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SSR marker-based genetic diversity assessment of turmeric (*Curcuma* spp.) germplasm lines for molecular characterization

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Turmeric *(Curcuma* spp.), a medicinal and economically important crop, exhibits significant genetic diversity, essential for breeding and conservation efforts. This study aimed to evaluate the genetic diversity of 30 turmeric germplasms using 20 simple sequence repeat (SSR) markers. Genomic DNA was isolated, and PCR amplification was performed with SSR primers. Out of 20 SSR markers, 18 exhibited polymorphism and reproducible banding patterns. The polymorphic information content (PIC) values ranged from 0.07 to 0.98, with an average of 0.61, indicating a high degree of polymorphism. Resolving power analysis identified the most informative markers for genetic differentiation. Jaccard's similarity coefficient varied from 0.13 to 0.95, highlighting significant genetic variation among the germplasms. Cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) grouped the germplasms into two major clusters, reflecting their genetic relatedness. These findings confirmed the effectiveness of SSR markers in assessing genetic diversity, providing valuable insights for turmeric breeding, conservation, and genetic resource management.

Keywords: *Curcuma longa*, Genetic diversity, Germplasm conservation, Polymorphism, SSR markers

Turmeric is a key plant of the Zingiberaceae family used for the diagnosis and treatment of many human diseases for more than 2500 years in different Asian countries[1]. As a species belonging to the genus Curcuma, it has been widely known for its use as a spice, food preservative, and medicinal agent. The genus Curcuma (Zingiberaceae) contains more than 80 discovered species in the Indo-Malayan region, of which about 40 species are indigenous to India[2]. Persistent taxonomic ambiguities within this genus can be solved with the aid of a combination of traditional taxonomic methods and molecular biology tools. Due to the growing demand for turmeric within the food and pharmaceutical industries, many studies have been done with the purpose of improving cultivation techniques[3,4]. However, for further productivity improvements, a comprehensive knowledge of the genetic diversity of crops is needed for the development of efficient breeding programs.

Among the techniques of molecular genetic fingerprinting, the polymorphism analysis based on DNA is considered to be the most effective method in finding different races and new characteristics. This technique is indispensable toensure a consistent variability while conserving genetic resources[5]. Although some research studies have explored morphologically and anatomically characterization of Curcuma species and cultivars, fewer studies have been conducted on its molecular characterization.

Molecular marker-based studies on turmeric (Curcuma longa) are a powerful weapon for improving the production of valuable metabolites through marker-assisted selection and genetic improvement. Microsatellites, which are also known as simple sequence repeats (SSRs), are subspecific DNA sequences consisting of short sequences of nucleotides of 1-6 base pairs in length, which are separated in tandem repeats. Compared to other molecular markers, SSRs have several advantages due to their ease of use, high level of informativeness, and co-dominance. They can be easily analyzed using the polymerase chain reaction (PCR) and gel electrophoresis. Furthermore, SSR loci are not only found in non-coding regions, but are also generally distributed in the coding regions of the genome. Compared to other molecular markers, SSRs have a number of advantages that make it very suitable for different applications. Their multi-allelic nature, great abundance, co-dominant inheritance, reproducibility,

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and applications throughout the genome contribute to their efficacy[7].

Sigrist *et al.*[8] studied the genetic diversity of 39 turmeric accessions of a Brazilian germplasm collection, with 18 genotypes from India and Puerto Rico, using 17 SSRs. Structure analysis showed two major groups. Senan *et al.*[9] isolated and characterized 21 polymorphic simple sequence repeat (SSR) loci, revealing their usefulness for genetic studies. Singh *et al.*[10] evaluated 10 genotypes using SSR primers and identified considerable polymorphism and two major clusters. Sahoo *et al.*[11] used EST-SSR markers to identify 8 elite cultivars and 88 accessions. Singh *et al.*[12], using nine SRRs in 30 genotypes, developed six highly informative markers and supported high levels of genetic variation. In *Catharanthus roseus*, Shokeen *et al.*[13] devised microsatellite markers for investigating the genetic polymorphism at different taxonomic levels. Moreover, preserving the genetic diversity of turmeric germplasm should be documented for conserving the elite genotypes with unique phytochemical and agronomic characteristics. Curcuminoid content and quality of the extract vary greatly between cultivars, and thus, genetic diversity of the parent lines helps in the breeding programmes for improvement in yield, stress tolerance, and concentration of the bioactive compounds.

These methods will lead to a better understanding of the genetic variation in this crop, help in the effective management of germplasm collections, and in the choice of genotypes in breeding programmes. Additionally, these genetic markers will be useful in the characterization of natural populations and landraces of turmeric, and could be an important source of characters for genetic enhancement of crops.

Materials and Methods

Collection of Leaf Samples

Fresh green leaves from 30 turmeric germplasm were collected, enveloped in aluminum foil, flash-frozen in liquid nitrogen, and preserved at -80°C. in the Biotechnology Department, S.V.P.U.A.&T., Meerut, for DNA isolation.

Isolation of Genomic DNA

Genomic DNA was isolated following the CTAB protocol[14]. Leaf tissue samples (0.30 g) were pulverized in liquid nitrogen and combined with 2.0 mL of pre-heated CTAB buffer. This mixture was incubated at 65°C for 1 h and subjected to phase separation using a chloroform: isoamyl alcohol (24:1) solution. After centrifugation, RNase treatment, isopropanol precipitation, ethanol washing, and air-drying, DNA pellets were dissolved in the TE buffer.

Quantitative and Qualitative Estimation of DNA

DNA concentration and purity were measured using a Biophotometer (Perkin Elmer UV/VIS Spectrometer Lambda 25) at 260 nm and 280 nm. A purity ratio (OD260/OD280) between 1.8-2.0 was considered optimal. DNA integrity was assessed using 0.8% agarose gel electrophoresis in 1X TAE electrophoresis buffer, dyed with ethidium bromide, and compared to a known DNA marker. DNA samples (7 µL) mixed with dye (3 µL) were run together with 1 Kb DNA ladder and electrophoresed at 50V for 2 h. DNA bands were visualized and analyzed with an Alpha Innotech (AlphaImager) System.

Amplification of genomic DNA through PCR using SSR primers

The genomic DNA of 30 turmeric germplasm was amplified using 20 SSR (Table 1) primers in a Thermal cycler. The primer working solutions were prepared and diluted in TE buffer solution. For amplification of DNA using SSR primers, a total of 25 µL reaction mixture was prepared, including Taq buffer, $MgCl_2$, dNTPs, primers, Taq polymerase, DNA, and water. InPCR process comprises 35 cycles, each consisting of denaturation at 94°C for 1 min, annealing at a temperature range of 50–60°C for 1 min, and elongation at 72°C for 1 min. The reaction was finalized with an extension step at 72°C for 10 min. The amplified DNA fragments were then separated on a 1.2% agarose gel using electrophoresis at 50V for 1 h. Finally, the bands were visualized using a gel documentation system.

