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Genetic Diversity of Tuberose (*Polianthes tuberosa* L.) Germplasm through Molecular Approaches to Obtain Desirable Plant Materials for Future Breeding Programs

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Received: 05 August 2025; Accepted: 10 October 2025; Published: 01 December 2025

ABSTRACT: The present study investigated the genetic diversity of 24 germplasms of *Polianthes tuberosa* L. via 16 inter simple sequence repeat (ISSR) marker techniques. The research findings revealed that the ISSR markers presented higher levels of band reproducibility and were more efficient at clustering germplasms. Among the 16 markers examined in this study, 12 had a complete polymorphism rate of 100%. The molecular analysis revealed a PIC ranging from 0.079 to 0.373, with a mean value of 0.30, whereas the range of the marker index was from 0.0001 to 0.409, with an average value of 0.03, and the primer resolving power ranged from 0.173 to 4.173, with a mean value of 2.02. The UPGMA clustering dendrogram indicated that all 24 germplasms were grouped into three main clusters. The study revealed a variable range of tree distances between 0.185 and 0.621, with the highest tree distance (0.621) detected between germplasms BR-24 and BR-1. Through these studies, the dissimilarity among the germplasms was evaluated, and diverse parents were identified for further crop improvement programs.

KEYWORDS: Dendrogram; genetic diversity; inter simple sequence repeats; polymorphism; tuberose

1 Introduction

Tuberose (*Polianthes tuberosa* L.), an ornamental geophyte of the plant family Amaryllidaceae [1], known for its elegant cut flower- and sweet-scented aroma, inherited its generic name from the Greek word



“*Polios*”, meaning white or shining and “*Anthos*”, meaning a flower [2]. It is of Mexican origin [3,4] and was distributed in various regions of the world throughout the 16th century as a cultivated plant, including India through Europe [5]. In India, tuberose is commonly known as Gulchari and Galshabbo in Hindi, Rajanigandha in Bengali and Nelasampangi in Telugu. The area under tuberose cultivation in India was 21,170 hectares, the production of loose flowers was 105.30 MT, and the production of cut flowers was 96.91 MT (Ministry of Agriculture and Farmers Welfare, Govt. of India, 2022–23). Long-distance shipment cannot attenuate the appearance of the inflorescence, for which it takes a front seat among the commercially valued ornamentals.

The genus *Polianthes* includes three types of inflorescences [6], i.e., single type having a single whorl of petals in florets mostly utilized in breeding programs as female parents [7], semidouble type having 2–5 whorls of petals in the florets and double type with >5 whorls of petals in the florets. It is diploid with a basic chromosome number of $n = x = 30$, of which 5 are large and the rest are small [8]. Assessing the genetic diversity of this commercial flowering ornamental is highly beneficial for studying the evolution of plants and their comparative genomics. This information can provide direction for sustaining the genetic integrity and diversity of tuberose germplasm collections from which breeders can develop new tuberose varieties with improved traits, such as enhanced fragrance, flower color, disease resistance or adaptability to different environments. To assess the genetic diversity for pre-determined breeding programmes, molecular marker-based approaches prove more authentic nowadays [9].

Molecular markers are specific nucleotide sequences that can be analysed by examining the variations in nucleotide sequences among different individuals [10,11]. For genetic diversity studies, different molecular methodologies are available, each of which differs in its informational content. ISSR involves the amplification of DNA segments located at a specific distance between 2 identical microsatellite repeat regions that are oriented in opposite directions [12]. The method employs microsatellites as primers in a single-primer PCR that amplifies numerous genomic loci, primarily focusing on simple sequence repeats of varying length. ISSRs possess the characteristic specificity of microsatellite markers but do not require any sequence information for the synthesis of primers, thereby benefiting from the advantage of being random markers [13]. ISSR markers can be used in genetic diversity analysis of tuberose because they do not need genome sequencing data, have high repeatability, and are fast, simple and inexpensive [14], which are capable of evaluating the genetic variability present either within a population or among potential parent plants. Breeders can ascertain the extent of genetic variation by examining the ISSR profiles of particular germplasms [15]. Keeping the above points in mind, this research investigation was carried out with the objective of performing a comprehensive evaluation to study the genetic diversity of tuberose through molecular markers and to select superior parents for hybridization among the twenty-four evaluated germplasms of tuberose.

2 Materials and Methods

2.1 Location and of the Study

The research investigation was conducted at the Instructional Farm, Department of Floriculture, Medicinal and Aromatic Plants, under the Faculty of Horticulture at Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India.

2.2 Plant Materials

The germplasms used for evaluation are listed in Table 1. The geographical distribution of the selected germplasm are presented in Fig. 1.

