Electronic Journal of Plant Breeding, 5(1): 52-57 (Mar 2014)
ISSN 0975-928X

Research Article

Genetic diversity in tuberose (*Polianthes tuberose* L.) using morphological and ISSR markers

P.Lalitha Kameswari[1], A. Girwani[2] and K.RadhaRani[2]
[1]Floricultural Research Station, Dr. YSR Horticultural University, Rajendranagar, Hyderabad-30
[2]College of Horticulture, Dr. YSR Horticultural University, Rajendranagar, Hyderabad
E-mail: lalitha_email@yahoo.com

(Received: 24 Feb 2014; Accepted: 10 Mar 2014)

Abstract
Tuberose is the most important bulbous plant grown both for cut flowers as well as for loose flowers. The flowers are more valued for their sweet and lingering pleasant fragrance. During the year 2012, both field and lab experiment was conducted to characterize the single category genotypes of tuberose collected from different states of India. Seven genotypes were subjected to morphological and molecular characterization. Results on morphological parameters clearly indicate significant differences among varieties for all quantitative characters except for vase life and floret length. Six ISSR primers viz., 808, 836, 840, 842, 855 and 857 were used for genetic diversity studies.A total of 62 bands were produced across seven genotypes, of which 53 bands were polymorphic, accounting for 85.48% polymorphism. The ISSR dendrogram grouped the genotypes into two major clusters. The genotypes Calcutta Single and Prajwal recorded the highest similarity index value of 0.706 which were considered closely related genotypes. Among the seven genotypes, Hyderabad Single and PhuleRajani are divergent from other geneotypes viz., Shringar, GK-T-C-4, ArkaNirantara, Prajwal and Calcutta Single.

Key words: Tuberose, genotypes, ISSR markers, genetic divergence

Introduction:
Tuberose (*Polianthes tuberose* L.) is a fragrant ornamental flower. It is native of Mexico (Trueblood, 1973). It occupies a prime position among commercial ornamental bulbous crops, because of 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. Hence it occupied a prime place in the flower market (Patil*et al.,* 1999). The long spikes of tuberose are used for vase decoration and bouquet preparation. The florets are used for making artistic garlands, ornaments and buttonhole use. The natural flower oil of tuberose is one of the most expensive perfumer's raw materials. In general there are only threetypes of cultivarsunder commercial cultivation i.e. Single, Semi Double and Double based on number of rows of petals they bear.

Identification of varieties or breeding lines is very important in any crop improvement programme especially in tuberose. Genetic diversity in this crop is meagre and there were no improved varieties of economic value until the hybrids 'Shringar', 'Suvasini', 'Prajwal' and 'Vaibhav' were released by the Indian Institute of Horticultural Research (IIHR), Bangalore. Traditionally, genetic diversity studies are based on differencesin morphological characters and qualitative traits (Schut*et al.,* 1997). It has been used as a powerful tool in the classification ofcultivars and also to study taxonomic status. Morphological traits continue to be the first step in the studies of geneticrelationships in most of the breeding programmes (Cox and Murphy, 1990; VanBueningen and Busch, 1997). Suchcharacteristics are often controlled by multiple genes and are subject to varyingdegrees of environmental modification and interaction. Most elite cultivated and breeding materials do not aboundwith readily observable morphological markers, a large number of which havedeleterious effects on agronomic performance (Smith, 1986). Hence, the most closely related cultivars are usually distinguished by DNA fingerprinting methods (Nybom, 1994). Compared to morphological and biochemicalcharacteristics, the DNA genome provides a significantly more powerful source ofgenetic polymorphism (Beckmann and Soller, 1986). These molecular markers are considered to be the most suitable means for estimatinggenetic diversity because of their abundant polymorphism and the fact that theyare independent of environment(Gepts, 1993).

Till date very little attempt has been made on molecular characterization of bulbous ornamental crops. Characterization of tuberose cultivars through molecular means is much desired, as there is much confusion in the naming of genotypes existing in different states of India as they are exclusively referred as single and double cultivars. It is probably expected that all the single types

developed from their corresponding states have evolved from single genotypes and all the local doubles probably from another. At present only few studies were recorded on the diversity analysis in tuberose using DNA markers. Hence, the present investigation was carried out with the objectives:(1) to study whether ISSR markers can be used for the characterization of genotypes for intellectual property rights, and (2) to employ thesemarkers for studying the genotypic variability among Indian tuberose varieties.