Diversity analysis using SSR markers

The genetic similarity among turmeric germplasm was estimated using Jaccard's similarity coefficient, which measures the proportion of shared polymorphic bands and excludes null alleles to prevent bias in co-dominant marker systems. A similarity matrix generated through NTSYS-PC software was used to construct a UPGMA dendrogram, which grouped the germplasm based on overall molecular resemblance. Furthermore, the Polymorphic Information Content (PIC) quantified the discriminatory potential of each SSR primer, and the Resolving Power(Rp)indicated the

Table 1 — SSR primer used in the present study for molecular analysis of turmeric germplasm

S. No	Forward and Reverse Primer 5′- 3′	Tm value	GC content
		Forward and Reverse Primer	Forward and reverse primer
1.	AAACCGCAAGAAAACTGAAG	57.00	40.00
	CTCTTCCCTGAACGATTCC		52.63
2.	TATGTGATGGTTGGGACG	59.00	50.00
	GTAGTGGAGGAAGACGCC		61.11
3.	GCACTACTTCCTTCTCGTTCAA	63.00	45.45
	CGTCGTAAAGATTAGCGTGTG		47.61
4.	TCAGGTTTCAGGGTGTAGAAG	60.00	47.61
	CCCAGCAAGATTTTACCAAG		45.00
5.	AGCAGTGCGTCTTTCATC	55.00	50.00
	CTCTTGTCACGGAACCTC		55.55
6.	AAGAAACTCCAACCACAATCC	62.00	55.00
	CTTGTCTTCCTCCTCCATTG		55.00
7.	AGCATGTGTCTAGCTCTTTGC	59.00	45.00
	AAGCAGTCGTTCCTCTACTGAC		50.00
8.	CATTGGGTGCCCACTTCC	57.00	47.36
	CCTCCCTGTCGCTCTCCTC		38.09
9.	AGTTGTGAAAGGGATAGAGTAGTTG	57.00	33.33
	AAGAAAGCAAATGCCAAGG		38.09
10.	CACCCTATGAGTGCTAACTGAAG	57.00	33.33
	ACCTGCACCACGATCAAC		38.09
11.	ACAGTCCCCTTCCCACTC	52.00	61.00
	TCTTGTTCCTATGCTCTACGC		41.00
12.	GTTCACAGCTTTAGCAGGGACAA	56.00	48.00
	CTCCTCTCCATATTCTCCATCTCG		50.00
13.	GCCAAGGTGGATCAGGTGAA	54.40	55.00
	TCAGCCGGAAATCTCGATGG		55.00
14.	AAGGTGGATCAGGTGAAGGC	53.00	55.00
	AGCCGGAAATCTCGATGGAC		55.00
15.	ACTGGACTGTCCGACAGCAT	56.30	55.00
	TCGTTTAGCGACAACGGATT		45.00
16.	CTCTCACGACGTCTTCCATCA	57.20	52.00
	AGACTCGCGTGTACAGAGCA		55.00
17.	ATCTGCTCTCCCATTATCTTG	54.50	42.85
	AAATCCTGGAAACTCATCCT		40.00
18.	AGAAGGAGAAGAAACGAAAGA	50.30	38.00
	TTTCGATAACAAGAAGTTGGA		33.00
19.	CTTAACCACTCTCCCAGTTG	51.20	50.00
	TAAAGAACCAATACTGGCAAA		33.00
20.	TGGCAACTTGATAAACAAGAC	51.90	38.09
	CAGCTTTGATGTCTGCTAGTT		42.85

efficiency of markers in distinguishing closely related genotypes. Together, these coefficients reflect the marker informativeness and the genetic structure of the germplasm.

The resolving power (Rp) of primers was evaluated using the method described by Prevost and Wilkinson[15], with the formula,

$$Rp = 1 - [2 \times (0.5 - p)]$$

where p represents the proportion of the 30 turmeric germplasms exhibiting the bands.Gene diversity was estimated based on the formula proposed by Anderson *et al.*[16]: $1 - \sum pi^2$. Twenty SSR primers were used, and the banding patterns were recorded as either present (1) or absent (0). Only

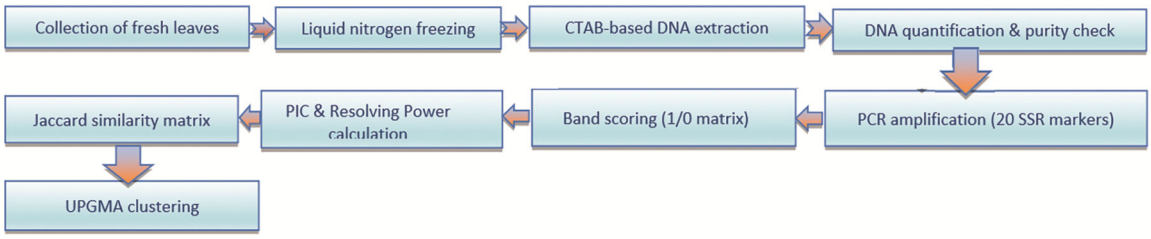

Fig. 1 — Pictorial Flowchart of Methodology

amplification products that were consistently reproducible were included in this analysis. A similarity matrix was generated using Jaccard's coefficient[17], incorporating data from all samples. The genetic distances among DNA accessions were calculated and analyzed in a pairwise manner using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA)[18]. Cluster analysis was performed using the software NTSYS-PC version 2.11s[19]. In some cases, bands were not detected, which may have resulted from inadequate homology between the primers and the DNA template or possible PCR amplification failure.

To provide a visual overview of the workflow, a pictorial flowchart summarizing the complete methodology used in this study has been included (Fig. 1). This flowchart outlines each step, beginning from sample collection to diversity analysis, ensuring clarity and reproducibility of the experimental procedure

Results

Genetic Diversity analysis

In the present study, to evaluate the genetic diversity of thirty turmeric germplasms at the molecular level, genomic DNA was isolated as described earlier. The extracted DNA was quantitatively assessed with a UV/VIS spectrophotometer. After that, Genomic DNA, dissolved in the TE buffer, was quantified by measuring absorbance at wavelengths of 260 nm and 280 nm. The result showed that the ratio of OD_{260}/OD_{280} of the DNA preparation from turmeric germplasm was found to be varied, from a lower 1.08 by germplasm 23 to a higher value of 2.33 by germplasm 3 (Table 2). Although DNA from most of the germplasm was 1.8 or more than. The ratio of OD_{260}/OD_{280} estimation method to check the purity of nucleic acid. A DNA sample is considered pure when the absorbance ratio was ≥1.8, indicating that the extracted DNA was of high purity and free from proteins, RNA or other contaminants. Concentration was measured in µg/ml, which was found to vary from a lower concentration of 3.35 µL in germplasm 17 to a higher concentration of 4.9 µL in germplasm 19.