Table 1: Tuberose germplasm utilized in the current study

Code	Genotype	Institute of release	Parentage	Source of collection	Characteristics
V ₁	Phule Rajani	MPKV, Rahuri	Local Single × Shringar	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	Moderate spike length (60–70 cm). Florets are white. Number of florets varies from 40–50 per spike.
V ₂	Hyderabad Single	ANGRAU, Hyderabad	Existing local genotype	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	Spike length is approximately 80–100 cm. Flowers are white in colour. This is highly susceptible to foliar nematode (<i>Aphelenchoides besseyi</i>) prevalent in Eastern India.
V ₃	Shringar	IIHR, Bangalore	Mexican Single × Pearl Double	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	Flower is attractive due to pinkish tinge. This is tolerant to root knot nematode (<i>Meloidogyne incognita</i>).
V ₄	Sikkim Selection	NBRI, Lucknow	Gamma ray induced mutant	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	The spike is the longest one (120 cm) reported thus far. It is a very good seed setter.
V ₅	Calcutta Single	Not available	Cultivated as a land race since the beginning of Tuberose cultivation in West Bengal	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	Spike length is approximately 80–100 cm. Flowers are white in colour. This is highly susceptible to foliar nematode (<i>Aphelenchoides besseyi</i>) prevalent in Eastern India.
V ₆	Arka Nirantara	IIHR, Bangalore	Shringar × IIHR-6	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	The flowers are white in colour and moderately fragrant.
V ₇	Hybrid GKTC-4	MPKV, Rahuri	Not available	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	Flower Spike curvature Absent.
V ₈	BR-1	BCKV, Mohanpur	Arka Nirantara × Prajwal	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	High floret yielder. It shows photothermoinsensitive behaviour.
V ₉	BR-2	BCKV, Mohanpur	Sikkim Local × Phule Rajani followed by clonal selection	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	Florets are bright white. Spike length is 40–50 cm. shows tolerance to lodging and is highly fragrant.
V ₁₀	Prajwal	IIHR, Bangalore	Shringar × Mexican Single	Bidhan Chandra Krishi VIswavidyalaya, West Bengal, India	Flower buds are slightly pinkish. It is recommended for loose flower and cut flower purpose. It is tolerant to foliar nematode.
V ₁₁	Mexican Single	Not available	Cultivated as a land race since the beginning of Tuberose cultivation in India	Ondisha University of Agriculture and Technology, Bhubaneswar, Odisha India	It bears florets on straight spikes, mildly fragrant, also called as—Mexican Everblooming.

(Continued)

Table 1 (continued)

Code	Genotype	Institute of release	Parentage	Source of collection	Characteristics
V ₁₂	Arka Sugandhi	IIHR, Bangalore	IIHR-6 × IIHR-5	Odisha University of Agriculture and Technology, Bhubaneswar, Odisha India	Small size spike with a greater number of single florets. Suitable for pot culture.
V ₁₃	BR-3	BCKV, Mohanpur	Sikkim Selection × Phule Rajani	Odisha University of Agriculture and Technology, Bhubaneswar, Odisha India	A single Tuberose cultivar with wide opening and having greenish florets during bud stage.
V ₁₄	Pratap Rajani	MPUAT, Udaipur	Not available	Odisha University of Agriculture and Technology, Bhubaneswar, Odisha India	It bears white coloured florets on long spikes.
V ₁₅	Pune Local Double	Not available (collected from OUAT, Bhubaneswar)	Existing double genotype	Odisha University of Agriculture and Technology, Bhubaneswar, Odisha India	A double Tuberose cultivar with pinkish tinge on florets during bud stage.
V ₁₆	Pearl Double	Not available	Introduced cultivar (cultivated in India since a long time)	Odisha University of Agriculture and Technology, Bhubaneswar, Odisha India	Flowers are pure white.
V ₁₇	BR-24	BCKV, Mohanpur	Arka Nirantara × Calcutta Double	Odisha University of Agriculture and Technology, Bhubaneswar, Odisha India	It is an early variety which shows greenish white colour flower.
V ₁₈	BR-19	BCKV, Mohanpur	Phule Rajani × Calcutta Double	Odisha University of Agriculture and Technology, Bhubaneswar, Odisha India	It is early variety produces 3–3.2 spikes/plant/year. It is good for cut flower production.
V ₁₉	BR-20	BCKV, Mohanpur	Phule Rajani × Calcutta Double	Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India	It is an early variety. Good for cut flower production and yielded 1.5–1.6 lakh spike/ha/year.
V ₂₀	BR-17	BCKV, Mohanpur	Arka Nirantara × Subhasini	Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India	Florets are pink colour having pleasant fragrance and shy opening and yields approximately 1.4–1.5 lakhs spikes/ha/year.
V ₂₁	BR-18	BCKV, Mohanpur	Phule Rajani × Vaibhav	Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India	A double Tuberose cultivar with wide opening and having normal white florets.
V ₂₂	Hyderabad Double	ANGRAU, Hyderabad	Existing double genotype	Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India	The variety is similar to Calcutta Double and suitable for cut flower production.
V ₂₃	Calcutta Double	Calcutta	Cultivated as a land race since the beginning of Tuberose cultivation in West Bengal	Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India	It is the most popular double type and florets are bright white in colour and moderately fragrant.
V ₂₄	Suvasini	IIHR, Bangalore	Mexican Single × Pearl Double	Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India	It is double type flower with pinkish tinge on the young florets. Spike yield is 25% higher than Pearl Double cultivar.