Material and Methods

Plant material**:** The plant material selected consisted of seven single category cultivars, viz., Hyderabad single, Calcutta single, PhuleRajani, Shringar, GK-T-C-4, ArkaNirantaraand Prajwalcollected from different states of India (Table 1). The experiment waslaid out in a randomised block design with three replications at Floricultural Research Station, Rajendranagar, Hyderabad.Morphological characterization was done using the parameters viz., plant height, number of leaves, days to spike emergence, days to flowering, rachis length, spike length, number of florets per spike, average flower weight, floret length, floret diameter, vase life, size of the bulb, weight and number of bulbs per plant.

Total genomic DNA was extracted by modified CTAB method (CetylTrimethyl Ammonium Bromide) (Murray and Thompson, 1980). Purity of DNA was assessed electrophoretically on 0.8% agarose gel. The concentration and quality of DNA was also estimated spectrophotometrically by using Nano Drop spectrophotometer at 260 nm.The template DNA samples were diluted to make the working solutions of 5ng/µl for PCR analysis. Thirty two ISSR primers (UBC primer set no. 9, University of British Columbia, Canada) were tested with *P.tuberosa* DNAs. Six ISSR primers were selected based on polymorphismand robustness of the bands obtained (Table 3).The PCR amplification for ISSR profiling was carried out in a total volume of 10 µlcontaining 1.0 µl of 5 ng template DNA, 1.0 µl of 10x PCR buffer (10 mMTris pH 9.0, 50 mMKCl, 15 mM $MgCl_2$), 0.2 µl of 25 mM $MgCl_2$, 0.6 µl of 200 M of each of the four dNTPs, 1.0 µl of 0.4 M ISSR primer and 0.2 µl of 0.6 Units *Taq*DNA polymerase (Bangalore Genei, India).

PCR amplifications were performed in a Gene Amp 9700 thermal cycler (Perkin Elmer Applied Biosystems)with initial denaturation at 94^0 C for 4 minutesfollowed by 35cycles of30 sec at 92^0 C, annealing temperature of 45^0C for 1 min and primer extension at 72^0C for 1 min and final extension at 72^0C for 7 min. The amplified products were electrophoretically separated on 1.7% agarose (Bangalore Genei, India) gels buffered with 1x TAE at 100 V for 3 h and visualized with ethidium bromide staining. The gel images were recorded using the Alpha InnotechFluorchem gel documentation system and the sizes of amplification products were determined by comparison to *Eco* RI and *Hind* III double digest (Bangalore Genei, India) as molecular weight standard. The reproducibility of the amplification was confirmed by repeating each experiment three times.

The banding pattern obtained from ISSRs in each genotype was scored as '1' to all the bands that could be reproducible and detected in the gel and '0' for the absence of band for each primer.Jaccard's similarity coefficient (J) was used to calculate similarity between pairs of genotypes. Where, [J = nx,y / (nt - nz)], nx, y = the number of bands common to genotype A and genotype B; nt the total number of bands presenting in all samples and nz = the number of bands not present in A and B but found in other samples.Cluster analysis was performed on molecularsimilarity matrices using the Unweighted Pair Group Method using Arithmetic Means (UPGMA) algorithm, from which dendrograms depicting similarity among genotypes were drawn and plotted using NTSYS-pc. 2.1 Software (Rohlf, 2000).

Results and Discussion

Significant difference among the cultivars was observed for all the quantitative characters studied except for vase life and length of the floret. The mean perforamnce of all the genotypes were mentioned in Table 2. Among the 32 ISSR primers used in the preliminary analysis, only six primers produced the bright, distinguishable, scorable and unambiguous bands.A total of 62 bands were produced across seven genotypes, of which 53 bands were polymorphic, accounting for 85.48% polymorphism. The number of bands varied from eight(ISSR-808) to thirteen (ISSR-855) depending on the primer with a mean value of 10.3 bands per primer. The amplicon sizes obtained with the ISSR primers ranged from 220bp to 2000bp. The percentage of polymorphism exhibited by ISSR primers ranged from 76.9% (ISSR-855) to 100% (ISSR-840). Primer amplification details as obtained for each ISSR primer are shown in Table 3.

