BFU 1409	1.333	1.308	0.160	0.200
23	BFU 1178	1.667	1.314	0.213	0.267
24	BFU 0308	0.000	1.000	0.000	0.000
25	BFU 0479	2.000	1.580	0.361	0.489
26	ZCMS 14	0.000	0.000	0.000	0.000
27	ZCMS 1	1.000	1.000	0.000	0.000

Na: mean number of different alleles, Ne: a mean number of effective alleles, h: diversity, and uh: unbiased diversity.

Although, the average number of alleles per primer was low, overall polymorphism was substantial. Effective allele numbers ranged from 1.000 to 1.731, and the primers BFU 0263 and BFU 0377 showed the highest values (Table 3). In contrast, each of the 10 primers (E.25, E.29, G.48, G.61, G.64, JN714388, JN714381, JN14362, BFU 0308, and ZCMS1) produced only one effective band. Expected heterozygosity (He) values ranged from 0.000 to 0.415, again with primers BFU 0263 and BFU 0377, contributing the topmost diversity indices, indicating considerable distinguishing power (Table 3). Liang *et al.* (2019) and Uddin *et al.* (2021) also observed the heterozygosity values of up to 0.52 in

Table 4. Polymorphism, diversity index (h and uh), and percentage of polymorphic loci (P %) in *Z. mauritiana* populations from Sumbawa Island based on SSR markers.

Population	N	P%	Na	Ne	I	h	uh
Sumbawa Barat (SB)	33.00	37.04%±4.45	1.222±0.134	1.258±0.071	0.217±0.057	0.148±0.039	0.185± 0.049
Kabupaten Sumbawa(KS)	29.00	25.93%±4.45	1.074±0.130	1.207±0.069	0.165±0.055	0.115±0.038	0.173± 0.057
Dompu (DP)	36.00	40.74%±4.45	1.333±0.119	1.259±0.067	0.229±0.055	0.154±0.038	0.193± 0.047

N: number of polymorphic loci; %P: percentage of polymorphic loci; Na: mean number of different alleles; Ne: mean number of effective alleles; I: Shannon's Information Index; h: diversity, and uh: unbiased diversity.

genotypes of the species *Z. mauritiana,* suggesting a moderate genetic diversity across the population.

Population structure and principal component analysis

The jujube population obtained from the district of Dompu (DP) exhibited the highest diversity, with 36 detected alleles and Na of 1.333 ± 0.119, I = 0.229 ± 0.055, h = 0.154 ± 0.038, uh = 0.193 ± 0.047, and polymorphic loci = 40.74% ± 4.45%. The district of West Sumbawa (SB)'s population followed closely with 33 alleles, Na = 1.222 ± 0.134, and polymorphic loci = 37.04% ± 4.45%. However, the district of Sumbawa Regency (KS) population showed the lowest diversity, with only 29 alleles, Na = 1.074 ± 0.130, and polymorphic loci = 25.93% ± 4.45% (Table 4). The results suggested that the district of Dompu could harbor more genetically diverse germplasm, which could be of considerable value for future breeding, conservation, and domestication efforts. These outcomes were more in line with the findings of Liang *et al*. (2019), who reported geographic variation in *Z. mauritiana* population diversity using SSR markers, and Sareen *et al*. (2023), who also reported significant population structure among *Ziziphus* species in India.

Principal component analysis (PCA) of 13 *Z. mauritiana* accessions from Sumbawa Island revealed an apparent population structure. The KS District accessions formed a tight and genetically uniform cluster, while the DP District accessions displayed a wide dispersion, indicating higher intra-population diversity and a partial overlap with the SB District accessions (Figure 3). Primers BFU0366, BFU1409, and BFU0977 contributed most to the variation, while primers E4.5, G.2, and G.3 showed minimal influence. These patterns align with diversity indices. The DP population provided the highest heterozygosity (h = 0.154), Shannon index (I = 0.229), and polymorphic loci (40.74%), followed by the SB population, while the KS population gave the lowest diversity (h = 0.115, P = 25.93%). The results confirmed that SSR-based diversity metrics were consistent with PCA clustering, proving effective for detecting population structure. The results were consistent with past findings, which authenticated the SSR markers' utility in showing genetic structure and ecotype differentiation in geographically diverse *Ziziphus* species (Liang *et al.,* 2019; Uddin *et al.,* 2021).

