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ORIGINAL ARTICLE

Analysis of Molecular Diversity in *Gerbera* Genotypes Revealed By RAPD Markers

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ABSTRACT
Random amplified polymorphic DNA (RAPD) markers were used to study the genetic diversity among seven genotypes of the genus Gerbera an important ornamental plant. Out of 20 RAPD decamers tested, only 13 amplified genomic DNA across all the 7 genotyes of Gerbera. A total of 52 bands were scored in all the genotypes. Our results suggested that genetic diversity in Gerbera genotypes using RAPD banding data may be useful for plant improvement and an efficient way to conserve genetic resources of Gerbera in addition to their ornamental and medicinal uses.
Key words: Gerbera , Molecular diversity, RAPD, PCR based DNA markers.

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INTRODUCTION

The Barberton/Transvaal daisy, or *Gerbera* is a flower with increasing commercial significance. *Gerbera* is one of the leading cut flowers and ranks among the top ten cut flowers of the world [1] [3]. It has wide applicability in the floral industry as cut flower and potted plant. The species, a perennial herb, is native to South Africa and Asia. It is an important commercial flower grown through the world in a wide range of climatic conditions. The genus consists of about forty species [2]. Out of the recorded species, only one species *Gerbera jamesonii* is under cultivation. Few important cultivators of *Gerbera* are *Cream Clementine, Maron Clementine, Delphi, Vesta, Uranus, Terra Queen, Dusty, Valantine, Diablo, mariso* and *Pascal.* Based on the flower heads, they may be grouped into single semi- double and double cultivars [4].
Like any ornamental plant *Gerberas* are produced exclusively for their aesthetic values. *Gerbera* is a member of the large sunflower family Asteraceae, as such, *Gerbera* offers great potential for comparative developmental research within a single genotype. Moreover, different *Gerbera* varieties show an impressive spectrum of colour patterns, directly displaying responses to developmental stages at all important morphological levels (flower type, flower organ and within organs). Further, *Gerbera* harbors an arsenal of Asteraceae type secondary metabolites, not present in other model plants. With powerful reverse genetics methods, a large collection of sequences and a new cDNA microarray, *Gerbera* has become a model plant of the sunflower family. *Gerbera* is one of the top selling cut flowers and pot plants in the world. *Gerbera viridifolia* is one of the species is referred to in the eastern cape as 'Iyeza lamazi' or the herb of milk and is probably used to stimulate milk production in cattle or as an ingredient of maas (the locally produced soured milk) [5],
[6]. The smoke of *G.viridifolia* is inhaled by the southern poeple to treat colds. Pounded leaf infusions of *G. ambigua* are used in zulu traditional medicine for tapeworm and stomach ache, whereas root infusions are taken orally for coughs. In Zimbabwe, root infusions are used for heart pain and abdominal pain in babies.
Present research work on analysis of genetic diversity of *Gerbera* genotypes using PCR based DNA markers will pave the way to understand economically important genus *Gerbera* and to elucidate the genetic variation in *Gerbera* genotypes.

MATERIAL AND METHODS

Plant material

The seedlings of 7 *Gerbera* genotypes i,e *Rosalin*, *Savannah*, *Winter queen*, *Goliath*, *Dana ellen, Dalma*, and *Sunway* were purchased from germlasm collections centre Pune. The collected fresh leaves were powdered in liquid nitrogen and stored in deep freezer
(-70^{0C}) till further processing.

DNA isolation

Genomic DNA was extracted from young leaves following the modified CTAB method [7].Quantification and purity of isolated DNA was checked through uv-spectrophotometer and agarose gel electrophoresis.