The banding pattern of ISSR markers scored in the form of binary data was used for computing Jaccard's similarity index values obtained for each pair wise comparison and are presented in the Table 4.The similarity coefficient based on six ISSR markers ranged from 0.300 to 0.706. Among the genotypes studied, the lowest similarity index (0.300) was recorded between Calcutta Single and Hyderabad Single and hence sonsidered as most divergent.The genotypes Calcutta Single and Prajwalrecorded the highest similarity index value of 0.706 which were considered closely related genotypes. The dendrogram based on ISSR

separated the seven genotypes into two main clusters at similarity index of 36% (Fig 1). The first cluster had five genotypes Shringar, GK-T-C-4, ArkaNirantara, Prajwal and Calcutta Single from Karnataka, Maharashtra and West Bengal. The second cluster consists of only two genotypes Hyderabad Single and PhuleRajani from Andhra Pradesh and Maharashtra respectively which were diverged at similarity coefficient value of 0.47.The genotype Shringar is also having close association with other four genotypes of cluster I at similarity coefficient of 0.62.

ISSR markers seem to produce more reliable and reproducible bands than RAPDs because of longer length of the primer and their high annealing temperature (Nagaoka and Ogihara, 1997). ISSRs are simple and quick method to check and estimate diversity level because of no prior sequence data is needed and a large number of markers can be generated(Zietkiewicz *et al*., 1994). They were proved to be useful in genetic diversity studies in chrysanthemum (Cai-hong *et al.,*2010).

Principal Component Analysis was done based on the Jaccard's similarity matrix obtained (Table 5). The first two principal components accounted for major variations. The First component accounts for 55.76% of all changes and second component accounted for 13.95% of principal changes. The description of the data was done using three dimensional pictorial graph and is represented in Fig.2. From the graph, it is evident that the tuberose genotypes were dispersed on the PC plot, which is a reflection of its genetic base. The results of PCA showed a clear cut separation. However, as depicted in figure some of the genotypes appear to be overlapping with each other depicting high similarity in these genotypes. It was clear from the analysis that the results obtained from PCA were in agreement with the dendrogram generated by UPGMA cluster analysis. This strengthened the ability and accuracy of the ISSR analysis applied to tuberose genotypes in the present study. Similarly among tuberose genotypes collected from different parts of India, the genetic relatedness was established with RAPD primers and found greatest similarity between Guwahati Double and Swarna Rekha though they are morphologically quite different (Jayoti Sarkar *et al.*, 2010). Grouping of the genotypes in dendrogram in some cases did not match their phenotypes.

From the above findings it can be concluded that, the Indian based tuberose genotypes showed both morphological and molecular diversity, of which only the genotypes Calcutta Single and Prajwalshowed more than 70% of similarity by which they can be considered as closely related genotypes. Among the seven genotypes, Hyderabad Single and PhuleRajani are divergent from other geneotypes viz., Shringar, GK-T-C-4, ArkaNirantara, Prajwal and Calcutta Single.

References

Beckmann, J. and Soller. 1986.Restriction Fragment Length polymorphisms in plant genetic improvement. Oxford surveys plant mol. *Biol. Cell Biol*., **3**: 197-250.

Cai-hongOuyang.,Qiao H E., Guo-xin Qin., Qi-gaoGuo., Guohui Han., Yong Wang., Bing Qin and Guo-lu Liang. 2010. Genetic Diversity of 25 Chrysanthemum [*Dendranthema* × *grandiflora* (Ramat.)Kitamura] Cultivars Revealed by ISSR Analysis.*Biochemical Systematics and Ecol.,* **38**(6):1160-1169.

Cox, T. S. AndMurphy, J. P. 1990. The effect of parental divergence on F2heterosis in winter wheat crosses. *Theor. Appl. Genet..,* **79**: 241-250.

Gepts, P. 1993. The use of molecular and biochemical markers in crop-evaluation studies. *Evolution. biol.*,**27**: 51-94.

JayotiSarkar., Misra, R. L., Bhat, K. V., Anita Singh and Sanjay K. Singh. 2010. Genetic diversity in tuberose (*Polianthestuberosa*) genotypes through Randomly Amplified Polymorphic DNA. *Indian J. Genet.*, **70**(2): 182-188.

Murray, M. and Thompson, W. 1980.The isolation of high weight plant DNA. *Nucleic Acids Res*., **8**:4321-4325.

Nagaoka, T. and Ogihara, Y. 1997. Applicability of Inter Simple Sequence Repeat polymorphisms in wheat for use as DNA markers in comparision to RFLP and RAPD markers. *Theor. Appl. Genet*., **94**: 597-602.