The DNA was qualitatively checked on a 0.8 % agarose gel (Fig. 2). The result showed the presence of bands of total genomic DNA with very little shearing. The isolated DNA was of good quality and in sufficient quantity, and was subjected to PCR amplification using an SSR marker.

Resolving power and Polymorphism information content

Molecular characterization of 30 turmeric germplasms was performed using simple sequence repeat (SSR) markers. Of the 20 SSR markers utilized, 18 produced polymorphic, scorable, and reproducible results, whereas two primers failed to amplify (Figs 3–7). Consequently, the successfully amplified primers were selected for further analysis.

The polymorphic information content (PIC) was calculated for all 18 SSR primers, which ranged from 0.07 for primer TU-SSR8 to 0.98 for primer TU-SSR1, with an average PIC value of 0.61 (Table 3). However, the SSR primers, TU-SSR 3, TU-SSR 4, TU-SSR 7, TUR S1, TUR B1, TUR P3, and TUR B10 also showed the higher PIC value 0.73, 0.87, 0.91, 0.89, 0.81, 0.95, and 0.93, respectively (Figure 9). A higher PIC value indicated that the primer pairs were more informative and capable of detecting greater genetic diversity. The primers, which exhibited a high PIC value, hold potential for future research in genetic resource management and taxonomy.

Resolving power of the 18 SSR primers ranged from 0.26 with primer TU-SSR1 to 3.33 with primer TURS2, with an average of 1.32 (Table 3). On the bases of resolving power and the ability of primers to differentiate all turmeric germplasm, the primers TURS2, TU-SSR 2, TU-SSR 3, TU-SSR 5, TU-SSR 6, TU-SSR 7, TU-SSR 8, TU-SSR 9, TU-SSR 10, TU-SSR 11, TU-SSR 12 and TUR S1, with resolving power 3.33, 1.46, 1.80, 1.53, 1.73, 1.0, 1.93, 1.33, 1.40, 1.66, 1.33 and 1.40 respectively, were found to be most informative more than 1.0 (Figs 8-10). Thus,

Table 2 — Spectrophotometric analysis of genomic DNA of 30 turmeric germplasm

S. no	Turmeric Genotypes	260 nm	280 nm	OD ratio 280/260	Concentration (µL)
1.	Genotype 1	0.82	0.51	1.61	4.10
2.	Genotype 2	0.85	0.52	1.63	4.25
3.	Genotype 3	0.84	0.36	2.33	4.20
4.	Genotype 4	0.76	0.42	1.81	3.80
5.	Genotype 5	0.78	0.42	1.86	3.90
6.	Genotype 6	0.84	0.39	2.15	4.20
7.	Genotype 7	0.84	0.42	2.00	4.20
8.	Genotype 8	0.90	0.44	2.05	4.50
9.	Genotype 9	0.86	0.46	1.86	4.30
10.	Genotype 10	0.93	0.48	1.93	4.65
11.	Genotype 11	0.78	0.43	1.81	3.90
12.	Genotype 12	0.80	0.52	1.54	4.00
13.	Genotype 13	0.74	0.40	1.85	3.70
14.	Genotype 14	0.94	0.43	2.19	4.70
15.	Genotype 15	0.72	0.66	1.90	3.60
16.	Genotype 16	0.86	0.39	2.21	4.30
17.	Genotype 17	0.67	0.47	1.43	3.35
18.	Genotype 18	0.85	0.38	2.24	4.25
19.	Genotype 19	0.98	0.79	1.24	4.90
20.	Genotype 20	0.83	0.42	1.98	4.15
21.	Genotype 21	0.95	0.79	1.20	4.75
22.	Genotype 22	0.75	0.41	1.83	3.75
23.	Genotype 23	0.90	0.83	1.08	4.50
24.	Genotype 24	0.79	0.41	1.92	3.95
25.	Genotype 25	0.95	0.58	1.64	4.75
26.	Genotype 26	0.94	0.50	1.88	4.70
27.	Genotype 27	0.93	0.58	1.60	4.65
28.	Genotype 28	0.80	0.41	1.95	4.00
29.	Genotype 29	0.95	0.57	1.67	4.75
30.	Genotype 30	0.96	0.53	1.81	4.80

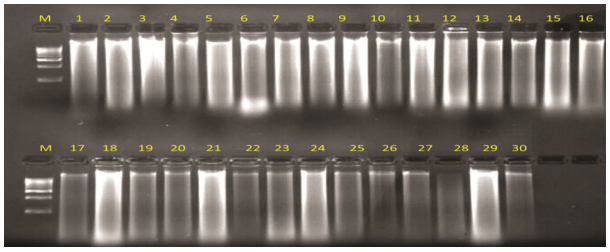

Fig. 2 — Genomic DNA isolated from 30 turmeric germplasm. Well M-1 kb marker, Well 1-30 – genomic DNA of turmeric germplasm

the significant value of resolving power indicated the ability of primers to resolve the different closely related genotypes of turmeric.

Jacchard coefficient of similarity

Jaccard's similarity coefficient was selected as it effectively measures shared polymorphic loci between genotypes without considering null alleles. UPGMA hierarchical clustering was utilized because it accurately represents evolutionary relationships within closely related germplasm.

The SSR primer banding patterns were scored as present (1) or absent (0) for each turmeric germplasm. Pairwise genetic similarities between samples were calculated using Jaccard's coefficient[17]. Statistical analyses were conducted using NTSYS-PC software (version 2.11s)[19], and a dendrogram was generated using the UPGMA method to cluster genotypes into distinct groups (18).

Genetic similarities were calculated using the Nei-Li similarity coefficient, revealing substantial genetic variation among all turmeric germplasms. The genetic similarity coefficient value among all the turmeric germplasm ranged from 0.13 to 0.95, with a mean of

