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RESEARCH ARTICLE

Exploring genetic diversity in Indian ginger (*Zingiber officinale* Rosc.) through microsatellite markers

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Abstract

Ginger (*Zingiber officinale* Rosc.) holds significant value as a rhizomatous spice known for its distinct taste and aroma. India is the worlds' largest producer, exporter and consumer of ginger. Local names commonly know many ginger cultivars and since the crop is propagated vegetatively, the chances of mixing are very high. This complicates the maintenance of purity and distinct characteristics of each variety. In the present study, 32 ginger genotypes were procured from various regions nationwide and assessed using 49 SSR (Simple Sequence Repeats) markers to evaluate their genetic diversity patterns. Among the 49 markers, 19 primers were amplified and produced 23 polymorphic bands, resulting in a polymorphism percentage of 52.63%. Additionally, the unweighted pair group method (UPGMA) cluster analysis grouped the genotypes into 5 distinct clusters, with similarity coefficients ranging from 0.31 to 1.00. This suggests that each genotype exhibits substantial variability. Genotypes Maran and Acc. 581 showed a similarity value 1.00, indicating perfect similarity (100%) in their genetic characteristics. These findings emphasize the critical role of SSR markers in germplasm conservation and highlight the potential for utilizing genetic diversity in breeding programs to develop improved ginger varieties with desirable traits.

Keywords

germplasm fingerprinting; Indian ginger; molecular characterization; phylogenetic diversity; SSR markers

Introduction

Ginger, botanically known as *Zingiber officinale* Rosc. (2n=22), is a member of the Zingiberaceae family and originates from Southeast Asia. The underground rhizome, renowned for its distinct flavour, aroma and pungency, is the ginger of commerce. This versatile crop holds immense cultural, culinary and medicinal significance. Rhizomes are marketed in different forms, including fresh, dried and powder. Being one of the oldest and most significant spices, its cultivation traces back to ancient civilization (1). Ginger was among the earliest oriental spices introduced to Europe and remains highly sought after today (2).

In India, over 75 recognized varieties of ginger are under cultivation, alongside approximately 500 indigenous varieties being maintained and

grown across various regions (3, 4). These cultivars are generally named after their respective localities or areas where they are grown. However, identifying distinct morphological traits among these varieties poses challenges, leading to confusion among farmers and breeders regarding their maintenance and utilization (5).

Since ginger is a vegetatively propagated crop, genotype mixing is highly likely. While genotypes are identified based on morphological traits, assessing these traits is often tricky and subjective, especially considering the close genetic relationships between most cultivars. Additionally, phenotyping traits are commonly influenced by environmental variations (6). Though molecular markers, primarily through Marker Assisted Selection (MAS), enhance breeding programs, they supplement the selection process based on morphological and quantitative traits. These traits remain crucial, especially in studying Quantitative Trait Loci (QTLs) for variety development in breeding programs.

Molecular marker technology is a robust tool for assessing genetic variation in ginger genotypes, revealing substantial differences at the DNA level. This approach provides a direct, reliable and efficient means for characterizing, conserving and managing germplasm unaffected by environmental factors (7). Several genetic markers assess genetic variations in asexually reproducing plants (8). Today, molecular markers are commonly employed to differentiate genotypes in horticultural crops (9). Among these, simple sequence repeat (SSR) markers stand out due to their reproducibility and effectiveness in understanding genetic diversity and identifying cultivars. This is attributed to their co-dominance and highly polymorphic behaviour (10-12).

SSRs are extensively utilized as versatile tools in plant breeding programs and evolutionary studies because they can reveal diversity among cultivars (13). Studies have demonstrated that SSRs in coding sequences (EST-SSRs) exhibit polymorphism across species, making them valuable for phylogenetic analysis (14, 15). For instance, twelve SSR markers were used in a recent study to evaluate the genetic diversity among 100 ginger genotypes (16). 13 SSR primers produced 160 polymorphic bands and 63.29% polymorphism (17). Considering the above context, this study utilized SSR markers to assess genetic differentiation among 32 ginger genotypes sourced from various regions across India. This is the first research effort to characterize extensive ginger germplasm diversity using molecular markers in Tamil Nadu, aiming to support the identification of genotypes with potential for commercial use and breeding programs.

