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Genetic Diversity in Tuberose (*Polianthes tuberosa* L.) Germplasm using Inter Simple Sequence Repeat (ISSR) Markers

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A B S T R A C T

Genetic variation among 21 Tuberose genotypes were evaluated using six inter simple sequence repeat (ISSR) markers. The results suggested that the ISSR markers produced much better reproducible bands and were more efficient in grouping germplasm. Polymorphic Information Content (PIC), Resolving Power (RP) and Marker Index (MI) for ISSR varied from 0.50-0.99, 1.61 - 3.80 and 0.99-4.57 respectively. The number of ISSR fragments generated per primer set ranged from 1 to 5 with fragment sizes varying from 180-1400 bp. A total of 19 polymorphic bands generated 100% polymorphism. All germplasm were clearly differentiated by their ISSR fingerprints. The Jaccard similarity indices (J) based on ISSR profiles were subjected to UPGMA cluster analysis. The dendogram generated by ISSR markers revealed three major groups and noted considerable amount of variation. Genotypes namely Hyderabad Single, Kalyani Single and Pearl double petaled cultivar were found to be more diverse in molecular analysis.

Introduction

Tuberose (*Polianthes tuberosa* L.) commonly known as Rajanigandha is native of Mexico and belongs to the family Amaryllidaceae. Among the bulbous flowering crops, it occupies a prime position due to its highly fragrant waxy flowers which can be used in various ways. It is commercially cultivated for cut flowers, loose flowers and also for extraction of its high valued natural flower oil. The flowers remain fresh for quite a long time and stand distance transportation.

Tuberose is diploid with chromosome number of 30, of which 5 are large and rest are small (Whitaker, 1934). The genus Tuberosa contains 12 species of which nine have white flower (Rose, 1903-05). In India, commercial tuberose cultivation is confined to one species *P. tuberosa*, which is basically a white-flowered type. To meet the increasing demands for modern cultivar in the world trade, a large number of cultivars are being grown for novel and desired traits. Hence, accurate identification and characterization of different genotypes are essential for enforcing the intellectual property rights (IPR) of breeders.

Morphological traits have long been used to estimate systematic relationships in crops (Chen *et al.,* 2004) and ornamentals (Wen and Hsiao, 2004). Though simple and irreplaceable, these descriptors suffer from many draw back and may be influenced of environment variation. Therefore, Molecular genetic diversity estimates are extremely useful for more accuracy, intellectual property protection particularly in the determination of essential derivation. These markers are highly polymorphic and have been successfully used in many bulbous flowering crops (Jingang *et al.,* 2008; Kiani *et al.,* 2012; Kameswari *et al.,* 2014 and Kumar *et al.,* 2016). At present only few studies are available with regard to diversity and genetic relationship in tuberose using DNA markers (Sarkar *et al.,* 2010; Kameswari *et al.,* 2014; Khandagale, 2014 and Bharti *et al.,* 2016). In view of above facts, the present study has been undertaken with the objective of an overall assessment of genetic diversity and genetic relationship among the twenty one cultivars of tuberose (fourteen each of single and seven double petaled respectively) using ISSR marker.

Materials and Methods

A total of 21 Tuberose cultivars representing majority of varieties under cultivation in India were analyzed using morphological traits and selected ISSR markers (Table 1). The materials were planted in randomized block design with 2 replications at Horticultural Research Centre of Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, U.P., India during 2016. The experimental soil was sandy loam in texture with 40.4, 24.2, 18.3 and 17.1% coarse sand, fine sand, silt and clay content, respectively. The soil had pH 7.9, EC 0.4 dS m^{-1} and an average bulk density of 1.55 Mg m^{-3} ha^{-1}.

Total genomic DNA was extracted from fresh and young leaves of tuberose by CTAB method with little modifications (Doyle and Doyle, 1990). The quality of DNA was checked on 0.8% agarose gel and DNA concentration was determined using a Bio-Rad's Smart Spec^{TM} Plus spectrophotometer (Bio-Rad Laboratories, Hercules, California, U.S.A.).

ISSR PCR amplification was carried out in 25 µl reaction volume containing 50 ng DNA, 1 × PCR buffer, 10 p mole primer, 200µM dNTPs, 2mM $MgCl_2$ and 1 U Taq polymerase. DNA amplification was carried out using the PTC Thermal Cycler (MJ Research Inc.). The amplification program included a denaturing step at 94^0C for 5 min, followed by 35 cycles with a denaturing step at 94^0C for 1 min, an annealing step at 53^0C for 1 min and an extension step at 72^0C for 2 min. After the last cycle, samples were kept at 72^0C for 5min. To identify the primers that produced clear, amplified bands, the amplification products were screened by electrophoresis on 1.5% agarose gels containing 0.002 ng/ml ethidium bromide in 0.5 Tris-borate buffers. The gels were examined under UV light and photographed. Amplification products were resolved by electrophoresis on 6% polyacrylamide gels. In order to evaluate the reproducibility of the DNA profile, DNA isolation and PCR reactions were carried out 3 times and only well-defined and reproducible bands were scored.