Relationships among the jujube cultivars

After conducting the SSR marker analysis and evaluating genetic diversity across populations, the research examined the genetic relationship among individual accessions of *Z. mauritiana*. Clustering analysis of the 13 jujube accessions from Sumbawa Island revealed two major clusters (Figure 4), which appeared consistent with PCA results (Figure 3). Cluster I comprised accessions KS3L1, DP1L2, DP1L3, KS1L1, KS2L2, SB1L2, and SB2L1, while cluster II consisted of accessions SB2L2, DP2L1, SB1LI, DP2L2, DP3L1, and SB2L3 (Figure 4). The phylogenetic structure confirmed that the District Dompu accessions exhibited a broad dispersion, indicating higher intra-population diversity. Conversely, the District KS accessions formed a tight cluster

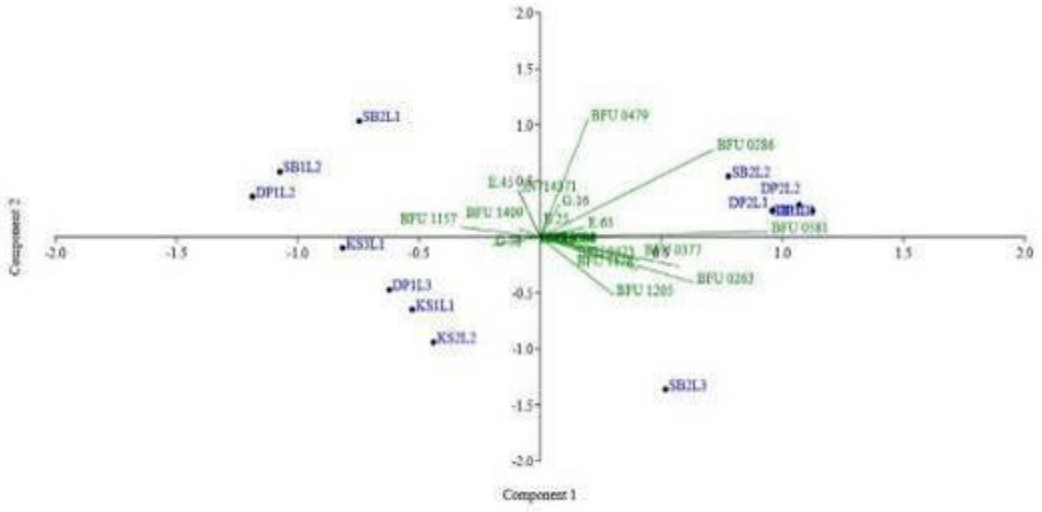

Figure 3. PCA-based genetic diversity assessment of 13 *Z. mauritiana* accessions based on SSR markers.

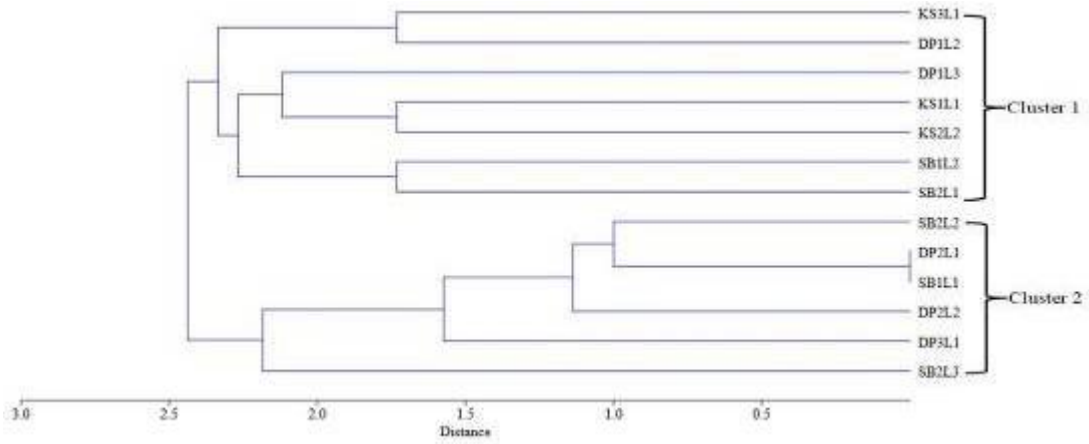

Figure 4. Genetic relationship of 13 *Z. mauritiana* accessions based on 27 SSR markers.

