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RESEARCH ARTICLE

Assessment of genetic diversity and population structure in gladiolus (*Gladiolus hybridus* Hort.) by ISSR markers

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Abstract ISSR (Inter simple sequence repeat) markers were used to assess the genetic diversity and population structure in 53 indigenous and exotic genotypes of gladiolus (*Gladiolus hybridus* Hort.). Molecular markers analysis showed PIC ranges from 0.42 (ISSR 861) to 0.99 (ISSR 855, ISSR 856 and ISSR 889) with an average 0.812, marker index ranged from 0.99 (ISSR 889) to 9.26 (ISSR 851) with an average 4.66 and resolving power of the primers ranged from 0.03 (ISSR 889) to 11.58 (ISSR 861) with an average value 3.80. The dendrogram based UPGMA clustering showed that all the 53 genotypes grouped into three main clusters. Nei's gene diversity (Na) varied from 0.929 to 1.717, effective number of alleles (Ne) varied from 1.262 to 1.369, Shannon's information index (I) ranged from 0.251 to 0.359 and gene diversity (He) was in the range from 0.167 to 0.229. Population structure analysis revealed three groups in which 32 genotypes were admixture types.

Keywords Gladiolus · Molecular diversity · Inter simple sequence repeats · Population structure

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Introduction

Gladiolus is a genus of perennial herbaceous bulbous flowering plants of high economic importance. Cultivated gladiolus (*Gladiolus hybridus* Hort.) is native of South Africa and belongs to the family Iridaceae. Most of the gladiolus (*Gladiolus grandiflorus* Hort.) are tetraploid (2n = 4x = 60), interspecific hybrids being cultivated for more than 260 years (Goldblatt 1996). The current number of species in the genus is 255 (Goldblatt and Manning 1998). Among these, 180 species are originally from South Africa (Duncan 1996, 2000; Goldblatt 1996; Goldblatt and Manning 1998; Goldblatt et al. 1993; Manning et al. 2002).

Several features like massive spikes with florets, brilliant colors, attractive shapes etc. makes it a queen of bulbous flowers. They are ideal for display, floral arrangement, interior decoration and making high quality bouquet (Lepcha et al. 2007). Among bulbous flowering plants, glaldiolus are ranked fifth to tulip (Tulip sp.), lily (*Lilium* sp.), fressia (*Fressia* sp.) and hippestrum (*Hippestrum* sp.) (Flower Council of Holland 2008). In the cut flower trade, they ranked fourth in the International market after the rose, carnation and chrysanthemum (Rathod et al. 2011). In India, commercial value of gladiolus has increased a lot because of its local and export market value.

Nowadays, most of the gladiolus cultivars are developed from inter-specific hybridization among several species. Hence, wide variation is exhibited among the cultivars for their growth, shape, spike length and floret colour. In India, efforts have been made on collection and evaluation of gladiolus germplasm in order to gather ample information for higher flower quality as well as flower yield and their contributing characters. Being ornamental, morphological characterization is highly recommended step in gladiolus for selection using flower, corm and vegetative traits etc.

However, environmental influence on these traits limits their sole usage in selection. Improvement of traits like flower color, fragrance, disease resistance etc. is lagging due to inadequate exploration of diversity present within different species of gladiolus. There is much needed effort required for gladiolus improvement and to explore unexplored diversity present within large gladiolus germplasm. However, performance of particular gladiolus genotype depends on the climatic conditions in which they were grown (Swaroop and Janakiram 2010). This suggests large scale evaluation of wild and cultivated gladiolus genotypes under certain agro-climatic conditions to explore and utilize tremendous genetic diversity. It would be highly desirable to prioritize in breeding programmes, genotypes with broader agro-climatic adaptability. Therefore, large-scale diversity evaluation in gladiolus would help to exploit uncharacterized genetic components and to test their efficacy for developing genotypes with resistance for different abiotic and biotic stresses. In last three decades, numerous plant genetic diversity studies have been conducted using wide range of molecular markers including traditional markers like RAPDs, SSRs, AFLP or next generation sequencing (NGS) methods etc. (Mondini et al. 2009). Molecular markers aids in proper selection and characterization of plant germplasm. Among several markers available, Inter Simple Sequence Repeats (ISSR) markers are highly reproducible, polymorphic, informative and quick to use (Bornet and Branchard 2001). For any diversity study, it is advantageous to include original parents of the progeny genotypes tested in order to evaluate possible hybrid vigour and distribution of diversity among progeny genotypes. In the present study, authors assess the genetic diversity and population structure in 53 commercial gladiolus genotypes, including some parental genotypes, collected from different parts of country, using discriminating power of ISSR markers. To the best of author's knowledge, present study is first report in which ISSR molecular-markers have been used for diversity and population structure analysis in gladiolus.

Materials and methods

Plant material

In total, 53 genotypes of gladiolus were obtained from different sources namely: Directorate of Floricultural Research IARI, New Delhi, Indian Institute of Horticultural Research, (IIHR) Bangalore, Punjab Agricultural University (PAU), Ludhiana and National Botanical Research Institute (NBRI), Lucknow. Details like genotypes name, flower colour, origin, and pedigree is provided in Table 1.