Materials and Methods

Plant material

Thirty-two ginger genotypes were procured from various regions across the country and maintained at the germplasm centre of the Hybrid Rice Evaluation Centre, Gudalur, in the Nilgiris district of Tamil Nadu and utilized in this study (Fig. 1 and Table 1). This centre is situated at an elevation of 1300 m above mean sea level (MSL) and 11.5° N latitude and 76.5° E longitude. The climate is tropical and moderately humid, with temperatures between 18-28 °C and relative humidity around 70%. The area receives an average annual rainfall of 2000 mm. The experimental plot has well-drained sandy loam soil.

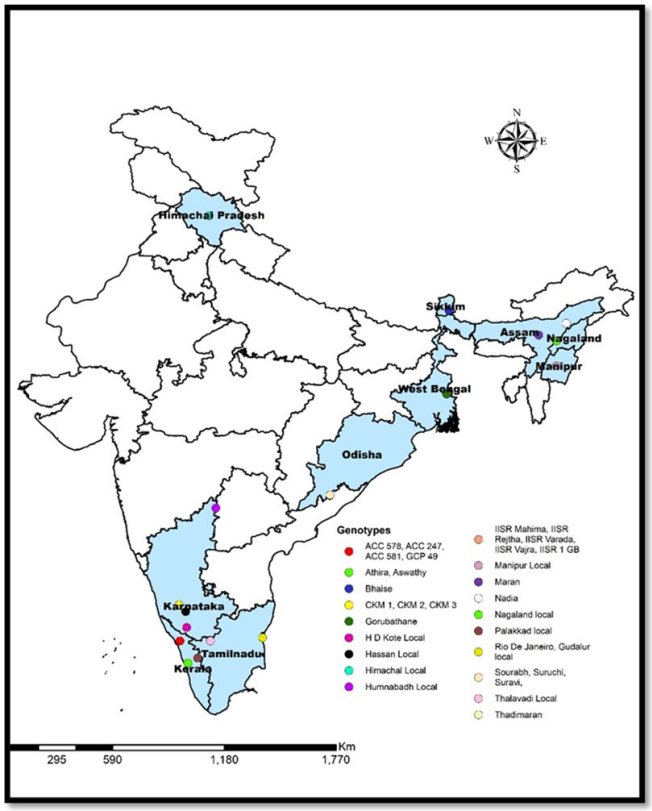

Fig. 1. Geographical map of India, indicating areas of the collection of the 32 ginger genotype.

Genomic DNA extraction

Leaves from plants aged 45 to 60 days were selected for DNA isolation. The genomic DNA extraction process followed the CTAB (Cetyltrimethylammonium bromide) method, as outlined (18). One gram of fresh, young leaf material was finely ground to initiate the isolation procedure using a pestle and mortar. Subsequently, the DNA was extracted using the CTAB extraction buffer. Following extraction, DNA quantification was done using a nanodrop spectrophotometer.

SSR analysis

Based on previous studies on ginger and turmeric, belonging to the family 'Zingiberaceae,' a set of SSR markers has been identified as reproducible and effective in assessing genetic diversity. In light of these findings, the same set of SSR markers was employed in the present study to evaluate the genetic diversity of ginger germplasm. 49 SSR primers were used, consisting of 4 genomic SSR primers, 6 EST SSR primers specific to ginger (19, 20) and 39 EST SSR primers derived from *Curcuma longa* (Table S1) (21). These primers were obtained from Bio-Serve Biotechnologies Pvt. Ltd., Hyderabad (Table S1). The PCR reaction mixture had a total volume of 20 µL and included the following components: 2.0 µL of dNTP (10 mM), 2 µL of primer (10 mM), 2.5 µL of 10X reaction buffer, 0.2 µL of Taq DNA polymerase (3 U/µL), 1.5 µL of template

