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Evaluation of genetic diversity in different genotypes of *Gerbera jamesonii* Bolus using random amplified polymorphic DNA (RAPD) markers

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Genetic diversity within *Gerbera jamesonii* Bolus is the key to genetic improvement of this important ornamental species. In the present study, genetic diversity of 12 accessions of gerbera was assessed through random amplified polymorphic DNA (RAPD) markers. A total of 40 RAPD primers belonging to OPD, OPE, OPF and OPG series of universal primers set were used out of which, only 10 primers produced clear, reproducible and scorable bands. Ten (10) decamer RAPD primers produced a total of 49 scorable bands from 12 genotypes of *G. jamesonii* Bolus, out of which 42 were polymorphic and seven were monomorphic. The percentage of polymorphism ranged from a maximum of 100.00% by OPE-02, OPE-14, OPF-18, OPG-18, OPG-16 and OPG-17 to a minimum of 50.00% by OPE-08. The RAPD profiles generated were further evaluated for studying the Jaccard's similarity coefficient. The average genetic similarity coefficient for the 12 accessions evaluated by Jaccard index was 0.66 ranging from 0.35 to 0.86. The RAPD amplification data were used to obtain similarity matrix and for generation of dendrogram using unweighted pair group method with arithmetic averages (UPGMA) method. Based on the dendrogram, all 12 genotypes could be distinctly divided into two clusters and the accession CF Orange was found most dissimilar from other accessions. This study shows that DNA based molecular marker RAPD is a powerful, less time consuming and cost effective molecular technique for assessment of genetic diversity among different genotypes of *G. jamesonii* Bolus. The availability of these gerbera RAPD markers would facilitate the use of molecular markers in gerbera breeding and genetic studies.

Key words: *Gerbera jamesonii* Bolus, molecular markers, genetic diversity, RAPD.

INTRODUCTION

Cultivated gerbera (*Gerbera jamesonii* Bolus) is one of the most important cut and pot flowers worldwide, ranking fifth, only after rose, carnation, chrysanthemum, and tulip, in the global cut flower trade (Bhatia et al., 2009; Teeri et al., 2006). It is valued for its unique and attractive flower forms and bright colours. Pioneered in the late ninetieth century in England, gerbera breeding has been very active in Netherlands, Denmark, Germany, United States, Israel and Japan (Kloos et al., 2005; Rogers and Tjia, 1990). Numerous cultivars have been released as cut

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Abbreviations: RAPD, Random amplified polymorphic DNA; **UPGMA,** unweighted pair group method with arithmetic averages.

flowers, pot flowers, or garden plants. Remarkably, no less than 1,150 gerbera cut flower cultivars were available from the Dutch flower auctions alone (Anonymous, 2001). Although different in important horticultural traits (e.g. flowering time, bloom height, count and longevity), many cultivars show only minor differences in leaf and flower morphology and are difficult to identify correctly based on morphology. This difficulty also exists in distinctness, uniformity and stability (DUS) tests of new gerbera cultivars for registration.

Traditionally, breeding of gerbera was based on hybridization among cultivars and phenotypic selection of novel or improved progeny, followed by clonal propagation of released cultivars. Increasingly, gerbera breeding is shifted toward seed propagated F1 hybrids for better uniformity and lower production costs (Rogers and Tjia, 1990), and incorporation of disease resistance and stress tolerance into new cultivars for better performance and sustainability (Deng and Harbaugh, 2008; Kloos et al., 2005). Molecular markers are being sought as a powerful tool to assist gerbera breeders' pursuit of these new breeding goals. Molecular markers have become an indispensable tool for breeding and cultivar development in many crops (Varshney et al., 2005). They are frequently required for correct identification of cultivars, accurate assessment of genetic relationships and diversity, efficient tagging and mapping of desirable genes, and early selection of superior genotypes. Apart from marker systems such as restriction fragment length polymorphism (RFLP), mini- and micro-satellites, RAPDs have proved to be very useful for the analysis of large numbers of genotypes (Debener et al., 1996).