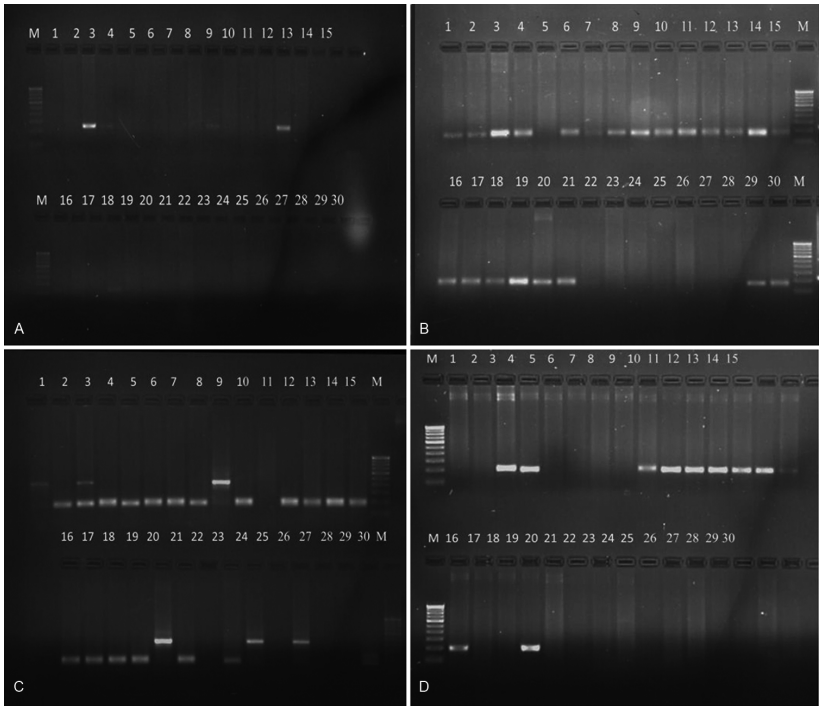

Fig. 3 — Amplification of 30 turmeric germplasm using SSR primer (A) TU SSR 1; (B) TU SSR 2; (C) TU SSR 3; and (D) TU SSR 4. Well M-1 kb marker, Well 1-30 – genomic DNA of turmeric germplasm

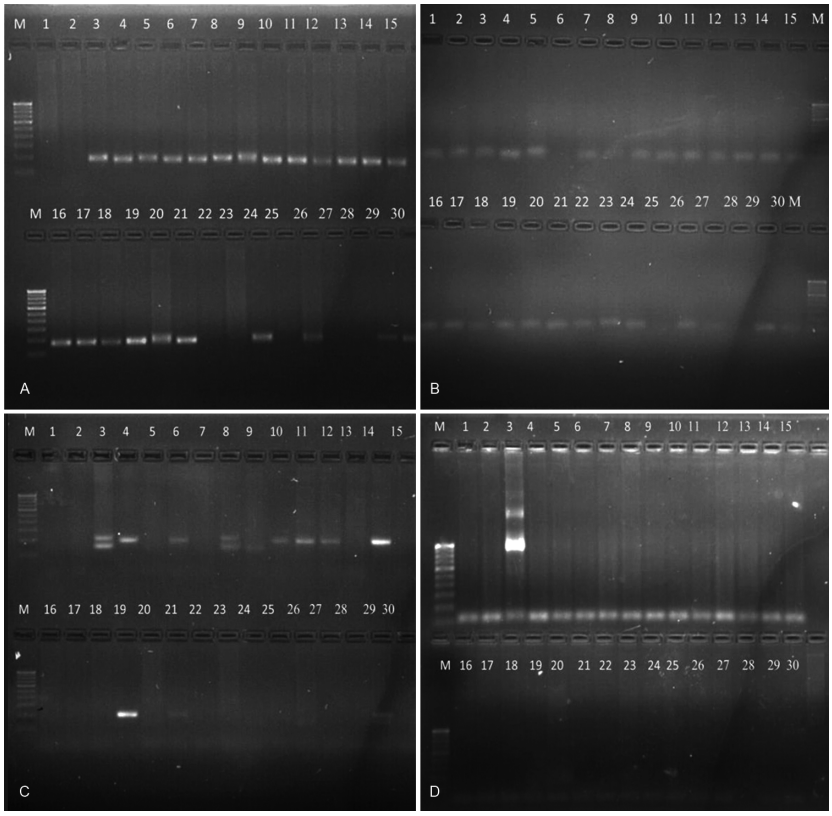

Fig. 4 — Amplification of turmeric germplasm using SSR primer: (A) primer TU SSR 5; (B) primer TU SSR 6; (C) primer TU SSR 7; and (D) primer TU SSR 8

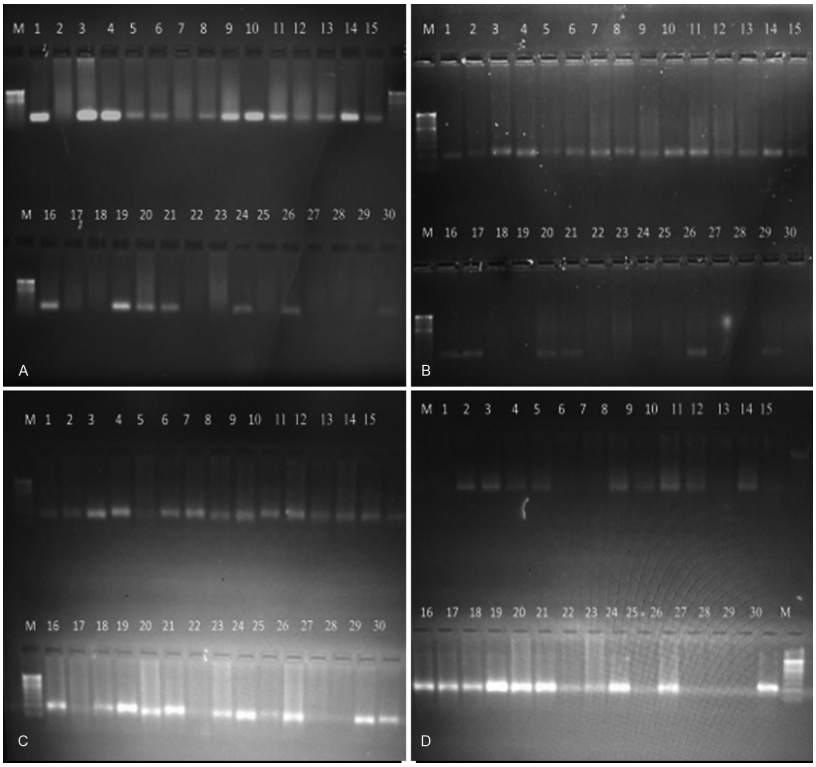

Fig. 5 — Amplification of turmeric germplasm using SSR primer (A) TU SSR 9; (B) primer TU SSR 10; (C) primer TU SSR 11; and (D) primer TU SSR 12