Table 1. List of 32 ginger germplasm and their source of collection

Sl. No.	Genotypes	Description	Longitude	Latitude
Released varieties				
1	IISR Mahima	Clonal selection, Released from ICAR-IISR, Kozhikode	75° 49' 22" E	11° 36' 20" *N*
2	IISR Rejatha	Clonal selection, Released from ICAR-IISR, Kozhikode	75° 49' 22" E	11° 36' 20" N
3	IISR Varada	Clonal selection, Released from ICAR-IISR, Kozhikode	75° 49' 22" E	11° 36' 20" N
4	IISR Vajra	Clonal selection, Released from ICAR-IISR, Kozhikode	75° 49' 22" E	11° 36' 20" *N*
5	IISR 1 GB	Clonal selection, Released from ICAR-IISR, Kozhikode	75° 49' 22" E	11° 36' 20" *N*
6	Sourabh	Mutant (V1S1 2) from HARS, OUAT, Pottangi, Odisha	*82° 57' 53.82" E*	*18° 33' 47.376"N*
7	Suruchi	Released from HARS, OUAT, Pottangi, Odisha	*82° 57' 53.82" E*	*18° 33' 47.376"N*
8	Suravi	An induced mutant of Rudrapur local, Released from HARS, OUAT, Pottangi, Odisha	*82° 57' 53.82" E*	*18° 33' 47.376"N*
9	Athira	Selection form somaclones of cultivar Maran, Released from KAU, Thrissur, Kerala	76° 12' 18.36" E	10° 32' 55.3" N
10	Aswathy	Single plant selection from somaclones of cultivar Rio-de-Janeiro. Released from KAU, Thrissur, Kerala	76° 12' 18.36" E	10° 32' 55.3" N
Promising lines				
11	Acc. 578	Promising germplasm collections, Sourced from National Active Germplasm Site (NAGS), ICAR-Indian Institute of Spices Research, Kerala	75° 49' 22" E	11° 36' 20" *N*
12	Acc. 247			
13	Acc. 581			
14	GCP 49			
Local types				
15	Maran	Landrace from Assam	92° 53' 15.7974" E	26° 11' 9.7212" N
16	Thandimaran	Landrace from Kerala	76° 15' E	10° 30' N
17	Rio De Janeiro	Popular ginger cultivar in South India, Collected from HREC, Gudalur	79° 45' 14.4" E	11° 45' 14.4" N
18	Gudalur Local	Local cultivars collected from Gudalur, Nilgiri district, Tamil Nadu	79° 45' 14.4" E	11° 45' 14.4" N
19	Nadia	Local cultivar, collected from Assam Agriculture University, Jorhat	94° 12' 3.5928" E	26° 45' 5.4888" N
20	Bhaise	Local cultivar, collected from KVK, Ranipool, North Eastern Sikkim	88° 36' 58.73" E	27° 21' 57.75" N
21	Nagaland Local	Local cultivar, collected from Dimapur, Nagaland	93° 44' 28.8" E	25° 54' 20.4" N
22	Manipur Local	Local cultivar, collected from Manipur	93° 43' E	24° 43' N
23	Himachal	Landrace from Himachal Pradesh	77° 12' E	31° 53' N
24	H D Kote Local	Local cultivar, collected from Mysore, Karnataka	76° 8' 38.5" E	12° 14' 32.3" N
25	Hassan Local	Local cultivar, collected from Hassan, Karnataka	76° 5' 48.1" E	12° 59' 52.3" N
26	Chikkamagalore Local-1	Local cultivar, collected from Chikkamagalore, Karnataka	75° 46' 44.7" E	13° 19' 41.7" N
27	Chikkamagalore Local-2	Local cultivar, collected from Chikkamagalore, Karnataka	75° 46' 44.7" E	13° 19' 41.7" N
28	Chikkamagalore Local-3	Local cultivar, collected from Chikkamagalore, Karnataka	75° 46' 44.7" E	13° 19' 41.7" N
29	Humnabadh Local	The popular local cultivar of North Karnataka. collected from KVK, Bidar, Karnataka	77° 32' 28.4" E	17° 56' 22.9" N
30	Thalavadi Local	Local cultivar, collected from Erode, Tamil Nadu	77° 16' 40.7" E	11° 36' 13.8" N
31	Gorubathane	Local cultivar, collected from BCKV, Mohanpur, West Bengal	88° 30' 51.6" E	23° 22' 20.4" N
32	Palakkad local	Local cultivar, collected from Palakkad, Kerala	76° 39' 59.6" E	10° 46' 52.8" N

DNA (30-40 ng μL^{-1}) and 11 µL of nuclease-free water. The PCR protocol, executed in a thermocycler, began with an initial denaturation at 94 ^{0}C for 5 min. This was followed by 35 cycles of 45 denaturation at 94 ^{0}C, 45 sec at 94 ^{0}C, 45 sec of annealing at 52-65 ^{0}C and 1 min extension at 72 ^{0}C. A final extension was performed at 72 ^{0}C for 20 min (7, 22). The amplified products were subsequently analyzed using 3% agarose gel electrophoresis. The gel, stained with ethidium bromide, was visualized using a Bio-Imaging system (Syngene, GBOXCHEMI, England).