PCR amplification

A set of 40 random decamer primers (Operon Technologies, Almeda, California, USA) were used to amplify the genomic DNA [8]. PCR reactions were conducted in a 20µl reaction mixture consisting of 100ng. geonomic DNA, 5pM / µl RAPD Primer, 10µM dNTPs, 0.5µl Taq. DNA polymerase (3U/ µl) (Bangalore Genei, Bangalore, India), 10 X PCR buffer and 1.5mM $MgCl_2$. DNA amplification was carried out using a Gradient palm Cycler (Corbett Research, Australia). Amplifications were carried out in a Gradient Palm Cycler with initial denaturation for 4 minutes at 94^{0}C and each cycle with 15 seconds at 4^{o}C, 15 seconds primer annealing at 35^{0}C, 1.15 minutes for extension at 72^{0}C. The reaction was continued for 40 cycles followed by 7 minutes at 72^{0}C to ensure the completeness of the primer extension. The PCR products were analyzed on 1.5 % (w/v) agarose gels in 1X TBE buffer under constant voltage of 100 v for 3 to 4 hours. Experiments were repeated several times to ensure the reproducibility of results and the best gels of the replicates were used for band scoring. The gels were stained with ethidium bromide solution and documented in a gel documentation system (Fig.1). A λ DNA, 500bp ladder (Banglore Genei, India) was used as molecular size marker.

Data Analysis

Polymorphic RAPD amplified product was considered to be a unit character and the populations were manually scored as binary data with presence of a band as "1" and absence as "0". Only clearly distinguishable DNA bands were used in the genetic analysis. The molecular size of the amplification products were calculated from a standard curve based on the known size of the DNA fragments of the ladder. Estimates of genetic similarity were calculated between all pairs of the species according to Jaccard's Similarity Index (1908) [9]. The matrix obtained was used to evaluate the genetic diversity among the genotypes of *Gerbera* with cluster analysis using an Unweighed Pair Group Method with Arithmetic Averages (UPGMA) [10]. All the statistical analysis were performed with an aid of NTSYS-PC computer program version 2.0 [11].The similarity matrix was calculated based on Jaccard's similarity coefficient.

In the present investigation, 7 genotypes of the genus *Gerbera* were used to assess interspecific genetic polymorphism. Out of 20 random decamer primers (operon) initially tested, only 13 primers were finally selected on the basis of robustness of the amplification, clarity and scorability of banding patterns, for genetic diversity analysis. These primers differed greatly in their efficiency for revealing polymorphism (Table.2). The estimates of pairwise genetic distance between the eight species of *Gerbera* is given in Table.3.

In all, 52 reproducible and scorable DNA fragments were amplified, 72 % of which were polymorphic and consistently generated from 13 primers across *Gerbera genotypes*. Primer OPB-8 yielded highest number of bands (15) while OPB-7 and OPB-13 amplified the lowest number of bands (2) with an average of 3.8 bands per primer (Table 2). The representative RAPD profiles of 7 genotypes of *Gerbera* are shown in (Fig 1). The approximate size of the largest fragment was 2.5kb, where as the smallest recognizable fragment was 0.5kb in size.

Seven accessions of *Gerbera* (Table 1) revealed 72% of polymorphism with 13 RAPD primers. A total of 52 bands were scored of which 37 bands are polymorphic. The number of bands ranging from 2 to 15 corresponding to an average of 3.8 bands per primer (Table 2). Jaccard's similarity matrix reveals that, the levels of genetic similarity between accessions ranged from 0.1 to 0.75 (Table 3). The maximum number of polymorphic bands (15) were yielded with primer OPB-8 and minimum (2 bands) from OPB-13 (Table 2). Dendrogram (Figure 2) reveals the formation of 2 major genetic groups among 7 accessions studied based on the genetic similarity index (Table 3), even though morphologically they were similar and inseparable. The first group is again classified into two sub groups, where the first sub group includes one accession *Sunway* while the second subgroup includes two subclusters, first subcluster includes three accessions *Savannah*, *Dana ellen* and *Rosalin*. The second subcluster includes two accessions *Winter queen* and *Goliath*. Maximum genetic similarity is between accessions *Winter queen* and *Goliath* (0.75). The second major group includes single accession *Dalma* . The denrogram (Fig 2) based on similarity

index (SI) (Table 2) showed distinct separation of the collected accessions though morphologically they were similar and inseparable. The dendrogram separated the accessions into two major groups, having 72% similarity.