DNA extraction, PCR amplification and cluster analysis

Genomic DNA extraction, PCR amplification and gel electrophoresis was performed as described earlier (Kumar et al. 2009). A set of 19 ISSR primers (Jingang et al. 2008; Shufang et al. 2010) were initially tested for amplification with selected genotypes (Table 2). Out of 19 primers, 17 primers that showed consistent and good amplification were further used for PCR amplification with 53 genotypes. The data generated by 17 ISSR primers on 53 genotypes were scored in binary matrix format and used for different statistical analysis. The 0–1 matrix data was subjected to calculate pairwise genetic similarity using Jaccard's coefficient (Jaccard 1908). The similarity matrix thus obtained was used to prepare dendrogram by unweighted pair group method of arithmetic averages (UPGMA) with the help of NTSYS-PC software version 2.02e (Rohlf 1993). Besides, PIC (polymorphism information content) (Botstein et al. 1980), marker index (MI) (Milbourne et al. 1997) and Resolving Power (Rp) (Prevost and Wilkinson 1999) were also calculated.

Population structure analysis

Gene diversity in population and subpopulation was measured by Nei (1973). Other genetic diversity estimates namely Na (number of different alleles), Ne (effective number of alleles) and I (Shannon's information index) were also calculated. Further, in order to estimate the number of subpopulations in the gladiolus germplasm, population STRUCTURE analysis was done using program STRUCTURE version 2.2 (Pritchard et al. 2000). The membership of each genotypes was tested for $K = 2$ to $K = 10$ with admixture model. Three independent runs were assessed for each fixed K and each run consisted of 30,000 burn-in period and 1,00,000 MCMC (Markov Chain Monte Carlo) iterations. The optimal value of K was determined by examination of the ΔK statistic and L (K) (Evanno et al. 2005) using Structure Harvester program (Earl and vonHoldt 2012).

Result

DNA profiling using IISRs

In the present study, 17 ISSR primers generated a total of 99 alleles among 53 genotypes which varied from one to seventeen with an average of 5.8 alleles per primer pair. Out of 99 alleles generated by the ISSR primers, 98 alleles were polymorphic and one was monomorphic, thus generating 99.01% polymorphism (Table 2, Fig. 1). ISSR 851

Table 1 Details of gladiolus genotypes studied

S. no.	Name of variety	Origin	Flower colour	Parental cross or source	Collection centre
1	Punjab Glance	India	Orange with yellow blotch	Happy End × Yellow Stone	PAU, Ludhiana, Punjab
2	Punjab Flame	India	Carmine with red blotch	Sylvia × White Prosperity	PAU, Ludhiana, Punjab
3	Punjab Pink	India	Pink	Suchitra × White Prosperity	PAU, Ludhiana, Punjab
4	Punjab Glad-1	India	Orange with yellow centre	Happy End × True Yellow	PAU, Ludhiana, Punjab
5	Pacifica	Exotic	Red group	Unknown	NBRI, Lucknow, UP
6	Orange Ginger	Exotic	Orange	Unknown	NBRI, Lucknow, UP
7	Prabha	India	Red group	Unknown	NBRI, Lucknow, UP
8	Sylvia	Exotic	Orange	Comm Koehl × Moorish Cherry	NBRI, Lucknow, UP
9	Aldebaran	Exotic	Yellow group	Unknown	NBRI, Lucknow, UP
10	Tiger Flame	Unknown	Red	Unknown	NBRI, Lucknow, UP
11	Victor	Exotic	Pink	Unknown	NBRI, Lucknow, UP
12	Pusa Shagun	India	Red	White Oak × Oscar	NBRI, Lucknow, UP
13	Regency	India	Red group	Ruffled Ebony × Ace of spades	NBRI, Lucknow, UP
14	Snow Princes	Exotic	White	Unknown	DFR, New Delhi
15	Inter Pearl	Unknown	Red group	Unknown	NBRI, Lucknow, UP
16	Yellow Stone	Exotic	Yellow	Unknown	DFR, New Delhi
17	Limoncilla	Exotic	Yellow lemon colour	Unknown	DFR, New Delhi
18	Pricilla	Exotic	Purple Pink	Diamond × Leana	DFR, New Delhi
19	Novalux	Exotic	Yellow group	Unknown	DFR, New Delhi
20	Gold Field	Exotic	Yellow	Unknown	DFR, New Delhi
21	Ocilla	Exotic	Cream white	Unknown	DFR, New Delhi
22	Punjab Dawn	India	Pink/peach	Suchitra × Melody	DFR, New Delhi
23	Chandni	India	Greenish white	Green Woodpecker × White Butterfly (1997)	DFR, New Delhi
24	Arka Darshan	India	Red purple with white blotch	Watermelon Pink × Shirley	IIHR, Bangalore, Karnataka
25	Arka Naveen	India	Purple–Violet group	Hybrid 74-39-1 × Tropic Sea	IIHR, Bangalore, Karnataka
26	Arka Shobha	India	Light Pink	Induced mutant from cv. wild Rose	IIHR, Bangalore, Karnataka
27	Jester Gold	Exotic	Yellow	Jester sport	DFR, New Delhi
28	Flavour Souvenir	Exotic	Yellow	Unknown	DFR, New Delhi
29	Forta Rosa	Exotic	Soft Pink	Unknown	DFR, New Delhi
30	Prince Margret Rosa	Exotic	Orange	Unknown	DFR, New Delhi
31	Arka Sagar	India	Pink with red and yellow blotch	Melody × Wild Rose	IIHR, Bangalore, Karnataka
32	Arka Poonam	India	Dresden Yellow	Geliber Herald' × 'R.N. 121′	IIHR, Bangalore, Karnataka
33	Arka Tilak	India	Red	Watermelon Pink × Lady John	IIHR, Bangalore, Karnataka
34	Arka Kum Kum	India	Red	Watermelon Pink × Lady John	IIHR, Bangalore, Karnataka
35	Arka Keshar	India	Yellow –Orange	Vink's Glory × Sagar	IIHR, Bangalore, Karnataka
36	Arka Gold	India	Yellow with red blotch	Green Bay × Gold Medal-412	IIHR, Bangalore, Karnataka
37	Pusa Sukanya	India	White with scarlet ring in the lip	Salmon Queen seedling selection	IARI, New Delhi
38	Wind Song	Exotic	Purple	Unknown	IARI, New Delhi
39	Hunting Song	Exotic	Scarlet red	Unknown	IARI, New Delhi