Data collection and statistical analysis

The SSR data generated for 32 genotypes were analyzed individually and collectively using statistical methods. The SSR products were visually evaluated and recorded based on the bands' presence (1) or absence (0). The genetic association was assessed by calculating the Jaccard's similarity coefficient using the marker data, following the Unweighted pair group method (UPGMA) and the dendrogram was generated using NTSYS-PC Ver. 2.2. software (23). The Polymorphic information content (PIC) was calculated using the formula proposed (24).

Results and Discussion

In this study, 49 SSR markers were employed to examine genetic diversity in 32 ginger genotypes. These SSR markers successfully identified polymorphic and monomorphic alleles in ginger genotypes, highlighting their effectiveness in assessing genetic variability among the genotypes. Primers were selected based on their ability to produce distinct, measurable and consistent amplified banding patterns. Out of the 49 SSR primers studied, 19 were successfully amplified, with each primer

showing different amplification patterns. The amplified products ranged in size from 50 to 1200 bp. Five of these 19 primers (ZOC-100, ZOC-28, Clon 16, Clon 01 and CLEST 07) exhibited 100% polymorphism, as detailed in Table 2. Out of the 41 total alleles, 23 were polymorphic. The primer GB -ZOM-103 detected the highest number of alleles (6), while primers such as ZOC-11, CSSR07, Clon 16, Clon 01, CSSR 24, CLEST SSR-02 and CLEST 07 produced the minimum number of alleles (one each). Similarly, comparable findings were noticed by using SSR markers to analyze the genetic variation and diversity within ginger collections (7). SSR primers demonstrated polymorphisms, indicating their effectiveness in detecting genetic variation in ginger genotypes. The high levels of polymorphism detected by the SSR markers indicate substantial genetic diversity among the genotypes studied (5, 7, 16). The number and frequency of alleles in a population affected the heterozygosity of the markers (6). The polymorphic information content (PIC), which measures gene diversity, averaged 0.70, ranging from 0.50 for the ZOC-100 primer to 0.98 for the GB-ZOM-103 primer (Table 3). Primers such as GB-ZOM-103, ZOC-11 and CSSR 07, which had PIC values exceeding 0.90, were highly influential in distinguishing ginger genotypes. The high allele polymorphism could be attributed to factors such as large genome size, outcrossing behaviour or inherent genetic diversity of the species. The primer GB-ZOM-103 exhibited the highest PIC value (0.98) with 6 alleles. A similar finding is consistent with the results (6, 7, 17, 21).

The SSR patterns were identified based on the appearance or disappearance of bands produced by these primers. The SSR patterns of each genotype were then compared with those of the others and an Euclidean distance matrix was computed for all 32 genotypes. The relationship among the genotypes was depicted in a dendrogram created using the UPGMA method (Fig. 2). From Jaccard's similarity coefficient, the dendrogram and the relatedness between the genotypes can be understood. This square matrix shows the similarity or distance between the 32 genotypes (Table 3). The similarity coefficient ranges from about 0.31 to 1.00, indicating a wide range of similarity among the genotypes. The genotypes near 0.31 showed less moderate similarity, while values closer to one indicate more similarity.

The genotypes were grouped into 5 main clusters, as illustrated in Fig. 2 Cluster I, II and III each contained a single genotype, such as Suravi, Hassan local and Gorubathane respectively and shared a similarity ranging