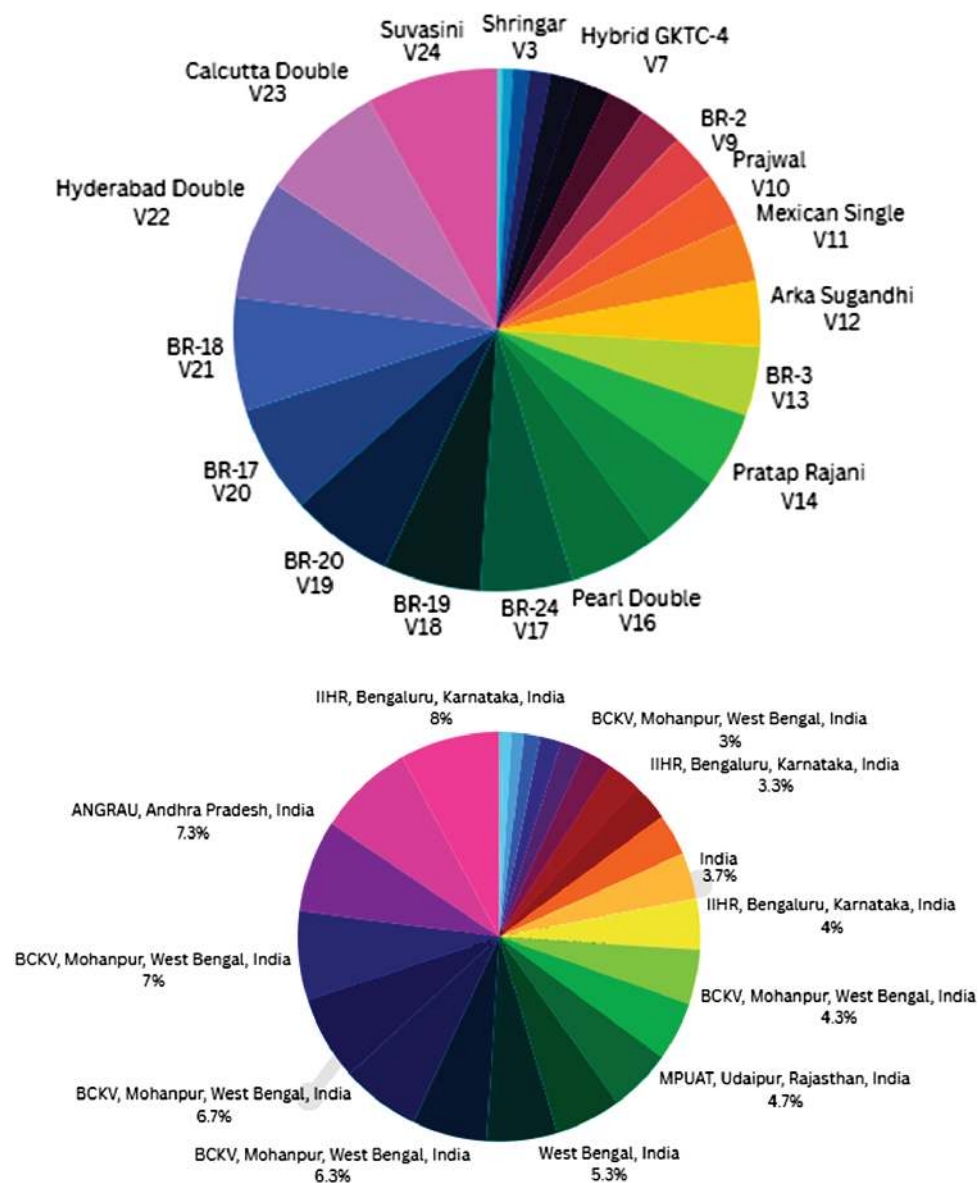


Figure 1: Germplasm and their geographical distribution used in the present study

2.3 Experimental Procedures

The molecular work was carried out at the Niche Area of Excellence Laboratory, UBKV. The total genomic DNA was extracted via a modified CTAB method [16]. The DNA concentration and purity were determined with a NanoDrop and UV-visible spectrophotometer at 260/280 nm (UV 1900i). The extracted DNA sample is known as a stock sample and was stored at -20°C . A total of 10 μL of stock sample and 90 μL of Milli-Q water were added, and the mixture was diluted to obtain a working sample. A total volume of 10 μL of PCR mixture (containing 5 μL of master mix, 1 μL of forward primer, 1 μL of reverse primer, 1 μL of genomic DNA and 2 μL of nuclease-free water) was prepared for PCR. After preparing for the reaction, the tubes were inserted into a thermal cycler (Veriti 96 Well Thermal-Applied Biosystems by Thermo Fischer Scientific Company). The DNA was amplified via polymerase chain reaction with 16 ISSR primer pairs (Table 2). For amplification of the DNA, initial denaturation at 94°C for 3 min, denaturation at 94°C for 30 s, annealing at

45°C–60°C (35 cycles), extension at 72°C for 10 min and a final extension at 72°C for 4 min were performed. The electrophoretically amplified products were separated on 3% agarose. The gels were buffered with 1X TAE buffer at 75 volts for 45 min to one hour. The gel was subsequently observed via a gel documentation system with a UV transilluminator (iBright CL750 image system; Invitrogen, Thermo Fischer Scientific Company).