with a low variation, and the District SB accessions displayed an intermediate variation and partial overlap with the population in the District DP. The results confirmed that SSR markers effectively captured the genetic structure of inter-island *Z. mauritiana* populations. Nikmatullah *et al.* (2023) reported cluster I accessions, such as S3L1, DP1L2, DP1L3, KS1L1, KS2L2, SB1L2, and SB2L1, typically have smaller, sour fruits and more thorns. Meanwhile, cluster II accessions tend to produce larger, sweeter fruits with fewer thorns, linking the traits to domestication.

The alignment between genetic and morphological data revealed that phenotypic variation is not solely environmentally driven but likely reflects the presence of multiple genotypes of *Z. mauritiana* on Sumbawa Island, Indonesia. The distinct clustering of KS accessions suggested a limited genetic variation, potentially due to localized vegetative and seed propagation and environmental homogeneity. Past studies

reported that genetic variation in plant populations typically arises through mutation, gene flow, and sexual reproduction (Chung *et al.,* 2023). These processes are constrained, such as in isolated and narrowly adapted populations, and the genetic diversity tends to decline (Ye *et al*., 2021).

In contrast, the broader dispersion of the jujube germplasm obtained from the DP District may reflect more diverse agroecological conditions, higher gene flow, and heterogeneous environments, as some ecotypes came from natural savanna and partially domesticated areas (Table 1). The SB jujube accessions, positioned between the two clusters, likely represent transitional genetic profiles shaped by historical seed exchange and overlapping ecological niches. This intermediate structure may have acquired formation from the historical exchange of ber planting materials and overlapping ecological niches. Overall, habitat heterogeneity and connectivity play a crucial role in shaping genetic diversity and population structure, with broader dispersion observed in ecologically diverse regions and transitional profiles emerging in zones of environmental overlap (Ye *et al*., 2021; Chung *et al*., 2023; Wu *et al*., 2023).

These results raise important questions about the domestication history and genetic introgression within local ber (*Z. mauritiana*) populations. Future research should aim to expand jujube sampling across broader ecological zones in Sumbawa and neighboring islands, incorporating high-resolution markers, such as SNPs, to resolve fine-scale genetic structure. Likewise, future studies need to integrate morphoagronomic, phytochemical, and ethnobotanical data to better understand the drivers of selection and local adaptation (Yildiz *et al*., 2022; Bidyananda *et al.*, 2024; Shaikh *et al*., 2024; Uddin *et al*., 2024b). Such integrative approaches will be essential for managing conservation strategies, supporting local breeding programs, and valorizing underutilized fruit plants' genetic resources in Indonesia.

This relevant study enunciated that SSR markers proved more effective in revealing genetic diversity and structure among the jujube (*Z. mauritiana*) accessions. The clustering and PCA analyses consistently grouped the ber accessions into two major clusters, reflecting distinct genotypic profiles (Figure 3). The two distinct jujube genotypes within the island's populations highlighted the complexity of *Z. mauritiana* domestication and local adaptation. The results provide a considerable base for targeted conservation, breeding, and valorization of region-specific jujube germplasm. Future research integrating high-resolution molecular markers, expanded geographic sampling, and morphoagronomic data will be essential to fully characterize the genetic landscape and support sustainable utilization of this underutilized ber fruit species in Indonesia.

CONCLUSIONS

The study confirmed the effectiveness of SSR markers in exploring the genetic diversity and structure of Indian jujube (*Z. mauritiana*) accessions across Sumbawa Island, Indonesia. Clustering and PCA analyses consistently revealed two major genotypic groups, reflecting distinct ecotypes shaped by local adaptation and domestication. The identification of two divergent genetic lineages underscores the species' complex evolutionary history, realizing the Dompu District as a reservoir of high genetic diversity. These insights provide a valuable basis for region-specific conservation, breeding, and sustainable utilization strategies. Future work should also incorporate high-resolution markers, broader geographic sampling, and morphoagronomic traits to deepen understanding and guide the valorization of this underutilized ber species in Indonesia.

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