Table 1 continued

S. no.	Name of variety	Origin	Flower colour	Parental cross or source	Collection centre
40	Friendship Sancere	Unknown	Snow white	Unknown	IARI, New Delhi
41	Shere Punjab	India	Pinkish orange	Suchitra × Melody	IARI, New Delhi
42	Arka Amar	India	Pink with White blotch	Watermelon Pink × Arka Aarti	IIHR, Bangalore, Karnataka
43	Pusa Suhagin	India	Florets ruby-red with barium yellow streak	Sylvia seedlings (1987)	IARI, New Delhi
44	Arun	India	Red	Sylvia × Fancy (1984)	IARI, New Delhi
45	Pusa Kiran	India	Florets are white with ray like red on throat	Ave open	IARI, New Delhi
46	Mohini	India	Floret colour red–purple	Ave × Christian Jane (2000)	IARI, New Delhi
47	Peter Pears	Exotic	Red–purple group	Salmoe × Maolete	IARI, New Delhi
48	Arka Aarti	India	Poppy-red with purple–red	Shirley × Melody	IARI, New Delhi
49	Legend Pink	Exotic	Pink	Unknown	IARI, New Delhi
50	Sancerre	Exotic	Snow White	Unknown	IARI, New Delhi
51	Novalux Yellow	India	Yellow group	unknown	IARI, New Delhi
52	American Beauty	Exotic	Pink/Peach	Unknown	IARI, New Delhi
53	White prosperity	Exotic	White	Unknown	IARI, New Delhi

Table 2 Details of the 17 ISSR primers used for diversity study on 53 gladiolus genotypes PIC (polymorphism information content), MI (marker index), RP (Resolving Power)

	Primer code	Sequence (5'–3')	PIC	RP	MI	No. of alleles	Polymorphic alleles	Monomorphic alleles	Polymorphism percentage
1	22,203	GAGAGAGAGAGAGAGAC	0.82	3.73	4.92	6	6	0	100
2	22,204	GAGAGAGAGAGAGAGAA	0.59	6.37	2.98	6	5	1	83.33
3	22,207	CTCTCTCTCTCTCTCTG	0.65	3.99	2.60	4	4	0	100
4	22,209	CACACACACACACACAA	0.81	2.90	3.27	4	4	0	100
5	22,215	TCTCTCTCTCTCTCTCC	0.55	6.45	2.79	5	5	0	100
6	22,218	ACACACACACACACACC	0.89	4.48	7.14	8	8	0	100
7	22,219	ACACACACACACACACG	0.84	4.67	7.56	9	9	0	100
8	834	AGAGAGAGAGAGAGAG(CT)T	0.78	5.20	5.52	7	7	0	100
9	836	AGAGAGAGAGAGAGAG(CT)A	0.96	1.92	5.78	6	6	0	100
10	840	GAGAGAGAGAGAGAGA(CT)T	0.79	2.33	3.16	4	4	0	100
11	851	GTGTGTGTGTGTGTGT(CT)G	0.92	4.45	9.26	10	10	0	100
12	855	ACACACACACACACAC(CT)T	0.99	0.22	3.99	4	4	0	100
13	856	ACACACACACACACAC(CT)A	0.99	0.41	3.98	4	4	0	100
14	857	ACACACACACACACAC(CT)G	0.94	3.58	8.51	9	9	0	100
15	861	ACCACCACCACCACCACC	0.42	11.58	3.37	8	8	0	100
16	866	CTCCTCCTCCTCCTCCTC	0.87	2.26	3.48	4	4	0	100
17	889	AGT)(CGT)(AGT)ACACACACACACAC	0.99	0.03	0.99	1	1	0	100
Average			0.812	3.80	4.66	5.82	5.76	–	99.02