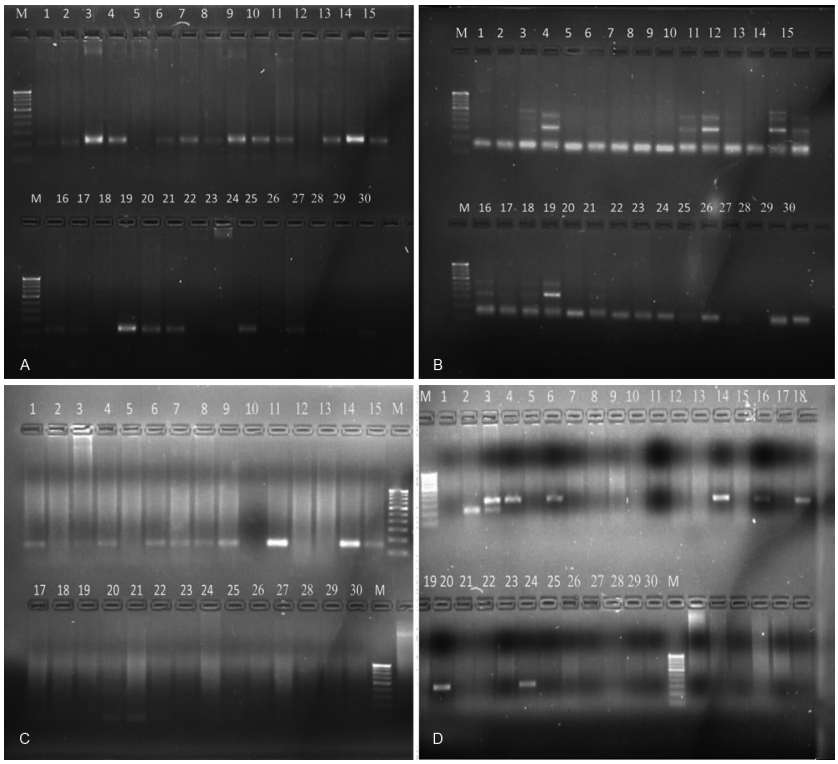

Fig. 6 — Amplification of 30 of turmeric germplasm using SSR primer (A) TUR S1; (B) primer TUR S2; (C) primer TUR B1; and (D) primer TUR P3

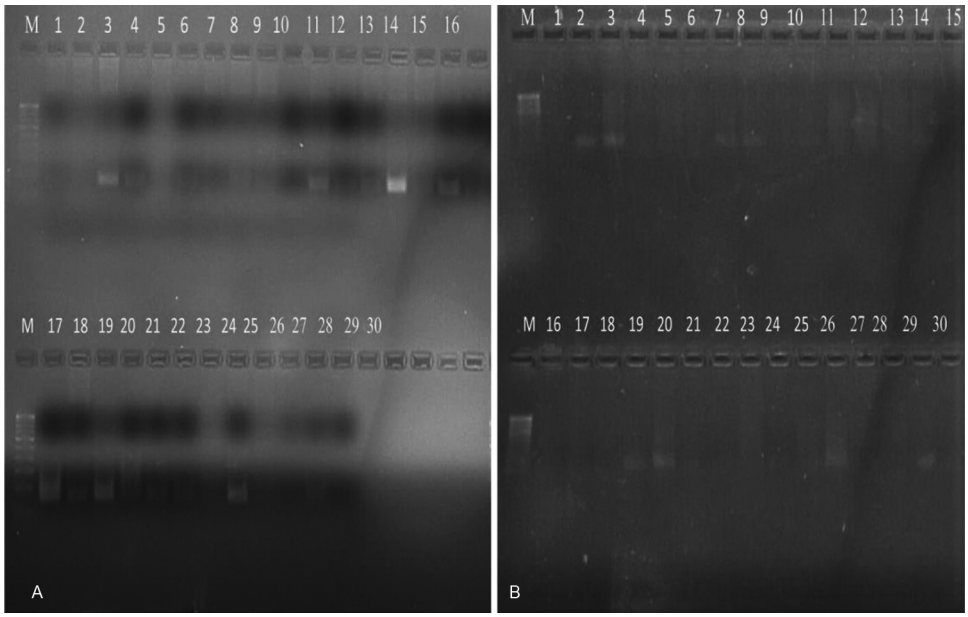

Fig. 7 — Amplification of 30 turmeric germplasm using SSR primer (A) TUR B9; and (B) primer TUR B10

Table 3 — Expected gene diversity and resolving power of the SSR primer used in the present study to amplify 30 turmeric germplasm

S. No	Primer code	Sequence	No of alleles	No of polymorphic Alleles	PIC	Resolving power	Polymorphic %
	TU-SSR1 F`	AAACCGCAAGAAAACTGAAG					
1	TU-SSR1 R`	CTCTTCCCTGAACGATTCC	1	1	0.98	0.26	100%
	TU-SSR2 F`	TATGTGATGGTTGGGACG					
2	TU-SSR2 R`	GTAGTGGAGGAAGACGCC	1	1	0.46	1.46	100%
	TU-SSR3 F`	GCACTACTTCCTTCTCGTTCAA					
3	TU-SSR3R`	CGTCGTAAAGATTAGCGTGTG	2	2	0.73	1.80	100%
	TU-SSR4 F`	TCAGGTTTCAGGGTGTAGAAG					
4	TU-SSR4 R`	CCCAGCAAGATTTTACCAAG	1	1	0.87	0.73	100%
	TU-SSR5F`	AGCAGTGCGTCTTTCATC					
5	TU-SSR5R`	CTCTTGTCACGGAACCTC	1	1	0.42	1.53	100%
	TU-SSR6 F`	CTATTAAGCGCAGTCCCCAG					
6	TU-SSR6 R`	AGTCTCTCGTGCGTTGCAGT	1	1	0.26	1.73	100%
	TU-SSR7 F`	GTGGTGATCCACTCAAGTTT					
7	TU-SSR7 R`	GTCCATCGACTTCGACTACT	2	2	0.91	1.0	100%
	TU-SSR8 F`	CTCTTTTGGGATCCAGACT					
8	TU-SSR8 R`	AGTTTAGAAGTTGCATGAGCA	1	1	0.07	1.93	100%
	TU-SSR9 F`	ATTCGTTGCAGTCAACTTTTA					
9	TU-SSR9 R`	GAGACAATGAATTTCAAGCAG	1	1	0.56	1.33	100%
	TU-SSR10F`	CACCCTATGAGTGCTAACTGAAG					
10	TU-SSR10 R`	ACCTGCACCACGATCAAC	1	1	0.51	1.40	100%
	TU-SSR11 F`	ACAGTCCCCTTCCCACTC					
11	TU-SSR11 R`	TCTTGTTCCTATGCTCTACGC	1	1	0.31	1.66	100%
	TU-SSR12 F`	GTTCACAGCTTTAGCAGGGACAA					
12	TU-SSR12 R`	CTCCTCTCCATATTCTCCATCTCG	1	1	0.56	1.33	100%

(*Contd.*)

Table 3 — Expected gene diversity and resolving power of the SSR primer used in the present study to amplify 30 turmeric germplasm (*Contd.*)

S. No	Primer code	Sequence	No of alleles	No of polymorphic Alleles	PIC	Resolving power	Polymorphic %
	TUR S1 F`	GCCAAGGTGGATCAGGTGAA					
13	TUR S1 R`	TCAGCCGGAAATCTCGATGG	1	1	0.89	1.40	100%
	TUR S2 F`	AAGGTGGATCAGGTGAAGGC					
14	TUR S2 R`	AGCCGGAAATCTCGATGGAC	4	4	0.44	3.33	100%
	TUR B1 F`	ATCTGCTCTCCCATTATCTTG					
15	TUR B1 R`	AAATCCTGGAAACTCATCCCT	1	1	0.81	0.66	100%
16	TUR P3 F	CTCTCACGACGTCTTCCATCA					
	TUR P3 R`	AGACTCGCGTGTACAGAGCA	2	2	0.95	0.86	100%
	TURB 9 F`	CTTAACCACTCTCCCAGTTG					
17	TURB 9 R`	TAAAGAACCAATACTGGCAAA	2	2	0.39	0.93	100%
	TURB 10 F`	TGGCAACTTGATAAACAAGAC					
18	TURB 10 R`	CAGCTTTGATGTCTGCTAGTT	1	1	0.93	0.53	100%