A set of 24 ISSR primers (University of British Columbia and IDT (Integrated DNA Technology) were initially tested out of these 06 primers, that showed consistently good amplification were used for final study with all 21 Tuberose cultivars. The 0-1 matrix data of ISSR analysis was subjected to calculate pair wise genetic similarity using Jaccard's coefficient (Jaccard, 1908). The similarity matrix thus obtained was subjected to prepare dendrogram by unweighted pair group

method of arithmetic averages (UPGMA) with the help using XLSTAT 2007 software (Addinsoft, 2007). Data generated by using 09 ISSR primers on 21 Tuberose genotypes were scored in binary format and further analysed as described previously (Kumar *et al.,* 2009). Besides this, PIC (polymorphism information content) described by Botstein *et al.,* (1980), marker index (MI) by Milbourne *et al.,* (1997) and Resolving Power (Rp) by Prevost and Wilkinson's, (1999) were also calculated.

Results and Discussion

In the present study, a total of 19 bands generated and all bands were observed as polymorphic by using nine ISSR primers. The size of bands scored in all the 21 genotypes were in the range of 180 to 1400 bp. The number of amplified bands generated by individual ISSR varied from 1- 4 bands with an average 3.16 bands per primer and the per cent of polymorphic bands was 100%. Kameswari *et al.,* (2014) used six ISSR primers to characterize seven tuberose genotypes and reported 85.48% polymorphism. Similarly Bharti *et al.,* (2016) used nine ISSR primers and found 92.50 % polymorphism which supports our finding of having higher polymorphism with ISSR marker system. Primer amplification details as obtained for each ISSR primer are shown in table 2. Data generated by using 06 ISSR ranged from 1 (ISSR-857) to 5 (ISSR-6F) with an average of 3.16 bands per primer (Figs. 1 and 2). Polymorphic information content varied from 0.50-0.99 with an average 0.82, resolving power vary from 1.61 to 3.80 with an average 2.38, while the marker Index varied from 0.99-4.57 with an average value of 2.62, respectively. Prevost and Wilkinson (1999) described the parameter like resolving power (Rp) as a measure of the discriminatory power of ISSR molecular marker. The values of resolving power in the study varied from 1.61 to 3.80 with an average 2.38. In another study, using ISSR markers to distinguish genotypes of cashew (Khandagale *et al.,* 2014) found Rp values between 0.91 to 4.55 an average 2.54. Both studies found a linear relationship between the ability to distinguish with genotypes and values of Rp.

All the 19 bands, generated by nine ISSR primers were used for genetic diversity studies. Moderate level genetic diversity was observed in the germplasm as indicated by the range of jaccard's dissimilarity co-efficient, 0.583 to 1.00 with an average 0.73 (Table 3). Combinations generated by Tuberose genotypes, the lowest dissimilarity (0.100) were found between the Prajwal and Suvasini and maximum dissimilarity (1.00) were observed between Kalyani Single and Pearl Double & Hyderabad Single. Kameswari (2014) also observed the similarity coefficient based on six ISSR markers ranged from 0.300 to 0.706. Hence, it is clear from the present study that some of the tuberose germplasms used in the study were the members of more restricted germplasm pool although cultivars randomly collected from the different locations. This could have happened due to highly heterozygous nature of this crop.

The UPGMA based on the clustering 21 tuberose genotypes were divided into three major groups (Fig. 3). Group I was the largest containing 11 cultivars, dissimilarity group II contained only 05 genotypes and group III contain 05 genotypes. The group I was further subdivided in three sub-group (I to III). The sub-group I Cluster I included six single petaled genotypes cultivars namely GKTC4, SVPUAT-1, Prajwal, SVPUAT-4 and Pragya Culum Local and Suvasini, is double cultivar.