RESULTS AND DISCUSSION

RAPD markers represent an efficient and inexpensive tool to generate molecular data and thus, have been used successfully in various taxonomic and phylogenetic studies,[15],[12],. High level of genetic similarity is expected among *Gerbera* genotypes due to similar geographical conditions. But, in contrast, they showed broad genetic base indicating earlier introduction of the species, and subsequently leading to accumulation of variation. The genetic differentiation of accessions of *Gerbera* genotypes could broadly be explained as a result of abiotic (geographical for example, hydrographic connections or climatic differentiation) and biotic (pollination between populations and seed dispersal) factors. The percentage polymorphism (72%) among *Gerbera genotypes* is comparatively much higher than other plants, for example, *Cathaya argyrophylla* (32%) [16],*Paeonia rockii* (33.3%) [17], a vulnerable medicinal plant *Oroxylum indicum* (49.61%)[15], *Costus speciosus* (35%) [18], this clearly indicates high level of genetic diversity within *Gerbera* genotypes. High level of genetic diversity in populations of *Gerbera* genotypes may be due to cross pollination by insects, dispersal of seeds, habitat changes. There is a close genetic similarity of 75% observed between accessions *Winter queen* and *Goliath* which clearly depict that, genetically the similarity may be because of the similar environmental conditions. In essence, the RAPD method used in this study displayed appreciable intra-population variation or molecular polymorphism, which is pre-existed in different collections. In spite of their morphological similarity, substantial polymorphism was observed among the accessions under study.The study revealed that, though the decamer primers are small in comparison to the large genome of *Gerbera jamsonii*, they produced appreciable amplicons sufficient to demarcate all accessions. The dendrogram also established genetic relatedness among different accessions and quantum of changes that occurred in the genome in the course of evolution. This study confirms the suitability of RAPD as a reliable, simple, easy to handle and elegant tool in molecular diagnosis of different accessions of *Gerbera jamsonii*. Currently, it is also proved that, the entries that were found to be similar in taxonomical classification based on morphological characteristics do have divergence at DNA level. Vast genetic variation is an indicative of the evolving nature of the taxa.

Table 1: Accession number and different genotypes of *Gerbera jamsoni* used for RAPD analysis

Sl.No.	Acc. No.	Genotypes of *Gerbera*
01	G.1	*Rosalin*
02	G.2	*Savannah,*
03	G.3	*Winter queen*
04	G.4	*Goliath,*
05	G.5	*Dana ellen*
06	G.6	*Dalma,*
07	G.7	*Sunway*

Table 2: Details of randomly selected decamer oligonucleotides

Primers	Nucleotide Sequence 5′ → 3′	No. of amplified bands	No. of polymorphic bands
OPA-02	**TGCCGAGCTG**	**5**	**3**
OPC-06	**GAACGGACTC**	**6**	**3**
OPD-02	**GGACCCAACC**	**6**	**4**
OPA-12	**TCGGCGATAG**	**-**	**-**
OPB-07	**GGTGACGCAG**	**5**	**2**
OPB-08	**GTCCACACGC**	**15**	**12**
OPB-10	**CTGCTGGGAC**	**-**	**-**
OPB-11	**GTAGACCCGT**	**7**	**6**
OPB-13	**TTCCCCCGCT**	**3**	**2**
OPB-16	**TTTGCCCGGA**	**-**	**-**
OPB-17	**AGGGAACGAG**	**-**	**-**
OPB-19	**ACCCCCGAAG**	**-**	**-**
OPB-20	**GGACCCTTAC**	**5**	**5**
	Total:	**52**	**37**

Table 3: Similarity matrix based on the Jaccard's Similarity Index

	1	2	3	4	5	6	7
1	1.000						
2	0.663	1.000					
3	0.538	0.357	1.000				
4	0.703	0.554	0.752	1.000			
5	0.578	0.653	0.271	0.470	1.000		
6	0.423	0.140	0.333	0.201	0.100	1.000	
7	0.535	0.491	0.423	0.594	0.491	0.167	1.000