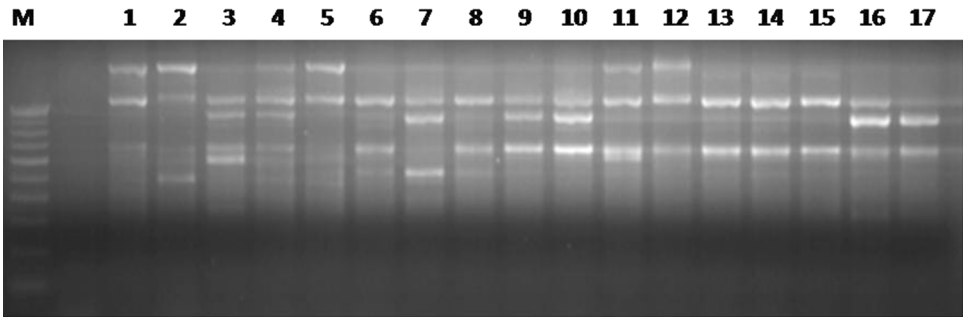

Fig. 1 Representative gel image depicting PCR amplification of 17 gladiolus genotypes (*1* Punjab Flame, *2* Punjab Pink, *3* Punjab Glad-1, *4* Pacifica, *5* Orange Ginger, *6* Prabha, *7* Sylvia, *8* Aldebaran, *9* Tiger Flame, *10* Victor, *11* Pusa Shagun, *12* Regency, *13* Snow Princes, *14* Inter Pearl, *15* Yellow Stone, *16* Limoncilla and *17* Pricilla) with primer ISSR 834. M represents 100 bp ladder

primer gave highest number of alleles (10 alleles) followed by, ISSR 22,219 and ISSR 857 (9 alleles each) and ISSR primers 889 gave minimum number (1 alleles) (Table 2). The PIC value ranged from 0.42 (ISSR 861) to 0.99 (ISSR 855, ISSR 856 and ISSR889) with an average of 0.812, marker index ranged from 0.99 (ISSR 889) to 9.26 (ISSR, 851) with an average 4.66 and resolving power of the primers ranged from 0.03 (ISSR 889) to 11.58 (ISSR 861) with an average value of 3.80. Prevost and Wilkinson (1999) described the parameter resolving power (RP) as a measure of the discriminatory power of ISSR molecular markers. The values of resolving power in present study ranged from 0.03 to 11.58. Genetic similarities (GS) were calculated using the Nei–Li similarity co-efficient. Significant genetic variation was found among all the gladiolus genotypes with the GS value ranging from 0.11 to 1.00. Of the 53 pair wise combinations, the highest genetic similarity of 0.82 was found between the genotype Novalux Yellow and American Beauty, followed by 0.80 between genotype Shagun and Regency and minimum genetic similarity was observed between Punjab Glance to White Prosperity.

Cluster analysis and relationship among the genotypes

The UPGMA based clustering as depicted by dendrogram (Fig. 2) showed that all the 53 gladiolus genotypes were grouped into two major groups (Group I to II) at the coefficient of GS = 0.14 (Fig. 2). the group II contains maximum number of 52 genotypes which can be further divided into two subgroups, namely, IIa and IIb. The group I and IIb comprise of a single genotype each namely "Arka Shobha" and "White Prosperity" respectively. The sub group IIa1 contain single genotype namely "Punjab Glance" whose parents were "Happy End" and "Yellow Stone" (Table 1). The sub group IIa2 consist of 50 genotypes. Among these, five genotypes namely "Punjab Glance", "Pusa Kiran", "Arka Amar", "Punjab Pink" and "Punjab Flame" were found to be the most distinct and present separately from other clustered genotypes.

Genetic diversity estimates

Among genetic diversity estimates, Na (number of different alleles) varied 0.929–1.717 with an average of 1.39, Ne (effective number of alleles) varied from 1.262 to 1.369 with an average of 1.24, similarly I (Shannon's information index) ranged from 0.251 to 0.359 with an average 0.25 and He (Nei 1973) gene diversity was in the range from 0.167 to 0.229 with an average 0.15 (Table 3).

Population STRUCTURE of gladiolus populations

Model based STRUCTURE' program was used to study the underlying population structure among the 53 gladiolus genotypes. The highest value ΔK was for K = 3, so the value of K = 3 was chosen for the final analysis of population structure (Fig. 3). Therefore, all 53 gladiolus genotypes were assigned to three clusters at $K = 3$ by their inferred genome fraction value. A genotype was assigned to a group if more than 75% of its genome fraction value is derived from that group (Fig. 4). Genotypes with membership probabilities < 0.75 were assigned to an admixed group. Out of 53 genotypes, 21 were assigned to three groups, and 32 genotypes were retained in the admixed clusters. Among three groups, red group contains 10 genotypes, green group comprised of seven (7) genotypes and finally blue group comprised of four (4) genotypes.