Table 2: Selection of molecular markers

Marker name	Sequence	Annealing temp	%Polymorphism	Reference & reports of polymorphism
ISSR 1	(GGC) ₅ AT	66	100	[17]
ISSR 2	(AAG) ₅ GC	48	100	[18]
ISSR 3	(AAG) ₅ TG	50	0	[17]
ISSR 4	(AAG) ₅ CC	50	100	[19]
ISSR 5	(AGC) ₅ CA	62	80	[20]
ISSR 6	(AGC) ₅ CG	52	100	[21]
ISSR 7	(AGC) ₅ TA	66	100	[22]
ISSR 8	(AGC) ₅ GA	62	100	[23]
ISSR 9	(GAC) ₆	66	75	[24]
ISSR 10	(GTGC) ₅	56	100	[25]
ISSR 11	(GACA) ₅	56	100	[26]
ISSR 12	(AGG) ₇	58	100	[27]
ISSR13	(GA) ₁₀ T	52	75	[28]
ISSR 14	T(GA) ₁₀	55	100	[29]
ISSR 15	(GTG) ₆	66	100	[24]
ISSR 16	(GGA) ₅	55	100	[30]

2.4 Data Analysis

For each individual, the molecular identification profile was determined by assigning a score of 1 or 0 to indicate the presence or lack of the band, respectively. The simple matching coefficient (SM) was computed via the binary matrix. SM is determined via the following formula: $SM = (a + d)/(a + b + c + d)$, where “a” represents the number of bands present in both individuals, “b” and “c” denote the count of bands present in just one individual, and “d” signifies the count of bands that are absent in both individuals. The evaluation of primer performance in distinguishing genotypes was conducted via several established metrics, including resolving power (RP), as proposed by [31]; the marker index (MI), as described by [32]; Shannon’s index (H'), as introduced by [33]; and the polymorphic information content, as defined by [34]. A cluster analysis utilizing the UPGMA method was performed to estimate the molecular dissimilarity and construct a dendrogram. The analysis was completed via a specific software package, DARwin 6.0.21, a software tool known for dissimilarity analysis and representation for Windows, which was developed by CIRAD [35]. The genotypes of Tuberose were clustered via the neighboring technique [35,36].

3 Results and Discussion

A total of 24 germplasms of tuberose were characterized via 16 ISSR markers. A list of different germplasms of tuberose and their characteristics is shown in Table 1. In the present study, 78 alleles were generated in total, of which 75 alleles were polymorphic [37] and three were monomorphic, thus generating

89.38% of the polymorphisms. A total of twelve primers were identified as 100% polymorphic among the 16 ISSR primers.

3.1 Band Size and Percentage of Polymorphic Alleles

The observed band sizes in all 24 germplasms ranged from 150 to 1200 base pairs (bp). The number of amplified alleles produced by each individual ISSR varied between 3 and 9 bands, with an average of 4.69 alleles per primer [38]. The percentage of polymorphic alleles ranged from 75% to 100% (Figs. 2 and 3). In another study reported by [6] that assessed the genetic characteristics of seven tuberose germplasms, six ISSR primers were used, which revealed a polymorphism rate of 85.48%. Another similar study [39,40] used nine ISSR primers and reported a polymorphism rate of 92.50%, which aligns with the current research observations of a higher level of polymorphism when the ISSR marker system was used.