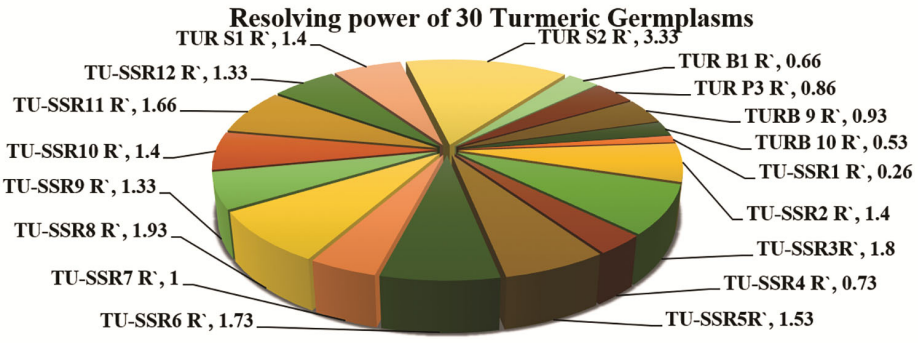

Fig. 8 — Resolving power of 30 turmeric germplasm

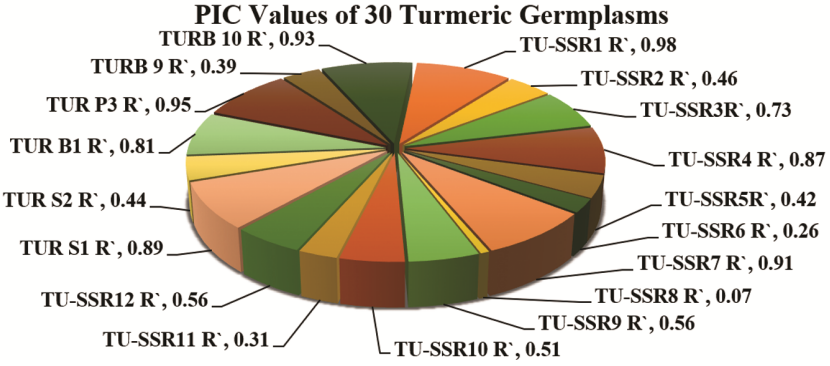

Fig. 9 — PIC value of 30 turmeric germplasm

0.54 (Table 4). The minimum genetic similarity coefficient was shown by turmeric germplasm 25 with germplasm 3.

The maximum genetic similarity coefficient was shown by germplasm 14 with germplasm 4, and germplasm 28 with germplasm 27. The distribution pattern of the similarity coefficient values is shown in (Fig. 11). The distribution pattern showed that the maximum turmeric germplasm showed 60-70% similarity, and the majority of turmeric germplasm showed similarity–50-80% similarity.

Cluster analysis of turmeric germplasm

A dendrogram was constructed using the UPGMA method based on a distance matrix expressed as a

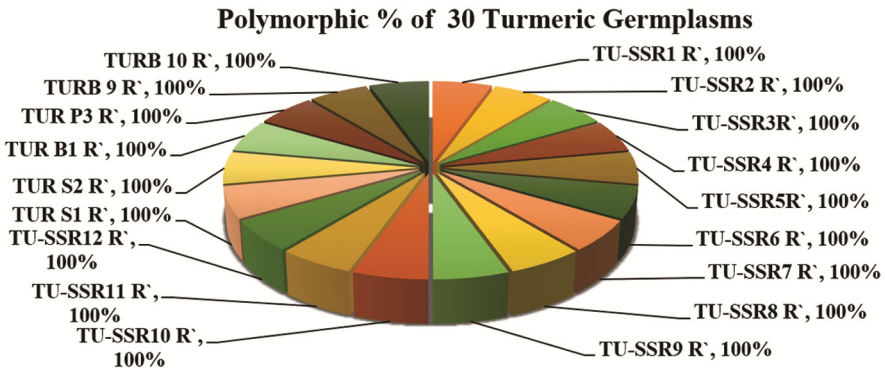

Fig. 10 — Polymorphic percentage of 30 turmeric germplasm

Table 4 — Similarity matrix based Jaccard Coefficient of similarity obtained from SSR primers in turmeric germplasm

	Tur 1	Tur2	Tur 3	Tur 4	Tur 5	Tur 6	Tur 7	Tur 8	Tur 9	Tur 10	Tur 11	Tur 12	Tur 13	Tur 14	Tur 15	Tur 16	Tur 17	Tur 18	Tur 19	Tur 20	Tur 21	Tur 22	Tur 23	Tur 24	Tur 25	Tur 26	Tur 27	Tur 28	Tur 29	Tur 30
Tur 1	1.00																													
Tur 2	0.72	1.00																												
Tur 3	0.45	0.45	1.00																											
Tur 4	0.54	0.54	0.77	1.00																										
Tur 5	0.68	0.68	0.31	0.50	1.00																									
Tur 6	0.72	0.63	0.54	0.72	0.59	1.00																								
Tur 7	0.81	0.81	0.45	0.54	0.68	0.72	1.00																							
Tur 8	0.68	0.68	0.68	0.68	0.63	0.77	0.77	1.00																						
Tur 9	0.77	0.59	0.59	0.59	0.63	0.59	0.68	0.72	1.00																					
Tur 10	0.59	0.59	0.68	0.81	0.63	0.59	0.59	0.63	0.63	1.00																				
Tur 11	0.59	0.50	0.68	0.68	0.54	0.68	0.59	0.72	0.72	0.81	1.00																			
Tur 12	0.72	0.63	0.45	0.63	0.77	0.72	0.72	0.68	0.68	0.77	0.68	1.00																		
Tur 13	0.72	0.72	0.54	0.72	0.77	0.72	0.72	0.77	0.77	0.77	0.77	0.81	1.00																	
Tur 14	0.59	0.59	0.77	0.95	0.54	0.77	0.59	0.72	0.63	0.81	0.90	0.68	0.77	1.00																
Tur 15	0.72	0.54	0.63	0.81	0.59	0.72	0.72	0.68	0.68	0.77	0.86	0.72	0.81	0.86	1.00															
Tur 16	0.59	0.68	0.68	0.86	0.63	0.68	0.59	0.63	0.63	0.81	0.81	0.68	0.86	0.90	0.86	1.00														
Tur 17	0.68	0.77	0.40	0.59	0.81	0.68	0.77	0.72	0.63	0.63	0.63	0.68	0.86	0.63	0.68	0.72	1.00													
Tur 18	0.50	0.68	0.50	0.68	0.63	0.59	0.59	0.54	0.45	0.63	0.63	0.59	0.68	0.72	0.68	0.81	0.72	1.00												
Tur 19	0.50	0.50	0.77	0.81	0.45	0.59	0.59	0.72	0.54	0.81	0.81	0.59	0.68	0.81	0.77	0.72	0.54	0.63	1.00											