Table.1 List of Tuberose genotype with their characters

S.No.	Genotype	Characteristics
1	Shringar	Single flowers on a sturdy spike a cross between Single x Double, (developed by IIHR)
2	SVPUAT-3	Double flowers on medium spike
3	Vaibhav	Semi-double flowers on medium spike, cross Mexican Single x IIHR-2, (developed by IIHR)
4	Prajwal	Single flowers on tall, stiff spikes; cross of Shringar x Mexican Single (developed by IIHR)
5	SVPUAT-4	Single flowers on a sturdy spike
6	SVPUAT-1	Pure single white flower with one row of corolla segment
7	Suvasini	A multi-whorled variety developed from cross between Single x Double, (developed by IIHR)
8	Mexican White Double	Creamy flower wit three row of corolla segments
9	Kalyani Single	Long single flowers, petals with creamy colour
10	Arka Nirantara	Single-flower type ,Single rows of petals, Flower Spike curvature Present
11	Sikkim Selection	Flowers are single but leaves are of variegated type
12	Hyderabad Double	More than three rows of corolla segment.
13	Mexican Single	Florets bearing single segment of corolla
14	GKTC-4	Single rows of petals , Flower Spike curvature Absent
15	Swarn Rekha	Doubled flowered type with golden yellow streak along the margin of leaf blade.
16	Phule Rajani	Single rows of corolla segment
17	Pearl Double	Flowers are pure white with more than three segments of corolla
18	Hyderabad Single	Single flower
19	SVPUAT-2	Single-flower type ,Single rows of petals
20	Pragya Culum Local	Single-flower type, long spike with white flower
21	Arka Sugandhi	Small size spike with more number of single florets

Table.2 ISSR Primer code, amplified bands, polymorphic alleles, Polymorphism %, PIC, RP and MI value of 21 tuberose genotypes

S No.	Primer Code	Primer Sequence	PIC	RP	MI	No. Bands	Polymorphic Band	Polymorphic %
1	ISSR 4F	AAGAAGAAGAAGAAGCC	0.509	2.76	1.018	2	2	100
2	ISSRUBC889	CATGGTGTTGGTCATTGTTCC	0.852	2.046	2.556	3	3	100
3	ISSR 6F	AGCAGCAGCAGCAGCCG	0.915	2.186	4.575	5	5	100
4	ISSR 857	ACACACACACACACACCTG	0.997	1.906	0.997	1	1	100
5	ISSR 840	GAGAGAGAGAGAGAGACTT	0.690	3.804	2.76	4	4	100
6	ISSR 855	ACACACACACACACACCTT	0.957	1.616	3.828	4	4	100
	Average		0.82	2.38	2.622	3.16	3.16	100

Table.3 Value of Jaccard's dissimilarity coefficient for 21 genotypes of tuberose

	Shringar	SVPUAT-3	Vaibhav	Prajwal	SVPUAT-4	SVPUAT-1	Suvasini	Mexican White Double	Kalyani Single	Arka Nirantara	Sikkim Selection	Hyderabad Double	Mexican Single	GKTC4	Swarna Rekha	Phule Rajani	Pearl Double	Hyderabad Single	SVPUAT-2	Pragya Culum Local	Arka Sugandhi
Shringar	0																				
SVPUAT-3	0.50	0																			
Vaibhav	0.83	0.33	0																		
Prajwal	0.70	0.40	0.60	0																	
SVPUAT-4	0.70	0.40	0.60	0.18	0																
SVPUAT-1	0.57	0.38	0.63	0.30	0.30	0															
Suvasini	0.67	0.33	0.56	0.10	0.10	0.22	0														
Mexican White Double	0.80	0.50	0.56	0.54	0.54	0.55	0.50	0													
Kalyani Single	1.00	1.00	1.00	0.83	0.83	0.78	0.82	0.92	0												
Arka Nirantara	0.60	0.75	0.86	0.60	0.60	0.43	0.56	0.70	0.67	0											
Sikkim Selection	0.71	0.50	0.57	0.40	0.40	0.38	0.33	0.50	0.75	0.33	0										
Hyderabad Double	0.78	0.73	0.80	0.80	0.71	0.85	0.79	0.58	1.00	0.91	0.83	0									
Mexican Single	0.90	0.60	0.50	0.71	0.71	0.75	0.69	0.30	1.00	0.91	0.73	0.40	0								
GKTC4	0.67	0.63	0.71	0.50	0.50	0.29	0.44	0.73	0.71	0.50	0.43	0.82	0.82	0							
Swarna Rekha	0.67	0.63	0.71	0.75	0.75	0.67	0.73	0.73	1.00	0.71	0.63	0.92	0.82	0.75	0						
Phule Rajani	0.78	0.60	0.67	0.71	0.62	0.75	0.69	0.58	1.00	0.91	0.73	0.22	0.40	0.70	0.82	0					
Pearl Double	1.00	0.83	0.75	0.90	0.90	0.86	0.89	0.89	1.00	1.00	0.83	1.00	0.88	0.80	0.80	0.88	0				
Hyderabad Single	0.83	0.57	0.67	0.73	0.60	0.63	0.70	0.82	1.00	1.00	0.89	0.80	0.80	0.71	0.88	0.67	0.75	0			
SVPUAT-2	0.80	0.50	0.25	0.70	0.70	0.57	0.67	0.67	1.00	0.83	0.71	0.90	0.63	0.67	0.67	0.78	0.67	0.60	0		
Pragya Culum Local	0.82	0.55	0.60	0.33	0.18	0.45	0.27	0.64	0.73	0.73	0.55	0.71	0.71	0.50	0.85	0.62	0.90	0.60	0.70	0	
Arka Sugandhi	0.78	0.60	0.67	0.71	0.62	0.64	0.69	0.58	1.00	0.91	0.83	0.40	0.40	0.70	0.82	0.22	0.88	0.50	0.63	0.62	0