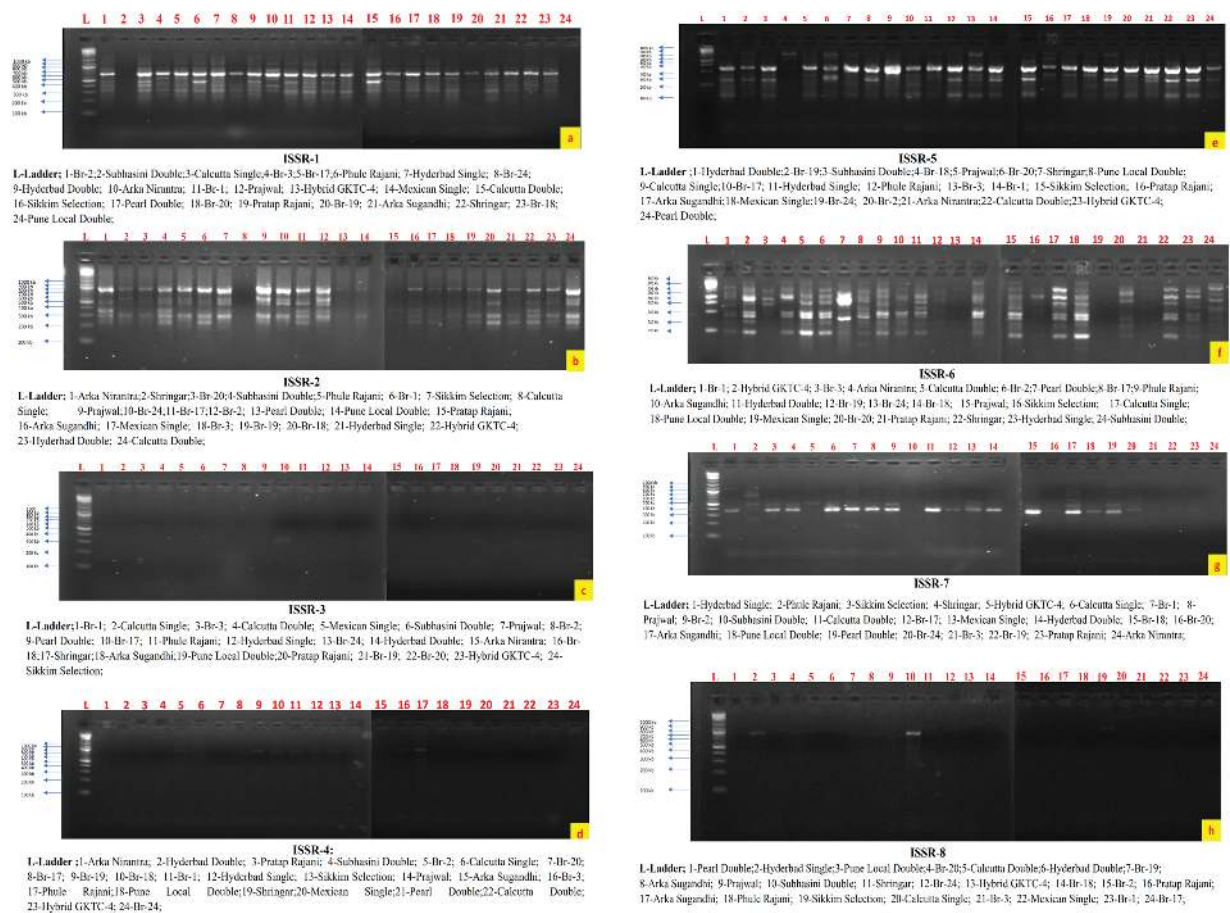


Figure 2: ISSR profiling of 24 germplasms of tuberose via a gel documentation system

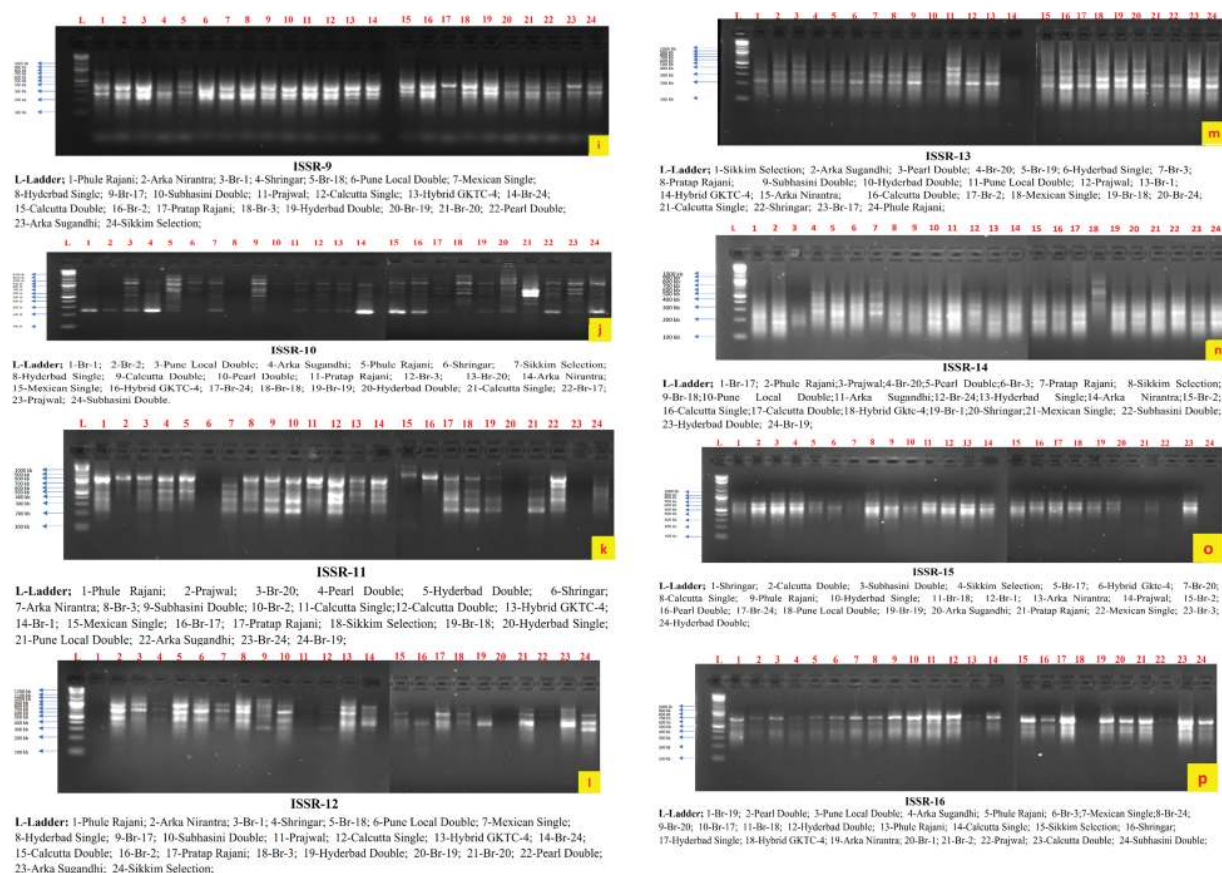


Figure 3: ISSR profiling of 24 germplasms of tuberose via a gel documentation system

3.2 Amplification Details for ISSR Primers

The amplification details for each ISSR primer are presented in [Tables 2 and 3](#). The data generated via the use of 16 ISSR primers ranged from ISSR-1 to ISSR-16, with an average of 4.68 bands per primer.