(*Contd.*)

Table 4 — Similarity matrix based Jaccard Coefficient of similarity obtained from SSR primers in turmeric germplasm (*Contd.*)

	Tur 1	Tur2	Tur 3	Tur 4	Tur 5	Tur 6	Tur 7	Tur 8	Tur 9	Tur 10	Tur 11	Tur 12	Tur 13	Tur 14	Tur 15	Tur 16	Tur 17	Tur 18	Tur 19	Tur 20	Tur 21	Tur 22	Tur 23	Tur 24	Tur 25	Tur 26	Tur 27	Tur 28	Tur 29	Tur 30
Tur 20	0.77	0.77	0.59	0.59	0.63	0.68	0.77	0.72	0.81	0.54	0.63	0.59	0.68	0.63	0.59	0.63	0.63	0.54	0.54	1.00										
Tur 21	0.77	0.68	0.59	0.77	0.72	0.86	0.77	0.90	0.72	0.72	0.81	0.77	0.86	0.81	0.77	0.72	0.81	0.63	0.72	0.72	1.00									
Tur 22	0.50	0.59	0.22	0.40	0.72	0.31	0.50	0.36	0.45	0.54	0.54	0.50	0.50	0.45	0.50	0.54	0.63	0.72	0.45	0.45	0.45	1.00								
Tur 23	0.68	0.77	0.22	0.40	0.81	0.50	0.68	0.54	0.54	0.54	0.45	0.68	0.68	0.45	0.50	0.54	0.72	0.72	0.45	0.54	0.63	0.81	1.00							
Tur 24	0.68	0.59	0.40	0.50	0.63	0.68	0.59	0.54	0.63	0.45	0.54	0.59	0.68	0.54	0.59	0.63	0.63	0.63	0.45	0.72	0.63	0.54	0.63	1.00						
Tur 25	0.59	0.68	0.13	0.31	0.72	0.50	0.59	0.45	0.54	0.45	0.45	0.59	0.59	0.36	0.40	0.45	0.63	0.63	0.36	0.54	0.54	0.81	0.90	0.63	1.00					
Tur 26	0.72	0.63	0.45	0.45	0.68	0.63	0.72	0.68	0.68	0.59	0.59	0.72	0.63	0.50	0.54	0.50	0.59	0.40	0.50	0.77	0.68	0.50	0.59	0.77	0.59	1.00				
Tur 27	0.59	0.59	0.45	0.22	0.72	0.50	0.59	0.36	0.45	0.36	0.36	0.59	0.50	0.27	0.40	0.36	0.63	0.54	0.27	0.45	0.45	0.81	0.81	0.63	0.90	0.59	1.00			
Tur 28	0.54	0.54	0	0.18	0.68	0.45	0.54	0.31	0.40	0.31	0.31	0.54	0.45	0.22	0.36	0.31	0.59	0.50	0.22	0.40	0.40	0.77	0.77	0.59	0.86	0.54	0.95	1.00		
Tur 29	0.72	0.81	0.45	0.54	0.68	0.72	0.90	0.77	0.59	0.68	0.59	0.81	0.72	0.59	0.63	0.59	0.77	0.59	0.59	0.68	0.77	0.50	0.68	0.59	0.59	0.81	0.59	0.54	1.00	
Tur 30	0.72	0.72	0.36	0.54	0.86	0.63	0.72	0.68	0.68	0.68	0.59	0.81	0.81	0.59	0.63	0.68	0.77	0.77	0.59	0.68	0.77	0.68	0.86	0.68	0.77	0.63	0.68	0.63	0.72	1.00

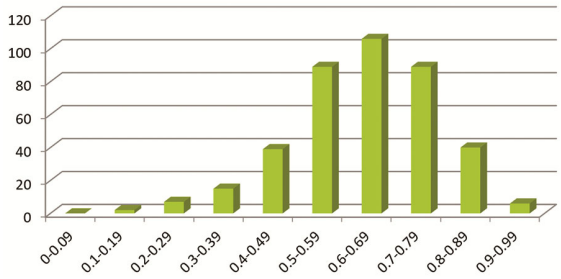

Fig. 11 — Histogram shows the distribution of genetic similarity among 30 turmeric germplasm

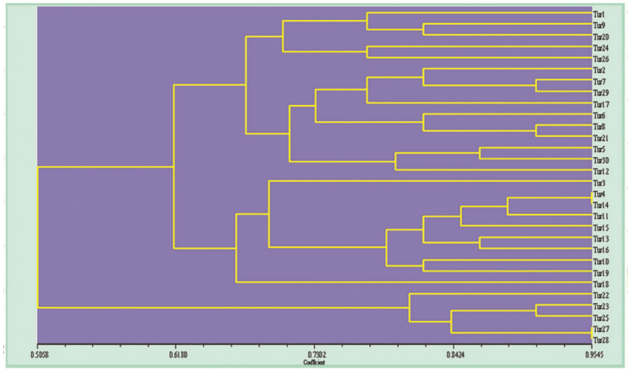

Fig. 12 — Dendrogram showing the cluster based on the grouping of 30 turmeric germplasm

similarity coefficient. The resulting UPGMA cluster analysis revealed that all germplasms were interconnected, demonstrating a high degree of genetic similarity, and were organized into two distinct clusters (Fig. 12). Cluster 1 grouped five germplasms, *viz*., germplasm 22, germplasm 23, germplasm 25, germplasm 27, and germplasm 28. The cluster II grouped 25 turmeric germplasm, which was further grouped into two sub-clusters designated as IIa and IIb. The sub-clusterIIa grouped 10 turmeric germplasms, of which nine germplasms, *i.e.,* germplasm 4, germplasm 11, germplasm 14, germplasm 15, germplasm 13, germplasm 16, germplasm 10, and germplasm 19 were grouped in one sub-cluster, whereas one germplasm, *i.e,* germplasm 18 did not group in the sub-cluster and remained as a separate branch of sub-cluster IIa.

The sub-cluster IIb grouped 15 turmeric germplasm, which was further subdivided into sub-clusters, *viz.,* b1 and b2. Of them, five turmeric germplasms, *viz.* germplasm 1, germplasm 9, germplasm 20, germplasm 24, and germplasm 26 grouped in sub-cluster b1. Whereas ten turmeric germplasms, *viz*. germplasm 2, germplasm 7, germplasm 29, germplasm 17, germplasm 6, germplasm 8 and germplasm 21, germplasm 5, germplasm 30, and germplasm 12, were grouped in sub-cluster b2 (Fig. 13).