Table 2. Polymorphism among the ginger genotypes detected by SSR markers

Primers	Repeat motifs	Primer sequence (5'-3')	Na	Ne	I	He	MB	PB	% MM	% PM	Allele range	PIC value
GB-ZOM-064	$(GGA)_5$	F-CGTAGGATCTTCCCGACC R-CGAGTGAACCCATGGAGA	1	1.98	0.69	0.50	1	0	100	0	250	0.81
GB-ZOM-040	$(GGC)_5$	F-TCTCCCTCTCGGATCCAT R-ATCCATTGCCTGATGGTG	2	1.85	0.65	0.46	1	1	50	50	210	0.75
GB-ZOM-103	$(CCT)_2$ (CTT) $(CCT)_4$	F-GCTGCGGACTAAATGCTG R-ACGCTAGGGAACAGGGAG	6	1.65	0.62	0.50	1	5	16.66	83.33	150-1200	0.98
ZOC 11	$(CTG)_9$	F-GGAGTATCTTCACCTCTGTGCC R-ACCCTCACCTTCTCCAAGC	1	1.82	0.64	0.45	1	0	100	0	250	0.93
ZOC-100	$(CGA)_9$	F-CATCCCACTGGAAGCGTACAAAC R-AGGTCGGAGGTGAAGTCTCTG	2	1.85	0.65	0.46	1	1	50	100	150-170	0.50
ZOC 98	$(CT)_{14}$	F-GTAGTCCCCAAACAGAAACTCG R-AGATCGAGGTGGTCAGCAAT	3	1.58	0.55	0.36	1	2	33.33	66.66	250-280	0.68
ZOC 28	$(GCCTTC)_4$	F-GCCTTCTTCGGAGTGTCCTT R-AACCAAAGCCTAATCCAAAACC	3	1.55	0.54	0.35	0	3	0	100	150-280	0.65
CSSR07	$(CCT)_4$	F-CGCAGCTGACACTTCTTCCT R-AAGTCCGGGAGTTCTAAAGG	1	1.82	0.64	0.45	1	0	100	0	120-300	0.93
Clon 16	$(AGA)_8$	F-TTGTGCCAAGTGAGGATTTG R-ACTCGCTTCTGCTCATCCAT	1	1.98	0.69	0.49	0	1	0	100	50-120	0.62
Clon 01	(TA)6TTG $(TC)_{16}$	F-ACTGGACTGTCCGAGAGCAT R-TCGTTTAGCGACAACGGATT	1	1.36	0.43	0.26	0	1	0	100	120-250	0.46
CSSR 18	$(AGA)_{13}$	F-CTTTTGGCTGATAAATGGAAGG R-AAGAAAGAACTGACATCCTCCG	2	1.98	0.69	0.49	1	1	50	50	250	0.59
CSSR 27	$(GGAG)_3$	F-TCTCCCGAGTGATTCTTTGA R-TCTTCCTCCATATCCCCTGA	1	1.00	0.45	0.42	1	0	100	0	180	0.81
CuMiSat-20	$(AC)_6$	F- CGATACGAGTCCATCTCTTCG R- CCTTGCTTTGGTGGCTAGAG	3	1.95	0.68	0.48	2	1	66.66	33.33	200	0.90
CLEST SSR-02	$(AAG)_{12}$	F-ACCGTAGCAAAGAAATAGGAC R-AAGGTGGAAGGAAACTCG	1	1.00	0.44	0.41	1	0	100	0	250	0.90
Clon 14	$(CTT)_7$	F-CTGCGGTCCAAGTACAAGATC R-CTAGCTGGTGGCGGTGGT	3	1.98	0.69	0.49	1	2	33.33	66.66	250	0.78
CLEST 07	$(AT)_8$...$(T)_{10}$	F-CGCAGCTGACACTTCTTCCT R-AAGTCCGGGAGTTCTAAAGG	1	1.82	0.64	0.45	0	1	0	100	150	0.90
Clon 09	$(AC)_{14}$	F-GGAGGAGGCAGTTGATTTGT R-GCTTTGGTGGCTAGAGATGC	3	1.65	0.58	0.39	2	1	66.66	33.33	50-120	0.65
Clon 15	$(AAG)_7$	F: GTCGCCCGATCTATTGTAGC R: GATCCATCCTCCCCTAAAGC	2	1.93	0.67	0.48	1	1	50	50	210	0.68
Clon 8	$(GT)_{10}$	F-CCGGTGAGGGTGATATCTTG R-AAGCTCAAGCTCAAGCCAAT	3	1.99	0.69	0.50	1	2	33.33	66.66	120-300	0.87
Total	-		**41**	**1.72**	**0.61**	**0.44**	**17**	**23**	**949.97**	**999.97**		**13.42**
Mean	-		**2.15**				**0.89**	**1.26**	**49.99**	**52.63**		**0.70**

Note: Na-Total number of alleles; Ne-Number of effective alleles; I; Shannons' index; He-Expected heterozygosity; MB-Monomorphic bands; PB-Polymorphic bands; % MB-Per cent monomorphism; % PB-Per cent polymorphism; PIC-Polymorphism Information Content