Fig.1 ISSR profiling pattern of 21 Tuberose genotype with ISSR-4 primer

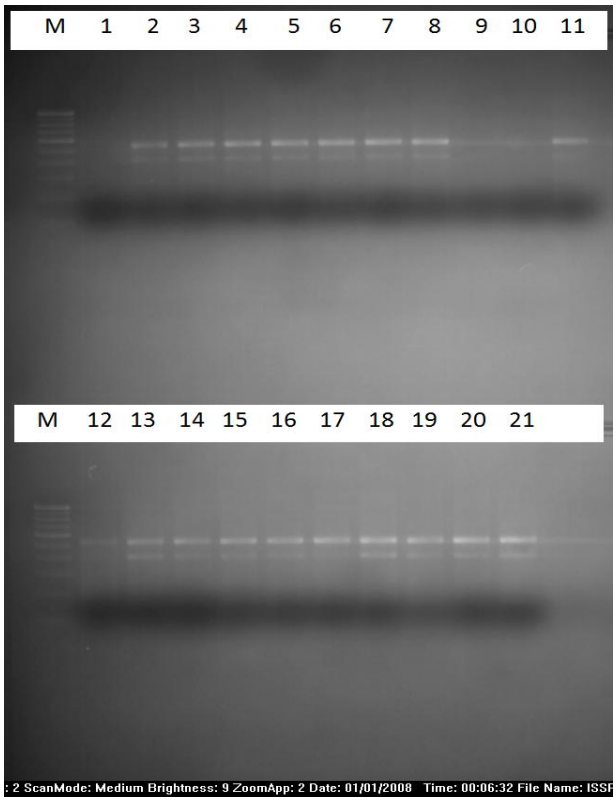


Fig.2 ISSR profiling pattern of 21 Tuberose genotype with ISSR-UBC-889 primer

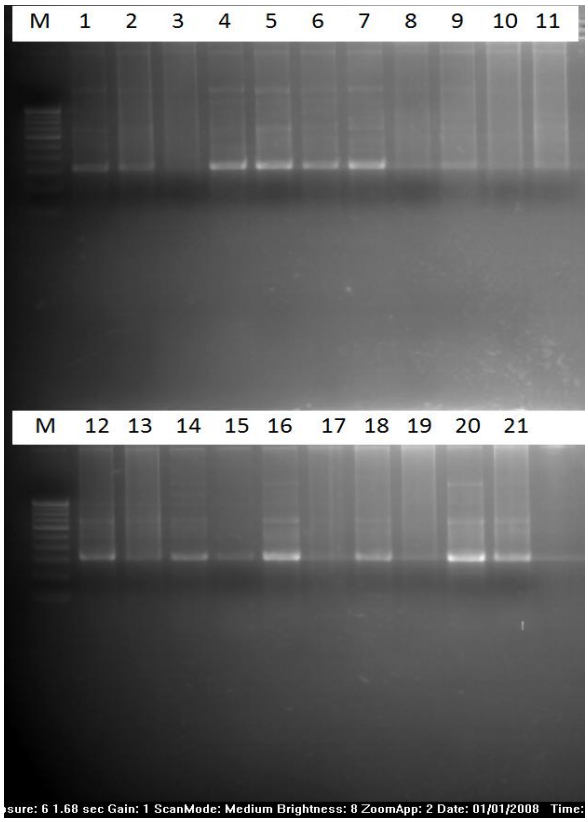
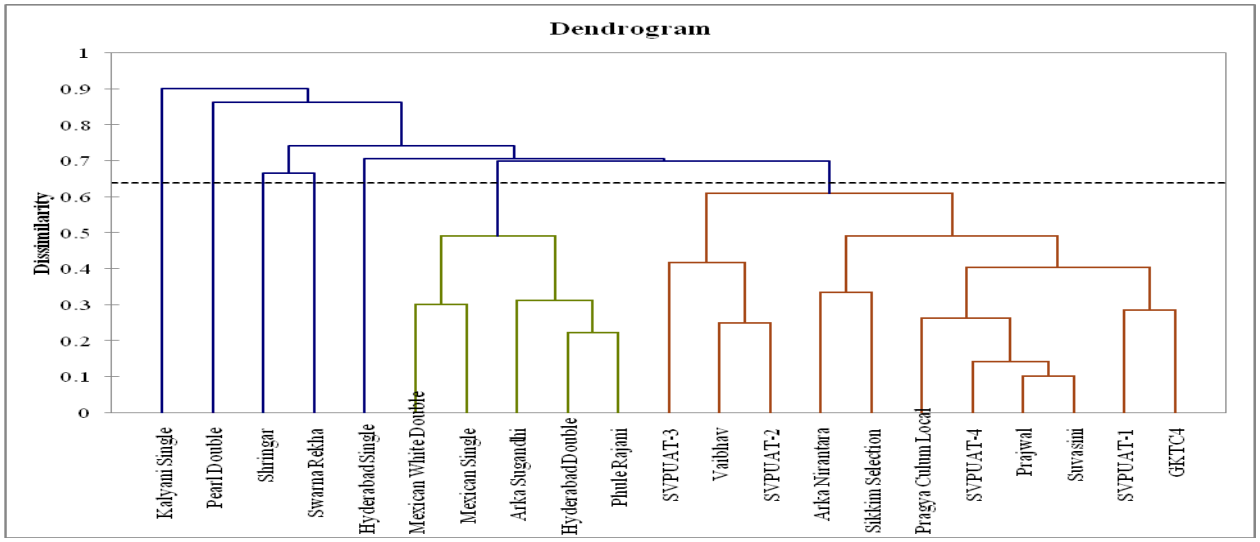


Fig.3 Dendrogram showing clustering of 21 tuberose varieties constructed using UPGMA based on Jaccard's similarity coefficient obtained from ISSR analysis



Sub-group II contained two genotypes that including two single petaled genotypes namely Sikkim Selection and Arka Nirantara, similarly sub-group III contained three genotypes including the cultivar SVPUAT-2 as single petaled and Vaibhav and SVPUAT-3 simultaneously semi double and double petaled cultivars. Group II was further subdivided into two sub-groups (I and II). The sub-group I had three cultivars out of which Phule Rajani and Arka Sugandhi is single petaled cultivar and Hyderabad Double is double petaled cultivar. Sub-group II having two cultivars included Mexican Single and Mexican White Double. Group III is the most important in this dendogram because in this group had three more diverse genotype including two single petaled cultivars namely Hyderabad Single and Kalyani Single and Pearl had double petaled cultivar.

Grouping of the genotypes in dendrogram in some cases did not match their phenotypes and most of the clusters had some mixed genotypes namely single and double. Several reasons may be attributed for these differences, but the most important fact is that the morphological expression is conditioned by stage of the plant, existing agricultural management practices and prevailing environmental conditions. Use of more number of polymorphic markers and accurate phenotyping may reduce it to a great extent.