Discussion

Molecular markers provide an effective tool to study the association and relationship among different genotypes. Several properties especially non responsive towards environmental, pleiotropic and epistatic effects make them highly advantageous and useful over traditional

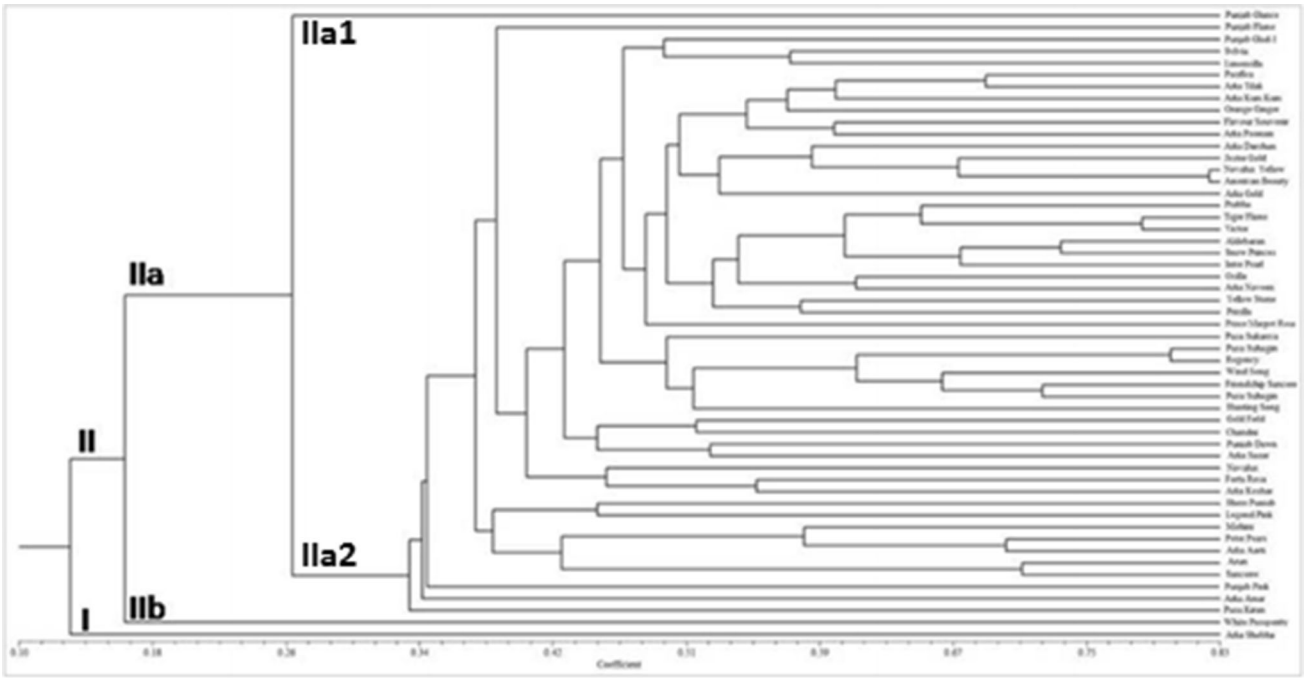

Fig. 2 Dendrogram of 53 gladilous genotypes generated by UPGMA clustering using Jaccards similarly matrix obtained from ISSR markers

Table 3 Different genetic diversity estimates for six populations (based on collection site) of gladiolus based on 99 loci

Population (sample size)	Na	Ne	I	He
Pop1 (4)	1.162 ± 0.095	1.262 ± 0.031	0.260 ± 0.026	0.167 ± 0.017
Pop2 (10)	1.343 ± 0.088	1.333 ± 0.038	0.295 ± 0.028	0.195 ± 0.020
Pop3 (13)	1.485 ± 0.084	1.326 ± 0.034	0.311 ± 0.026	0.200 ± 0.018
Pop4 (10)	1.717 ± 0.067	1.369 ± 0.034	0.359 ± 0.023	0.229 ± 0.017
Pop5 (12)	1.253 ± 0.097	1.319 ± 0.038	0.359 ± 0.028	0.186 ± 0.020
Pop6 (4)	0.929 ± 0.099	1.292 ± 0.037	0.251 ± 0.029	0.170 ± 0.020
Mean	1.39 ± 0.015	1.24 ± 0.005	0.25 ± 0.004	0.15 ± 0.003

Na number of different alleles, *Ne* effective number of alleles, *I* Shannon's information index *He* Nei's (1973) gene diversity

morphological or phenotypic markers (Mondini et al. 2009). Polymorphic genetic markers have wide potential applications in plant improvement programmes as a means for varietal and parentage identification, evaluation of polymorphic genetic loci affecting quantitative economic traits and genetic mapping. In the present study, authors selected ISSR markers for diversity evaluation because of their easy application and results interpretation. These markers are highly polymorphic and have been successfully used in many bulbous crops (Jingang et al. 2008; Kiani et al. 2012; Kameswari et al. 2014). In earlier studies, di-nucleotide based ISSR primers have been used in genotyping of some important boulbous flowering plant with high reproducibility and sufficient polymorphism (Kameswari et al. 2014). Rp values of markers suggested that the set of ISSR primers used were capable of distinguishing different gladiolus genotypes. Results observed in present study suggested the use of MI and PIC parameters to compare the information content of polymorphic ISSR and the use of Rp to select the most informative ISSR marker for best selection among different genotypes.