Table 3: Marker efficiency parameters for genetic diversity studies of tuberose

ISSR Locus	Sequence	Tm°C	PIC	NTA	NPA	%PP	HI	MI	DP	RP
ISSR 1	(GGC) ₅ AT	66	0.331	7	7	100	0.409	0.409	0.348	0.824
ISSR 2	(AAG) ₅ GC	48	0.347	6	6	100	0.448	0.012	0.565	2.608
ISSR 3	(AAG) ₅ TG	50	0	0	0	0	0	0	0	0
ISSR 4	(AAG) ₅ CC	50	0.079	3	3	100	0.083	0.0001	0.999	0.173
ISSR 5	(AGC) ₅ CA	62	0.311	5	4	80	0.385	0.012	0.455	2.086
ISSR 6	(AGC) ₅ CG	52	0.301	9	9	100	0.369	0.012	0.430	3.826
ISSR 7	(AGC) ₅ TA	66	0.355	6	6	100	0.462	0.007	0.870	4.173
ISSR 8	(AGC) ₅ GA	62	0.282	3	3	100	0.340	0.003	0.956	0.869
ISSR 9	(GAC) ₆	66	0.259	4	3	75	0.305	0.010	0.343	1.130
ISSR 10	(GTGC) ₅	56	0.373	8	8	100	0.496	0.009	0.790	4.000
ISSR 11	(GACA) ₅	56	0.350	5	5	100	0.453	0.006	0.881	2.521

(Continued)

Table 3 (continued)

ISSR Locus	Sequence	Tm°C	PIC	NTA	NPA	%PP	HI	MI	DP	RP
ISSR 12	(AGG) ₇	58	0.373	6	6	100	0.496	0.009	0.789	3.565
ISSR13	(GA) ₁₀ T	52	0.345	4	3	75	0.444	0.012	0.558	1.217
ISSR 14	T(GA) ₁₀	55	0.364	6	6	100	0.480	0.008	0.842	3.217
ISSR 15	(GTG) ₆	66	0.373	3	3	100	0.496	0.011	0.710	1.217
ISSR 16	(GGA) ₅	55	0.297	3	3	100	0.363	0.012	0.425	0.956

Note: Tm°C = Annealing temperature, PIC = Polymorphic information content, NTA = Number of total alleles, NPA = Number of polymorphic alleles, % PP = Percent polymorphism, HI = Heterozygosity index, MI = Marker index. DP = Discriminating power and RP = Resolving power.

3.3 Polymorphic Information Content (PIC)

The PIC ranged from 0.079 (ISSR 4) to 0.373 (ISSR 10, ISSR 12, ISSR 15), with an average value of 0.30 [41]. The PIC is employed to assess the discriminatory capacity of molecular markers and to estimate the genetic diversity of germplasms. The observed differences in polymorphic information content values across various tuberosa germplasms can be attributed to both the genetic diversity inherent in the germplasms and the variability in the number of tested ISSR loci [31].

3.4 Resolving Power (RP)

The resolving power demonstrated variability, ranging from 0.173 (ISSR 4) to 4.173 (ISSR 7), with an average value of 2.02 [42,43]. The obtained resolving power values indicate that the set of ISSR primers used in the study have the ability to differentiate between germplasms. Additionally, the efficiency of ISSR [30] markers within the range of RPs of 4.6 (ISSR 857) and 12.5 (ISSR 841) in the fingerprinting of potato cultivars has been reported.

3.5 Marker Index (MI)

Similarly, the marker index (MI) ranged from 0.0001 (ISSR 4) to 0.409 (ISSR 1), with a mean value of 0.03. In the present analysis, primers, namely, ISSR1, ISSR 2, ISSR 5, ISSR 6, ISSR 10, ISSR 12, and ISSR 14, which have above-average PICs, MIs and RPs, presented relatively high efficiency. Dinucleotide-based ISSR primers have been used to group germplasms of certain significant bulbous blooming plants and have demonstrated a good level of repeatability and an adequate degree of polymorphism [6]. Thus, the ISSR marker system. This type of marker was determined to be more productive in analysing diversity among closely related individuals because it targets different sites in the genome [12].

3.6 UPGMA Clustering

The 24 tuberosa genotypes were clustered via the UPGMA method, resulting in the formation of three distinct clusters: Cluster I, Cluster II and Cluster III, which are listed in Table 4 and Fig. 4.

Table 4: Clustering of tuberose genotypes via UPGMA

Sl. No.	Clusters and sub clusters	Germnplasm	Name of the Germplasm
1	Cluster-I	Cluster I <i>a</i>	2 Calcutta Single and BR-20
		Cluster I <i>b</i>	4 Hyderabad Single, Shringar, BR-17 and BR-18
2	Cluster-II	Cluster II <i>a</i>	2 Suvasini and Arka Nirantara
		Cluster II <i>b</i>	6 BR-2, BR-1, Hyderabad Double, Calcutta Double, Phule Rajani and Prajwal
3	Cluster-III	Cluster III <i>a</i>	3 Pune Local Double, Pearl Double and Arka Sugandhi
		Cluster III <i>b</i>	7 BR-24, Mexican Single, Sikkim Selection, BR-19, Hybrid GKTC-4, BR-3 and Pratap Rajani

Cluster-I comprises six cultivars, whereas cluster-II has eight germplasms with dissimilarities, and cluster-III comprises ten germplasms. Cluster I was separated into two subclusters, namely, *I a* and *I b*. Subcluster *I a* comprises two distinct germplasms, specifically Calcutta Single and BR-20. On the other hand, Subcluster *I b* was separated into subclusters consisting of the germplasms Hyderabad Single, Shringar, BR-17 and BR-18. Cluster II was subsequently separated into two subclusters, namely, *Ila* and *Iib*. Subcluster *Ila* was composed of two germplasms, namely, Suvasini and Arka Nirantara. Subcluster *Iib* was further subdivided into subclusters, comprising the germplasms BR-2, BR-1, Hyderabad Double, Calcutta Double, Phule Rajani and Prajwal. Cluster III was subdivided into 2 subclusters, namely, *IIla* and *IIlb*. Subcluster *IIla* was then subdivided into subclusters, encompassing the germplasms Pune Local Double, Pearl Double and Arka Sugandhi. Subcluster *IIlb* was further subdivided into subclusters, consisting of the germplasms BR-24, Mexican Single, Sikkim Selection, BR-19, Hybrid GKTC-4, BR-3 and Pratap Rajani.