Nybom, H. 1994. DNA fingerprinting a useful tool in fruit breeding. *Euphytica.*, **77**:56-64.

Patil, P. R., Reddy, B. S., Patil, S.R.andKulkarni, B. S. 1999.Effect of community planting and fertilizer levels on growth and flower yield of tuberose (*Polyanthestuberosa* L) cv. Double. *South Indian Hort*.,**47**: 335-338.

Rohlf, F. J. 2000. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.1 Exceter Software, New York, USA.

Schut, J.W., Qi, X.andStam, P. 1997. Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. *Theor. Appl. Genet.,* **95**:1161–1168.

Smith, J. S. C. 1986. Biochemical fingerprints of cultivars using reversed-phasehigh performance liquid chromatography and isozyme electrophoresis: *AReview: Seed Sci. Tech.*, **14**: 753-768.

Trueblood, E.W.E. 1973 "Omixochitl" -the tuberose (*Polianthestuberosa*). *Econ. Bot.*,**27:** 157-173.

Van Bueningen, L.T., and Busch, R. H. 1997. Genetic diversity among North American spring wheat cultivars: I. Analysis of the coefficient of parentage matrix. *Crop Sci.,* **37**:570–579.

Zietkiewicz. E., Rafalski, A. and Labuda, D. 1994 Genome fingerprinting by Simple Sequence Repeat (SSR) Anchored polymerase chain reaction Amplification. *Genomics.,* **10:**176-183.

Table 1. List of selected genotypes selected for the present investigation

S. No	Name of the genotype	Place of origin
1	Hyderabad Single	Andhra Pradesh
2	Calcutta Single	West Bengal
3	PhuleRajani	Maharastra
4	Shringar	Karnataka
5	GK-T-C-4	Maharastra
6	ArkaNirantara	Karnataka
7	Prajwal	Karnataka

Table 2 Mean performance of tuberose genotypes based on vegetative parameters

Sl.No	Varieties	Plant Height (cm)	No. of leaves	Days to spike emergence	Days to flowering	Rachis length (cm)	Spike length (cm)	Vase life (days)	No. of florets/spike	Average floret weight (g)	Floret length (cm)	Floret diameter (cm)	Weight of bulbs(g)/ plant	No. of bulbs/ plant	Size of bulbs(cm)
1	Hyderabad Single	48.21	39.09	62.34	72.65	23.48	64.99	6.53	45.81	2.01	5.57	3.02	239.11	49.11	1.40
2	Calcutta Single	49.48	30.72	64.36	75.67	34.71	84.58	6.52	36.26	1.78	5.41	2.87	275.11	29.56	1.52
3	PhuleRajani	45.18	32.62	65.20	74.04	26.31	64.08	6.32	43.29	2.55	5.41	2.74	378.00	78.22	1.63
4	Shringar	46.41	35.11	65.43	75.15	22.51	61.63	6.39	39.20	2.37	5.51	3.58	253.00	48.00	1.32
5	GK-T-C-4	41.05	25.97	68.16	78.88	24.64	63.70	7.02	42.20	2.10	5.83	3.52	296.27	30.21	1.55
6	ArkaNirantara	54.73	20.58	80.27	89.44	21.92	67.47	6.70	44.86	2.40	6.15	3.72	394.12	34.35	1.83
7	Prajwal	57.32	26.38	67.30	84.61	31.34	85.24	6.80	36.73	2.77	6.00	3.90	320.00	39.22	1.36
	Mean	48.91	30.07	67.58	78.63	26.42	70.24	6.61	41.19	2.28	5.70	3.34	307.94	44.10	1.52
	S.E	1.74	2.99	1.40	2.63	0.61	1.36	NS	1.64	0.06	NS	0.26	0.63	0.54	0.05
	C.D at 5%	5.35	9.21	4.30	8.09	1.87	4.19	---	5.06	0.19	---	0.79	1.93	1.67	0.16

Table 3.Nucleotide sequences and polymorphism of the ISSR primers for tuberose genotypes