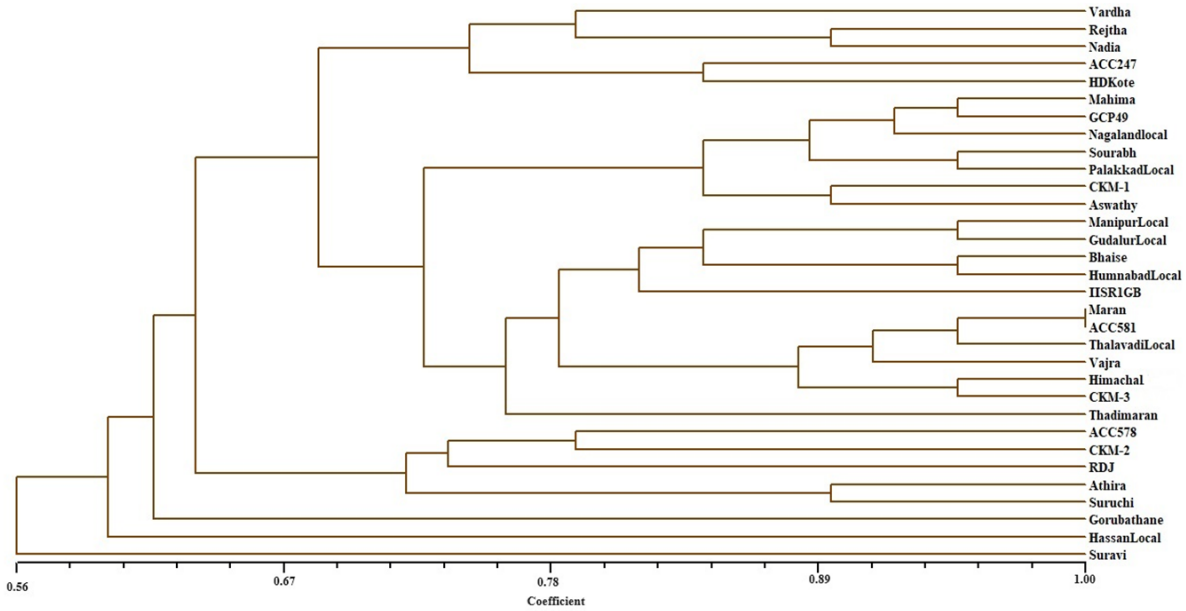

Fig. 2. UPGMA dendrogram based on SSR markers using Jaccards' similarity coefficient.