In the present study ISSR profile was used for diversity analysis and relationship among different gladiolus genotypes. The ISSR polymorphism obtained in the present study is 99% which is more than that reported by Jingang et al. (2008) who found 93% polymorphism using same ISSR markers in different gladiolus collection. Results showed that primers namely ISSR 851, ISSR 22,219, ISSR 857, ISSR 861 and ISSR 22,218 that have above average PIC, MI and Rp, were to be more efficient. Previously researchers have compared the genetic diversity in other floricultural bulbous crops, with pedigree information (Patra et al. 2008; Cui et al. 2014; Anderson et al. 2010). In the present study, genotype Arka Shobha, belonging to

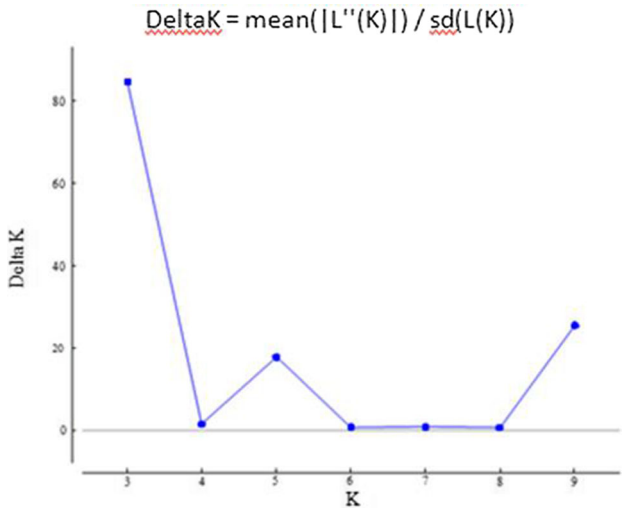

Fig. 3 Highest ΔK value for the gladiolus genotype at $K = 3$

group I, is an induced mutant cultivar from wild rose and found to be most distinct genotype. Pedigree of White Prosperity from group II is unknown. Pusa Kiran had been developed from Ave open with white colored florets, Arka Amar is a cross of Water melon × Arka Aarti. Punjab Pink and Punjab Flame shared common parent i.e. White Prosperity. Punjab Pink has been developed by hybridizing Suchitra and White Prosperity while Punjab Flame is the progeny from Sylvia × White Prosperity cross. Clustering of some genotypes could be proved by their parental relationship. Two closely clustered genotypes namely "Punjab Down" and "Arka Sagar" share common parent i.e. genotype "Melody". Punjab Down is a cross between Suchitra and Melody, while Arka Sagar is a cross between Melody and Wild Rose. Another genotype named "Arka Aarti" although shared common parent i.e. Melody but found to be far present in different subcluster. Likewise, Arka Tilak, Arka Kumkum and Arka Darshan shared common parent i.e. Watermelon Pink. These three genotypes are present close to each other. Arka Tilak and Arka Kumkum both originated from same parental cross i.e. Watermelon Pink x Lady John, while Arka Darshan originated from a cross between Watermelon Pink x Shirley. Likewise genotype namely "Pusa Suhagin" is present near to genotype named "Sylvia". Pusa Suhagin is found to have been originated from Sylvia seedlings (Table 1). It has also been observed from the present study that majority of the exotic genotypes like Sylvia, Aldebaran, Limoncilla, Pricilla, Snow Princes, Ocilla, Yellow Stone and Prince Margret Rosa were found to be clustered together. From clustering pattern, it seems that up to some extent parental-progeny relationship exists among the genotypes and some related genotypes clustered together. The results were consistent with Pragya et al. (2010) and Ranjan et al. (2010) who had also observed the parental relationship among the gladiolus genotypes by using RAPD and AFLP markers.