The concept of tree distance is utilized to measure the genetic variation between different germplasms quantitatively (Table 4). The range of tree distance, which helps in investigating the spatial distribution and patterns of genetic diversity within the population or group of tuberose germplasms, varied between 0.185 and 0.621, and the obtained results are comparable to the information generated in tuberose [44] and in *Gladiolus* [45]. The greatest tree distance (0.621) was detected between germplasms BR-24 and BR-1, followed by BR-18 and BR-24, with a tree distance of 0.598, whereas the lowest tree distance (0.185) was 0.185 between germplasms Mexican Single and Phule Rajani, indicating an extensive range of germplasms within the study area (Table 5).

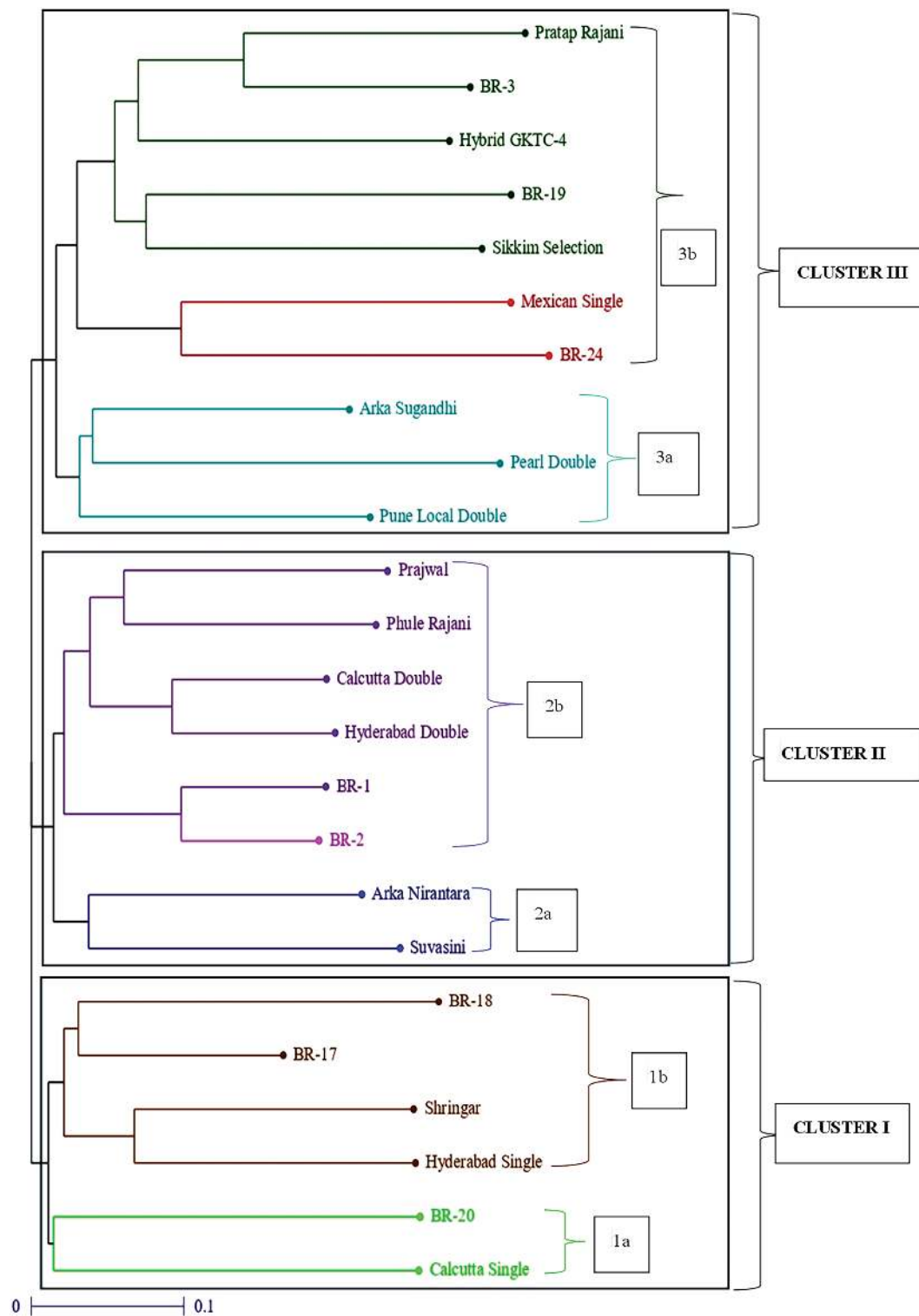


Figure 4: Dendrogram constructed via UPGMA for 24 tuberose genotypes [Colour lines representing number of germplasm in three cluster groups (from down to top), i.e., light green (1a), brown (1b), Royal blue (2a), Purple (2b), sea green (3a), Red, dark green (3b)]