Fig.1. *Gerbera* genotypes , A. *Dana ellen* and *B. Dalma*

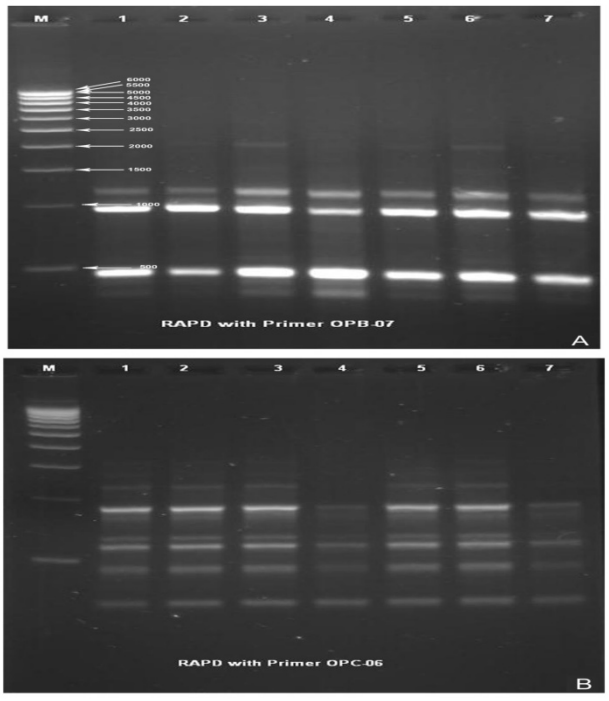

Fig. 2. RAPD profiles of 7 *Gerbera* genotypes generated by primer (A) OPC-6 and (B) OPB- 7. M- indicates the DNA molecular size marker (500 bp ladder). Lane numbers corresponds to the species serial number given in Table 1.

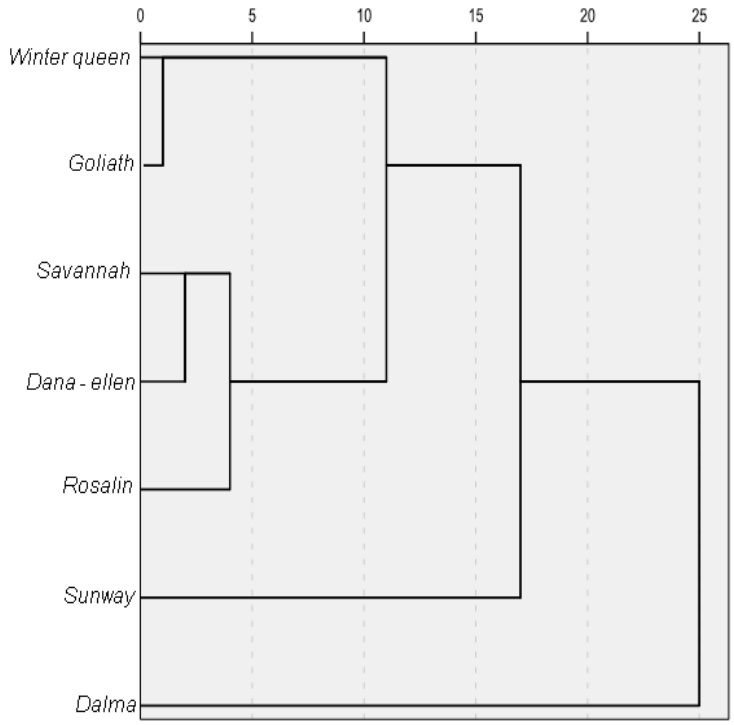

Fig.3. Dendrogram showing the genetic relationship among 7 *Gerbera* genotypes generated by RAPD data using the UPGMA method.

CONCLUSION

The study was undertaken to evaluate the extent and range of genetic diversity available in *Gerbera* genotypes. Accurate estimates of diversity are a prerequisite for optimizing sampling strategies and for conserving the genetic resources. The high diversity revealed by RAPD is in agreement with the conclusion that, out breeding plants retains considerable variability [19],[13],[14].Variation within the species suggests that, this species has large effective population size or large mutation rate due to longer generation.

As most of the *Gerbera* genotypes are used as an ornamental plant, the presence of a unique RAPD marker among the various*Gerbera* genotypes indicate the utility of the approach for DNA fingerprinting purposes. RAPD fingerprinting has a number of potential applications and include the determination of cultivar purity, efficient use and management of genetic resources particularly the establishment of property rights (plant variety protection and patenting). This study represents only the first step in using RAPD markers as a tool to develop species specific SCAR DNA markers for molecular analysis of individual *Gerbera* genotypes.

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