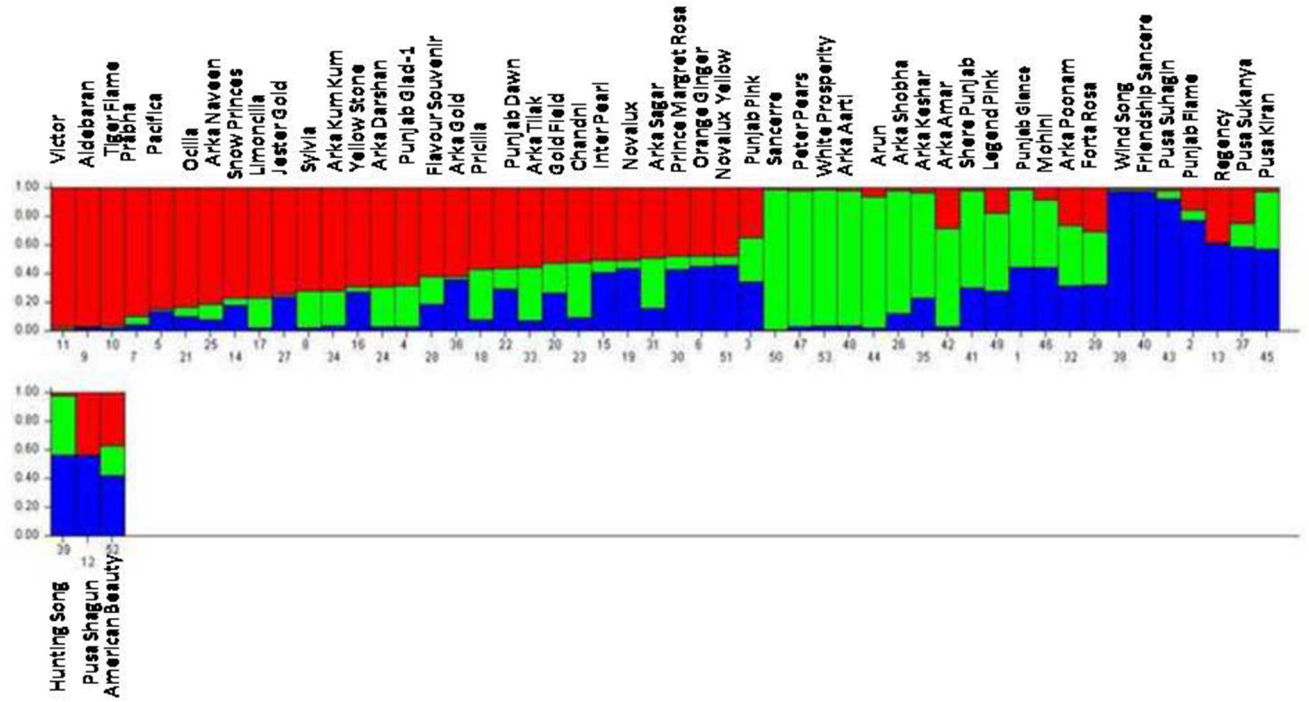

Fig. 4 STRUCTURE plot of 53 gladiolus genotypes with K = 3. The different colour bars referred to three different genetic groups/pools respectively (color figure online)

STRUCTURE results showed the presence of mixed populations among three clusters with majority of the mixtures in cluster A. The mixed population could be attributed to various reasons like breeding/domestication history, high level of heterozygosity etc. It is believed that the cultivated gladiolus, *Gladiolus grandiflorus* now known as *G. hybridus* Hort. developed from a number of wild species viz *G. Papilioentus, G. Netalensis, G. Opposityflorus, G. Papilio and G. Saundersii* (Banard 1972; Imanishi 1989). Garden gladiolus varieties of today has emerged from diverse genetic parentage that are heteroploids ranging from 2n = 30 to 180 (Bhajantri and Patil 2013). Nowadays, most of the gladiolus genotypes are developed from inter-specific hybridization among several species. Thus modern genotypes are the results of complex inter specific crosses. They are heterozygous and tetraploid and the knowledge of the hereditary transmission of numerous characteristics is poor (Cantor and Chis 2009). Results of population structure analysis and UPGMA clustering partially corroborates each other. Not all but many genotypes were assigned to same cluster by population structure analysis and Jaccard's similarity coefficients based dendrogram (Figs. 2, 4). For example genotypes namely Sancerre, White Prosperity, Arka Aarti, Peter Pears, Arun, Arka Amar, Arka Shobha etc. assigned to same cluster by population structure analysis and UPGMA clustering.

Implication for future gladiolus improvement programme

Improvement of floricultural crop like gladiolus depends on the evaluation and characterization of genetically diverse plant material. The genetic relationship among genotypes, observed in the present study could be useful for designing particular breeding programme specially selection of parents. Clustered presence of exotic genotypes in the present study indicates that these genotypes harbor different genetic components that could be useful for the improvement of indigenous and development of novel genotypes. Genotypes documented in the present study can be useful for breeders to broaden the genetic base. Genotypes namely Arka Shobha, Punjab Glance, White Prosperity, Pusa Kiran etc. could be used as parents in a particular breeding depending on their interesting desirable economic traits and hybridization potential.