Table 3. Similarity matrix of ginger genotypes generated by SSR markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	1.00																															
2	0.84	1.00																														
3	0.73	0.57	1.00																													
4	0.73	0.68	0.89	1.00																												
5	0.68	0.63	0.94	0.94	1.00																											
6	0.68	0.73	0.73	0.73	0.78	1.00																										
7	0.63	0.57	0.89	0.89	0.94	0.73	1.00																									
8	0.68	0.63	0.84	0.84	0.89	0.68	0.84	1.00																								
9	0.78	0.73	0.84	0.84	0.89	0.78	0.84	0.89	1.00																							
10	0.68	0.73	0.73	0.73	0.78	0.78	0.84	0.78	0.78	1.00																						
11	0.68	0.73	0.52	0.52	0.57	0.78	0.52	0.68	0.68	0.68	1.00																					
12	0.68	0.73	0.63	0.73	0.68	0.78	0.63	0.68	0.68	0.78	0.68	1.00																				
13	0.57	0.63	0.52	0.52	0.57	0.78	0.63	0.57	0.68	0.78	0.68	0.68	1.00																			
14	0.68	0.73	0.73	0.73	0.78	0.78	0.73	0.78	0.78	0.89	0.68	0.78	0.78	1.00																		
15	0.68	0.73	0.73	0.73	0.78	0.78	0.84	0.78	0.78	1.00	0.68	0.78	0.78	0.89	1.00																	
16	0.73	0.78	0.68	0.68	0.73	0.94	0.68	0.73	0.84	0.84	0.84	0.84	0.84	0.84	0.84	1.00																
17	0.73	0.68	0.68	0.78	0.73	0.73	0.78	0.73	0.84	0.73	0.63	0.73	0.73	0.63	0.73	0.78	1.00															
18	0.57	0.73	0.42	0.42	0.47	0.57	0.52	0.47	0.57	0.68	0.57	0.57	0.68	0.68	0.68	0.63	0.52	1.00														
19	0.73	0.78	0.57	0.57	0.63	0.73	0.57	0.73	0.73	0.73	0.84	0.63	0.63	0.73	0.73	0.78	0.57	0.73	1.00													
20	0.68	0.63	0.52	0.63	0.57	0.47	0.63	0.68	0.68	0.57	0.57	0.47	0.47	0.47	0.57	0.52	0.73	0.57	0.73	1.00												
21	0.57	0.63	0.52	0.52	0.57	0.78	0.63	0.68	0.68	0.78	0.78	0.68	0.78	0.68	0.78	0.84	0.73	0.68	0.84	0.68	1.00											
22	0.63	0.57	0.57	0.68	0.63	0.52	0.68	0.73	0.73	0.63	0.63	0.63	0.52	0.52	0.63	0.57	0.79	0.52	0.57	0.73	0.63	1.00										
23	0.73	0.89	0.57	0.68	0.63	0.73	0.57	0.73	0.73	0.73	0.73	0.73	0.63	0.73	0.73	0.78	0.68	0.63	0.78	0.63	0.73	0.68	1.00									
24	0.63	0.68	0.57	0.57	0.63	0.84	0.68	0.73	0.73	0.84	0.84	0.73	0.84	0.73	0.84	0.89	0.78	0.63	0.78	0.63	0.94	0.68	0.78	1.00								
25	0.68	0.63	0.63	0.63	0.68	0.57	0.73	0.78	0.78	0.68	0.68	0.47	0.57	0.57	0.68	0.63	0.73	0.68	0.84	0.89	0.78	0.73	0.63	0.73	1.00							
26	0.47	0.52	0.52	0.63	0.57	0.47	0.63	0.68	0.57	0.57	0.47	0.68	0.47	0.47	0.57	0.52	0.73	0.57	0.52	0.68	0.68	0.73	0.63	0.63	0.68	1.00						
27	0.36	0.52	0.52	0.52	0.57	0.68	0.52	0.57	0.47	0.57	0.57	0.57	0.57	0.57	0.57	0.63	0.42	0.47	0.63	0.47	0.68	0.31	0.63	0.63	0.47	0.57	1.00					
28	0.57	0.52	0.84	0.84	0.89	0.78	0.94	0.78	0.78	0.78	0.57	0.68	0.68	0.68	0.78	0.73	0.84	0.47	0.52	0.57	0.68	0.63	0.52	0.73	0.68	0.68	0.57	1.00				
29	0.63	0.68	0.78	0.78	0.84	0.84	0.89	0.73	0.73	0.94	0.63	0.73	0.73	0.84	0.94	0.78	0.68	0.63	0.68	0.52	0.73	0.57	0.68	0.78	0.63	0.52	0.63	0.84	1.00			
30	0.57	0.73	0.73	0.73	0.78	0.78	0.84	0.68	0.68	0.89	0.57	0.68	0.68	0.78	0.89	0.73	0.63	0.68	0.63	0.47	0.68	0.52	0.73	0.73	0.57	0.57	0.68	0.78	0.94	1.00		
31	0.73	0.78	0.78	0.78	0.84	0.84	0.78	0.84	0.84	0.94	0.73	0.84	0.73	0.94	0.94	0.89	0.68	0.63	0.78	0.52	0.73	0.57	0.78	0.78	0.63	0.52	0.63	0.73	0.89	0.84	1.00	
32	0.68	0.63	0.63	0.63	0.57	0.68	0.63	0.47	0.57	0.68	0.47	0.57	0.57	0.57	0.68	0.63	0.63	0.57	0.63	0.68	0.68	0.52	0.63	0.63	0.57	0.47	0.57	0.57	0.73	0.68	0.63	1.00