The main advantage of random amplified polymorphic DNA (RAPD) markers over other molecular markers, in particular to markers involving DNA-DNA hybridization techniques, is the low technical input and small quantity of DNA needed for the analysis. The present study has been planned to evaluate genetic diversity among 12 accessions of cultivated gerbera genotypes using DNA based molecular marker RAPD.

MATERIALS AND METHODS

Plant material

The present investigation was conducted at Department of Plant Molecular Biology and Biotechnology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India during 2011-12. Twelve (12) genotypes of tissue cultured plantlets viz., Stanza, Fana, CF Gold, Diego, Cherany, CF Orange, Lion, Venezia, Torbin, Jaffana, Kento and Ice Queen, were planted in 2009, two years before the commencement of the present study at greenhouse complex of ACHF, NAU, Navsari. Eight week old plantlets of these 12 genotypes were procured from Germini Agro Pvt. Ltd., Pune, Maharashtra, India.

DNA isolation and RAPD analysis

The genomic DNA was extracted from the young leaves following the cetyldimethyl ammonium bromide (CTAB) method of (Keim et al., 1988) with some modifications. Fresh 10 days old leaves (1 g) from each genotype were powdered in liquid nitrogen using pre-cooled pestle and mortar. The resulting powder was transferred to a 30 ml test tube and extracted for 45 min at 65°C with 5 ml of pre-warmed (65°C) extraction buffer. Equal volume of chloroform: isoamyl alcohol (24:1) was added and transferred in new centrifuge tube. The mixture was centrifuged at 5000 rpm for 10 min at 10°C. This process was repeated and the aqueous phase was transferred into another tube containing equal volume of chilled isopropanol. The sample was incubated at -20°C for 1 h to precipitate nucleic acid. Tubes were centrifuged to collect precipitate. The pellete was washed with 70% ethanol, vacuum dried, dissolved in Tris-EDTA (TE) buffer, pH 8.0, containing RNAase (50 μg ml^{-1}), and incubated for 1 h at 37°C for RNA degradation. Degraded RNA was then removed with equal volume of chloroform: isoamyl alcohol (24:1). DNA was precipitated with equal volume of chilled isopropanol and incubated at -20°C for 30 min, followed by centrifugation at 10,000 rpm for 10 min. The pellete of DNA was washed twice with 70% ethanol, dried at room temperature and resuspended in 100 μl TE buffer.

The reaction volume of 25 μl was subjected to amplification through PCR in a thermal cycler (Eppendorf, Germany) along with a control (without genomic DNA). Prior to amplification, reaction mixture was gently tapped and spun briefly. The genomic DNA was amplified using random primers of OPB, OPC, OPD, OPE, OPF, OPG and OPI series (Operon Tech., California, USA). The PCR reactions for RAPD were carried out in a 25 μl of reaction mixture as described by William et al. (1990). The reaction buffer consisted of 2.5 μl of 10X Taq Buffer with 15 mM $MgCl_2$, 0.4 μl dNTPs mix (2.5 mM each), 0.6 μl Taq DNA polymerase (3 U μl^{-1}), 2 μl genomic DNA (30 ng) and 17.5 μl sterile distilled water. The primers showing polymorphic bands were then used for analyzing the genetic diversity. PCR amplification was performed in a DNA thermal cycler and amplified products were resolved by electrophoresis on 1.5% agarose gel in tris-borate EDTA (TBE) buffer stained with 0.5 μg/ml ethidium bromide and photographs were taken by MultiDoc Digital Imaging System UVP.

Data scoring and analysis

Consistently, well-resolved fragments in the size range of 100 bp to 2.5 kb were manually scored. Each band was treated as a marker. The scoring of bands was done on the basis of their presence (1) or absence (0) in the gel. The genetic associations were evaluated by calculating the Jaccard's similarity coefficient for pair-wise comparisons based on the proportion of shared bands produced by the primers. The similarity matrix was subjected to the cluster analysis of unweighted pair group method with arithmetic averages (UPGMA) and a dendrogram was generated by using NTSYS-pc version 2.1 software (Rohlf, 2000).