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References

Anderson NO, Younis A, Sun Y (2010) Inter simple sequence repeats distinguish genetic differences in easter lily 'Nellie White' clonal ramets within and among bulb growers over years. J Am Soc Hortic Sci 135(5):445–455

Banard TT (1972) On hybrid and hybridization. In: Lewis GJ, Obermeyer AA, Barnard TT (eds) Gladiolus a revision of the south african species. J of South Afric Bot, Supplement, 10: 304–310

Bhajantri A, Patil VS (2013) Studies on ethyl methane sulphonate (EMS) induced mutations for enhancing variability of gladiolus varieties (*Gladiolus hybridus* Hort.) in M1V2 generation. Karnataka J Agric Sci 26:403–407

Bornet B, Branchard M (2001) Non-anchored inter simple sequence repeat (ISSR) markers reproducible and specific tool for genome fingerprinting. Plant Mol Biol Rep 22:427–432

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Cantor M, Chis LM (2009) New gladiolus cultivars homologated at USAMV Cluj-Napoca. Bull UASVM Hortic 66:504–509

Cui GF, Wu LF, Wang XN, Jia WJ, Duan Q, Ma LL, Jiang XL, Wang JH (2014) Analysis of genetic relationships and identification of lily cultivars based on inter-simple sequence repeat markers. Genet Mol Res 13:5778–5786

Duncan G (1996) Growing South African bulbous plants, National Botanical Institute. Cape Town, S. Africa. ISBN 1-874907-15-3

Duncan G (2000) Grow bulbs: a guide to the species cultivation and propagation of South African Bulbs, National Botanical Institute, Kirstenbosch Gardening Series. Claremont, S. Africa. ISBN 0-900048-53-0

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Flower Council of Holland (2008) Facts and figures. http://www.flowercouncil.org/us/marketinformation/. Accessed 14 June 2009

Goldblatt P (1996) Gladiolus in tropical Africa: systematics, biology & evoluation. Timber Press, Portland, Oregon. ISBN 10: 0-8819-233-38/0-88192-333-8

Goldblatt P, Manning J (1998) Gladiolus in Southern Africa: timber press. Portland. ISBN 10: 1874950326

Goldblatt P, Takei M, Razzaq ZA (1993) Chromosome cytology in tropical African gladiolus (Iridaceae). Ann Mo Bot Gard 80:461–470

Imanishi H (1989) Gladiolus. In: Mastsuo T (ed) Collected data of plant genetic resources. Kodansya. Scientific, Tokyo, pp 1077–1080

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull Soc Vaud Nat 44:223–270

Jingang W, Ying G, Daidi C, Shenkui L, Chuanpin Y (2008) ISSR analysis of 26 general species of *Gladiolus hybridus* Hort J Northeast Agric Univ 15(4):6–10

Kameswari PL, Girwani A, Radh-Rani K (2014) Genetic diversity in tuberose (*Polianthes tuberosa* L.) using morphological and ISSR markers. Electron J Plant Breed 5(1):52–57

Kiani M, Memariani F, Zarghami H (2012) Molecular analysis of species of Tulipa L. from Iran based on ISSR marker. Plant Syst Evol 298:1515–1522

Kumar V, Sharma S, Sharma AK, Sharma S, Bhat KV (2009) Comparative analysis of diversity based on morpho-agronomic traits and microsatellite markers in common bean. Euphytica 170:249–262

Lepcha B, Nautiyal MC, Rao VK (2007) Variability studies in gladiolus under mid hill conditions of Uttrakhand. J Ornam Hortic 10(3):169–172

Manning J, Goldblatt P, Snijman D (2002) The color encyclopedia of Cape Bulbs, Timber Press. Portland. ISBN 9780881925470

Milbourne D, Meyer R, Bradshaw JE, Baird E, Bonar N, Provan J, Powell W, Waugh R (1997) Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. Mol Breed 3(2):127–136

Mondini L, Noorani A, Pagnotta MA (2009) Assessing plant genetic diversity by molecular tools. Diversity 1:19–35

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci 70:3321–3323

Patra B, Acharya L, Mukherjee AK, Panda MK, Panda MC (2008) Molecular characterization of ten cultivars of Canna lilies (*Canna* Linn.) using PCR based molecular markers (RAPDs and ISSRs). Int J Integr Biol 2:129–137

Pragya Bhat KV, Misra RL, Ranjan JK (2010) Analysis of diversity and relationships among gladiolus cultivars using morphological and RAPD markers. Indian J Agric Sci 80(90):766–772

Prevost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR finger printing of potato cultivars. Theor Appl Genet 98:107–112

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Ranjan P, Bhat KV, Mishra RL, Singh SK, Ranjan JK (2010) Genetic relationship of gladiolus cultivars inferred from fluorescence based AFLP markers. Sci Hortic 123:562–567

Rathod DM, Chawla SL, Ahur TR, Patel MA (2011) Effect of planting time and chamicals on growth, flowering and yield of gladiolus (*Gladiolus grandiflorus*) cv. American beauty. J Ornam Hortic 14(1&2):24–27

Rohlf FJ (1993) NTSYS-PC: numerical taxonomy and multivariate analysis system. Version 2.02e, Exeter Software, Setauket, New York

Shufang G, Huijuin FU, Jingang W (2010) ISSR analysis of M1 generation of *Gladiolus hybridus* Hort. treated by EMS. J Northeast Agric Univ 17(2):22–26

Swaroop K, Janakiram T (2010) Divergence studies in gladiolus. Indian J Hortic 67:352–355