Table 5: Tree distance of 24 diverse genotypes of Tuberose

Germplasm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1																							
2	0.408	1																						
3	0.443	0.497	1																					
4	0.470	0.524	0.534	1																				
5	0.365	0.419	0.404	0.456	1																			
6	0.368	0.437	0.472	0.500	0.394	1																		
7	0.431	0.485	0.470	0.523	0.374	0.461	1																	
8	0.532	0.586	0.595	0.556	0.518	0.561	0.584	1																
9	0.349	0.418	0.453	0.481	0.375	0.348	0.441	0.542	1															
10	0.381	0.383	0.469	0.497	0.391	0.410	0.458	0.558	0.391	1														
11	0.185	0.413	0.447	0.475	0.369	0.372	0.436	0.536	0.353	0.385	1													
12	0.377	0.447	0.481	0.509	0.403	0.339	0.470	0.570	0.357	0.419	0.381	1												
13	0.458	0.512	0.522	0.422	0.444	0.488	0.511	0.544	0.468	0.485	0.463	0.497	1											
14	0.506	0.560	0.570	0.531	0.492	0.535	0.559	0.458	0.516	0.533	0.511	0.545	0.519	1										
15	0.342	0.411	0.446	0.474	0.368	0.341	0.434	0.535	0.208	0.384	0.346	0.350	0.462	0.509	1									
16	0.485	0.539	0.549	0.479	0.471	0.515	0.538	0.571	0.495	0.512	0.490	0.524	0.467	0.545	0.488	1								
17	0.504	0.558	0.568	0.560	0.490	0.534	0.557	0.621	0.515	0.531	0.509	0.543	0.548	0.596	0.508	0.575	1							
18	0.450	0.504	0.481	0.542	0.411	0.479	0.478	0.603	0.460	0.477	0.455	0.488	0.530	0.577	0.453	0.557	0.576	1						
19	0.506	0.560	0.570	0.333	0.492	0.536	0.559	0.592	0.516	0.533	0.511	0.545	0.458	0.567	0.510	0.515	0.596	0.578	1					
20	0.508	0.562	0.572	0.502	0.494	0.537	0.561	0.594	0.518	0.535	0.513	0.547	0.490	0.568	0.511	0.460	0.598	0.580	0.538	1				
21	0.400	0.454	0.464	0.456	0.386	0.429	0.452	0.517	0.410	0.427	0.404	0.438	0.444	0.491	0.403	0.471	0.436	0.471	0.492	0.494	1			
22	0.432	0.487	0.471	0.524	0.375	0.462	0.367	0.585	0.443	0.459	0.437	0.471	0.512	0.560	0.436	0.539	0.558	0.479	0.560	0.562	0.454	1		
23	0.466	0.520	0.505	0.558	0.371	0.496	0.476	0.619	0.476	0.493	0.471	0.505	0.546	0.594	0.469	0.573	0.592	0.513	0.594	0.596	0.487	0.477	1	
24	0.417	0.471	0.481	0.473	0.403	0.446	0.469	0.534	0.427	0.444	0.421	0.455	0.461	0.508	0.420	0.488	0.469	0.488	0.509	0.511	0.365	0.471	0.504	1

Note: 1. Phule Rajani, 2. Hyderabad Single, 3. Shringar, 4. Sikkim Selection, 5. Calcutta Single, 6. Arka Nirantara, 7. Hybrid GKTC-4, 8. BR-1, 9. BR-2, 10. Prajwal, 11. Mexican Single, 12. Arka Sugandhi, 13. BR-3, 14. Pratap Rajani, 15. Pune Local Double, 16. Pearl Double, 17. BR-24, 18. BR-19, 19. BR-20, 20. BR-17, 21. BR-18, 22. Hyderabad Double, 23. Calcutta Double, 24. Suvasini.

4 Conclusion

Among the examined markers, ISSR 6 [NTA-9, NPA-9 and 100% polymorphism] presented the highest level of polymorphism in comparison with the other markers. The ISSR, i.e., ISSR 10, ISSR 12 and ISSR 15, had a maximum PIC value of 0.374. Among the 16 markers examined in the present investigation, 12 had a complete polymorphism rate of 100%. The UPGMA dendrogram yielded three primary clusters, each of which was subsequently subdivided into subclusters. The germplasms BR-1 and BR-24 presented a significant level of diversity, as indicated by a tree distance of 0.621 in the dendrogram analysis. These findings indicate that these two germplasms possess an extensive range of genetic variations that render them distinct and make them suitable for inclusion in a productive tuberose breeding programme. In the present study, ISSR marker-related observations supported the genetic diversity of the tuberose germplasms studied.

Acknowledgement: The authors are thankful to Bidhan Chandra Krishi Viswavidyalaya and Odisha University of Agriculture and Technology for providing the germplasm of Tuberose and to the Niche Area of Excellence Laboratory, Uttar Banga Krishi Viswavidyalaya, for extending the facilities to conduct the research trial. The authors also extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2025-30).

Funding Statement: This research was funded by Uttar Banga Krishi Viswavidyalaya, India; Bangladesh Wheat and Maize Research Institute, Dinajpur 5200, Bangladesh. The study was also funded by Taif University, Saudi Arabia, Project No. (TU-DSPP-2025-30).

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Availability of Data and Materials: The data may be available upon request to the corresponding authors.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

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