Note: 1-IISR Varada; 2-IISR Rejatha; 3- IISR Mahima; 4- *Nagaland local*; 5-GCP-49; 6-*Manipur local*; 7 Sourabh; 8-*Chikkamagalore local- 1*; 9-Aswathy; 10-Maran; 11-Acc. 247; 12-Thadimaran; 13- IISR 1 GB; 14-IISR Vajra; 15-Acc. 581; 16-*Gudalur Local*; 17-Acc. 578; 18-*Hassan local*; 19-*HD Kote local*; 20-Athira; 21-Bhaise; 22-*Chikkamagalore local*-2; 23-Nadia; 24-*Humnabad local*; 25-Suruchi; 26-Rio De Janeiro; 27-Suravi; 28-*Palakkad Local*;29-Himachal; 30-*Chikkamagalore local*-3; 31-*Thalavadi local*; 32-Gorubathan

from 0.56% to 0.61%. Cluster IV was further divided into subclusters A and B with a similarity of 72%. Cluster A comprised 2 genotypes (Athira and Suruchi) sharing a similarity of approximately 89%. Cluster B included 3 genotypes, Acc. 578, CKM-2 (Chikkamagalore local-2) and Rio De Janeiro, with a similarity of 73%. Cluster V, the largest, comprised 24 genotypes and was further subdivided into Cluster C and D, which exhibited 68% similarity. Cluster C was further split into Cluster E and F, with a similarity of 73%. Cluster E entailed 11 genotypes, *viz.*, Manipur Local, Gudalur Local, Bhaise, Humnabad Local, IISR 1GB, Maran, Acc. 581, Thalavadi Local, IISR Vajra, Himachal and CKM-3 (Chikkamagalore Local-3). Among 11 genotypes, Maran and Acc. 581 showed a similarity value 1.00, indicating perfect similarity (100%) in their genetic characteristics. Seven genotypes, such as

Mahima, GCP-49, Nagaland Local, Sourabh, Palakkad Local, CKM-1 (Chikkamagalore Local-1) and Aswathy, fall under Cluster F sharing a similarity of 84%. Cluster D was additionally divided into Cluster G and H, which shared a similarity of around 74%. Cluster G included 2 genotypes (Acc. 247 and HD Kote Local) that shared 84% similarity, while Cluster H included 3 genotypes (IISR Varada, IISR Rejatha and Nadia) with a similarity of 89%.

Notably, 2 genotypes (Maran and Acc. 578) in Cluster IV exhibited a very close relationship, indicating high similarity in their genetic characteristics. Genotypes within the same cluster demonstrate closer genetic relationships, while those in different clusters or that diverge early (Suravi, Hassan Local and Goribathane) may represent unique or highly divergent varieties. These unique varieties hold potential value for breeding programs to introduce new traits. Interestingly, most genotypes from nine states did not cluster exclusively by geographic origin. Instead, they formed mixed groups, with genotypes from various states intermingling. This finding underscores that the genetic diversity among ginger varieties is not primarily determined by their geographical source. Consequently, ginger genotypes have minimal or no location specificity (5-7, 16).

Genetic variation is determined by geographical origin and human-driven selection processes and the spread of desirable genetic variants plays a crucial role in determining the genetic makeup of plant populations (25). Accordingly, this diverse genetic material has the potential to enrich the local genetic resources and provide valuable insights for future breeding efforts. Genotypes that form distinct groups are promising germplasms that can be utilized to broaden the genetic base (26). Conversely, genetically similar genotypes are valuable in breeding programs for maintaining consistency, stabilizing desirable traits and providing a reliable foundation for developing improved crop varieties.

Conclusion

Using SSR markers has revealed significant genetic variation in ginger genotypes sourced from diverse agricultural and climatic regions across the country. Of the nineteen SSR primers tested, primers ZOC-100, ZOC-28, Clon 16, Clon 01 and CLEST 07 exhibited 100% polymorphism. Primers such as GB-ZOM-103, ZOC-11 and CSSR 07, with PIC values exceeding 0.90, were highly influential in distinguishing ginger genotypes. Regardless of their collection sites or geographical origins, the 32 ginger genotypes clustered into distinct groups. This clustering highlights each genotype's considerable variability and potential, influenced by genetic similarities, human selection or environmental adaptation to local climates. These findings are invaluable for breeders and germplasm curators in characterizing, conserving and protecting the ginger gene pool and implementing targeted crop improvement programs.

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Authors' contributions

BAV conducted the experiment, recorded observations and analyzed the data. KV guided the research, formulated the data and approved the final manuscript. MM reviewed, edited, summarized and revised the manuscript. NS, VP and SPT assisted with editing and revising the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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Supplementary data

Table S1. A list of 49 SSR primers was used to study the genetic diversity of ginger genotypes

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