S. No	ISSR Primer	Annealing temperature (^{0}C)	DNA repeats	No. of total bands	Polymorphic bands(%)	Size range of amplified product(bp)
1	808	52^{0}C	$(AG)_8C$	8	7(87.5)	250-1550
2	836	53^{0}C	$(AG)_8YA$	9	7(77.7)	250-2000
3	840	53^{0}C	$(GA)_8YT$	9	9(100)	300-2000
4	842	55^{0}C	$(GA)_8YG$	11	9(81.8)	420-1900
5	855	53^{0}C	$(AC)_8YT$	13	10(76.9)	250-1600
6	857	55^{0}C	$(AC)_8YG$	12	11(91.7)	220-1550

Table 4. Jaccard's similarity matrix among seven genotypes of tuberose based on ISSR data

	Shringar	GK-T-C-4	Arka Nirantara	Phule Rajani	Prajwal	Hyderabad Single	Calcutta Single
Shringar	1.000						
GK-T-C-4	0.451	1.000					
ArkaNirantara	0.511	0.591	1.000				
PhuleRajani	0.366	0.349	0.405	1.000			
Prajwal	0.580	0.688	0.667	0.340	1.000		
Hyderabad Single	0.425	0.341	0.359	0.464	0.362	1.000	
Calcutta Single	0.538	0.577	0.617	0.333	**0.706**	**0.300**	1.000

Fig: 1 Dendrogram showing the genetic relationship among tuberose genotypes using UPGMA analysis of ISSR data

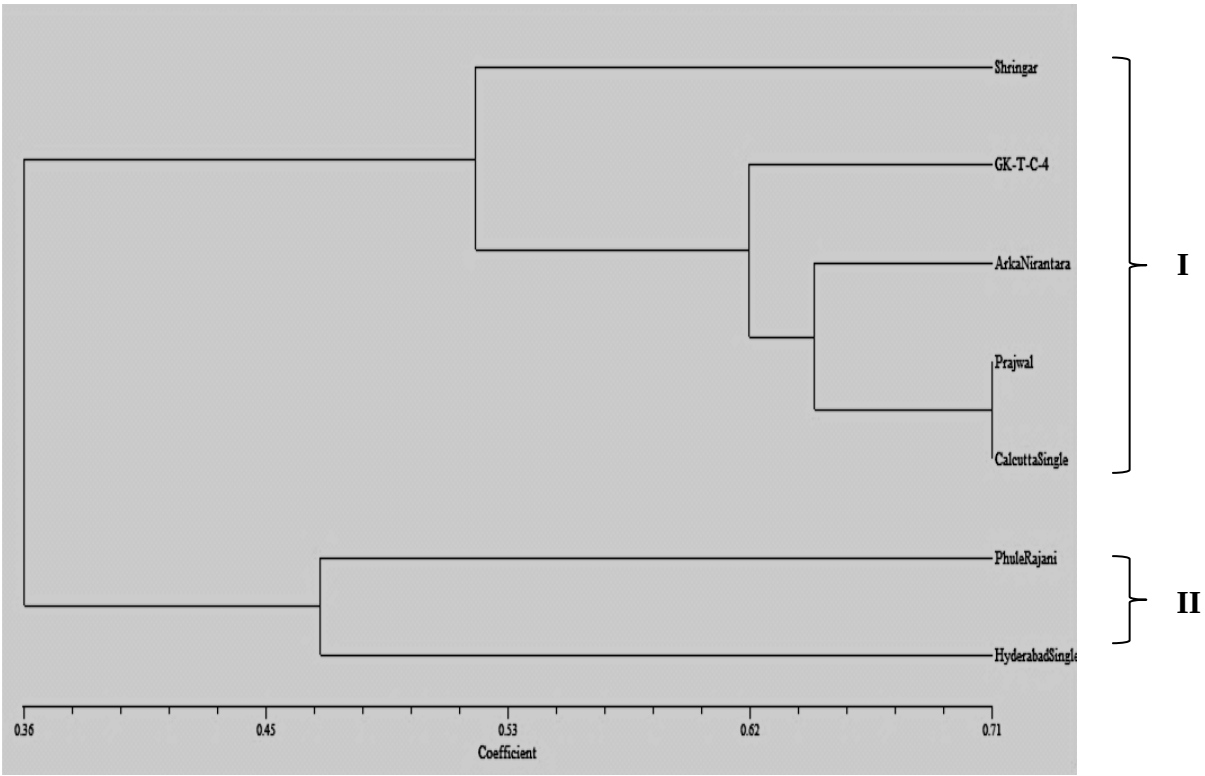

Fig 2 The relative position of tuberose genotypes based on ISSR markers (Three dimensional)