These differences might have arisen due to several reasons, but the most important is that the genetic (or structural) origin of each marker is different, while morphological expression (phenotype) is conditioned by the state of the plant, agricultural management and environmental conditions. Similar observation was also made by Dhanraj *et al.*, (2002) and Thimmappaiah *et al.*, (2009) in cashew. From the present study, it has been observed that ISSRs are simple and quick method for estimating the genetic diversity analysis in tuberose. Similar findings have been reported by Zietkiewicz *et al.*, (1994). ISSR markers were proved to be useful in genetic diversity studies in other floricultural crops like chrysanthemum (Cai-hong *et al.*, 2010; Baliyan *et al.*, 2014), lilium (Guo *et al.*, 2011; Xi *et al.*, 2012; Zhao *et al.*, 2014) and Zinnia (Ye *et al.*, 2008).

In conclusion among the 21 cultivars analyzed with ISSR marker, three main groups were recognized by UPGMA based on Jaccard's similarity coefficient. The first group contained 11 cultivars; the second group included 05 cultivars and third group contain 05 cultivars respectively. In the present study, ISSR provided good insist of genetic diversity available in tuberose germplasm. It was also suggested that ISSR analysis is less time consuming, less expensive, more reliable, reproducible and generate more reliable, reproducible and generate more polymorphism. Due to unique ISSR fingerprints, it can be useful for determination of cultivar purity and efficient use and management of genetic resources collection.

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