Discussion

During recent years, molecular markers have usually been used as a suitable approach for cultivar identification since it is more rapid and cost-

GROUP	SUB-GROUP		Germplasm
I			Germplasm 22, Germplasm 23, Germplasm 25 Germplasm 27 and Germplasm 28
II	II A		Germplasm 4, Germplasm 11, Germplasm 14, Germplasm 15, Germplasm 13, Germplasm 16, Germplasm 10 and Germplasm 19
	II B	B 1	Germplasm 1, Germplasm 9, Germplasm 20, Germplasm 24 and Germplasm 26
		B 2	Germplasm 2, Germplasm 7, Germplasm 29, Germplasm 17, Germplasm 6, Germplasm 8 and Germplasm 21, Germplasm 5, Germplasm 30 and Germplasm 12
III			Germplasm 18

Fig. 13 — Grouping of 30 turmeric germplasm

effective[20]. Systematic knowledge about the *Curcuma* genus has encountered the problems caused by a different mode of multiplication (sexual and asexual), which results in very little variation in morphology between and within the taxa[21-23]. Molecular markers are also useful to differentiate the turmeric cultivars with similar morphological characters. The findings from this study will contribute to elucidating genetic relationships and assessing the extent of genetic diversity. The efficiency of diversity analysis and the power of the molecular markers mainly depend on the rate of polymorphism detected amongst the genotypes. In the present investigation, 18 SSR markers were used to characterize the 30 turmeric germplasm, which was found to be distributed throughout the turmeric genotypes; it seems to be important to reveal gene information and its product. Basak *et al.*[24] have done their study to determine the ability of ISSR and RAPD markers for genotyping of turmeric cultivars in India. They reported 43.37% to 62.65% polymorphism in turmeric cultivars, with the combined data of both markers. Whereas in the present study, polymorphism is observed in the range of 13% to 95This extensive genetic variability indicates that the morphological differences observed among cultivars could be attributed to somatic mutations that are not detectable using SSR markers. Consequently, examining additional loci is essential to accurately identify and distinguish these germplasms. Furthermore, the low similarity observed among them likely arises from evolutionary mechanisms, including elevated mutation rates, replication slippage, and unequal crossover.

High genetic polymorphism is the prerequisite of any marker system to study genetic variation[25]. Previous molecular studies have documented a substantial degree of genetic diversity among the various turmeric germplasms[23,26,27].

The studies of diversity analysis of turmeric cultivars are beneficial to identify the ecotype and to understand the intra diversity present among. Furthermore, the studies will be helpful for plan conservation strategies for optimal utilization of the species. The present study is a maiden attempt for molecular characterization of turmeric germplasm using SSR markers. The findings revealed significant genetic divergence among turmeric germplasm, demonstrating the effectiveness of SSR markers in assessing and defining genetic diversity within the species.

The UPGMA-based cluster was generated using SSR data, showed relationships among 30 germplasm. All of the turmeric germplasm were clustered together except germplasm 3 and germplasm 18. The distribution pattern of the similarity coefficient showed that the maximum turmeric germplasm had

60-70% similarity, and the majority of turmeric germplasm had a similarity of 50-80%. This revealed that the turmeric germplasm was diverse due to differences in species, their ecotype, and origin.

Similar to the present study,Siju*et al*.[28]evaluate genetic diversity in 20 turmeric varieties using 18 newly designed SSR markers. They reported the levels of polymorphism depending on its discriminating power, which ranged from 0.19 to 0.70. They also reported 0.6 to average discriminating power of SSR markers,which is helpful for the future utility to generate SSR markers for diversity analysis in turmeric. The authentic knowledge of genetic variability among turmeric germplasm is significant to establish the core collection of germplasm and impairing work for breeding.

Singh *et al*.[29] utilized nine SSR markers to assess the genetic relatedness among thirty turmeric genotypes.Molecular analysis of the turmeric germplasm using simple sequence repeat (SSR) markers has continuously shown large genetic variation. In investigations with about thirty accessions, SSR markers have been demonstrated to be highly reliable because of loci amplification of multiple loci. 18 markers have shown clear and reproducible polymorphic bands. The polymorphic information content (PIC) of these markers usually ranges over a wide range of values, for example, 0.07 to almost 1.0; the average is about 0.6, therefore reflecting high informativeness. Analyses like resolving power and similarity coefficient calculation (Jacard's similarity) have shown wide genetic divergence among genotypes with similarity index variation from low (0.13) to very high (0.95).

Cluster analyses using approaches such as UPGMA often separate germplasm into two or more genetically coherent groups, reflecting their relatedness and having a spatial or breeding correlation to their geographic and/or breeding history. Such evidence emphasizes the power of using SSR markers for mapping of genetic diversity in different populations of turmeric for the identification of clear genotypes and population structures. These insights add to breeding strategies, conservation planning, and germplasm management directly, helping derive relationships important for picking better lines of choice for yield, bioactive compounds content, and stress resilience. The widespread use and repeatability of SSR-based diversity profiling make this technique a mainstay of turmeric genetic research and improvement programs, and facilitate the sustainable use of the genetic resources of this valuable crop.

Conclusion

For molecular analysis, 20 SSR markers were used to characterize the 30 turmeric germplasm. The polymorphic information content (PIC) varied from a minimum of 0.07 to 0.98 with an average PIC value of 0.61. Resolving power of the SSR primers ranged from 0.26 to 3.33 with an average of 1.32. Based on resolving power, the primers TU-SSR 2, TU-SSR 3, TU-SSR 5, TU-SSR 6, TU-SSR 7, TU-SSR 8, TU-SSR 9, TU-SSR 10, TU-SSR 11, TU-SSR 12, and TUR S1 were also found informative because they have resolving power more than 1.0.

The genetic similarity coefficient value among all the turmeric germplasm ranged from 0.13 to 0.95, with a mean of 0.54. The minimum genetic similarity coefficient was shown by turmeric germplasm 25 with germplasm 3. Whereas, the maximum genetic similarity coefficient was shown by germplasm 14 with germplasm 4 and germplasm 28 with germplasm The UPGMA-based clustering analysis revealed that all germplasms were interconnected and displayed a high degree of genetic similarity. The distribution pattern showed that the maximum turmeric germplasm shows 60-70% similarity.

The study demonstrates that SSR markers are a reliable and powerful tool for assessing genetic diversity and characterizing turmeric germplasms. The identified genetic variability provides valuable insights for germplasm conservation, breeding programs, and crop improvement strategies. Future studies incorporating additional molecular markers and more extensive germplasm collections will further enhance the understanding of turmeric’s genetic diversity and facilitate its sustainable utilization.

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Conflict of interest

All authors declare no conflict of interest.

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