RESULTS

Forty (40) RAPD primers (Operon Technologies Inc., Germany) belonging to OPD, OPE, OPF and OPG series of universal primers set were used for initial screening. However, primers from OPD showed no amplification, it may be due to absence of complementary sequence in the genome. Ten primers viz., OPE-02, OPE-08, OPE-13, OPE-14, OPF-18, OPG-03, OPG-10, OPG-13, OPG-16 and OPG-17 were selected for evaluating molecular differences existing among genotypes. The nucleotide sequences of each primer are shown in Table 1. The number of scorable bands for each RAPD primer varied from 2 (OPE-02) to 6 (OPE-08, OPE-13, OPG-03 and OPG-10) as shown in Table 1. Ten (10) RAPD primers produced 49 distinct and scorable bands, with an

Table 1. Details of amplification obtained with different RAPD primers in different varieties of gerbera (*Gerbera jamesonii* Bolus).

Name of primer	Sequence details	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism (%)	Total number of bands amplified
OPE 02	5'-GGTGCGGGAA-3'	2	0	2	100.00	17
OPE 08	5'-TCACCACGGT-3'	6	3	3	50.00	51
OPE 13	5'-CCCGATTCGG-3'	6	1	5	83.33	32
OPE 14	5'-TGCGGCTGAG-3'	5	0	5	100.00	40
OPF 18	5'-TTCCCGGGTT-3'	5	0	5	100.00	42
OPG 03	5'-GAGCCCTCCA-3'	6	0	6	100.00	49
OPG 10	5'-AGGGCCGTCT-3'	6	1	5	83.33	49
OPG 13	5'-CTCTCCGCCA-3'	5	2	3	60.00	41
OPG 16	5'-AGCGTCCTCC-3'	3	0	3	100.00	28
OPG 17	5'-ACGACCGACA-3'	5	0	5	100.00	23
Total		49	7	42	-	372

Table 2. Jaccard's similarity coefficient among different varieties of gerbera (*Gerbera jamesonii* Bolus) based on the RAPD data.

	Stanza	Fana	CF Gold	Diego	Cherany	CF Orange	Lion	Venezia	Torbin	Jaffana	Kento	Ice Queen
Stanza	1.000											
Fana	0.718	1.000										
CF Gold	0.600	0.725	1.000									
Diego	0.634	0.800	0.725	1.000								
Cherany	0.639	0.641	0.488	0.641	1.000							
CF Orange	0.485	0.421	0.457	0.459	0.394	1.000						
Lion	0.667	0.707	0.675	0.707	0.632	0.486	1.000					
Venezia	0.711	0.795	0.763	0.750	0.590	0.486	0.744	1.000				
Torbin	0.692	0.775	0.744	0.821	0.615	0.472	0.816	0.865	1.000			
Jaffana	0.610	0.690	0.659	0.651	0.537	0.472	0.683	0.725	0.707	1.000		
Kento	0.559	0.487	0.571	0.450	0.351	0.429	0.556	0.556	0.500	0.500	1.000	
Ice Queen	0.488	0.610	0.537	0.571	0.415	0.371	0.641	0.600	0.625	0.548	0.625	1.000

average of 4.9 bands per primer. Each primer generated a unique set of amplification products (Figure 1). A perusal of data shown in Table 1 reveals that 10 decamer primers produced a total of 49 scorable bands in the 12 genotypes of *G. jamesonii* Bolus, out of which 42 were polymorphic and seven were monomorphic. The percentage of polymorphism ranged from a maximum of 100.00% by OPE-02, OPE-14, OPF-18, OPG-18, OPG-16 and OPG-17 to a minimum of 50.00% by OPE-08.

The RAPD profiles generated were further evaluated for studying the Jaccard's similarity coefficient (Table 2). The RAPD amplification data were used to obtain similarity matrix (Table 2) and for generation of dendrogram (Figure 2) using UPGMA method. The dendrogram obtained clearly indicated two distinct major clusters I and II. The genotype (CF Orange) found in second cluster was having minimum similarity with other genotypes while rest of the genotypes were laid in first cluster. It is evident from Table

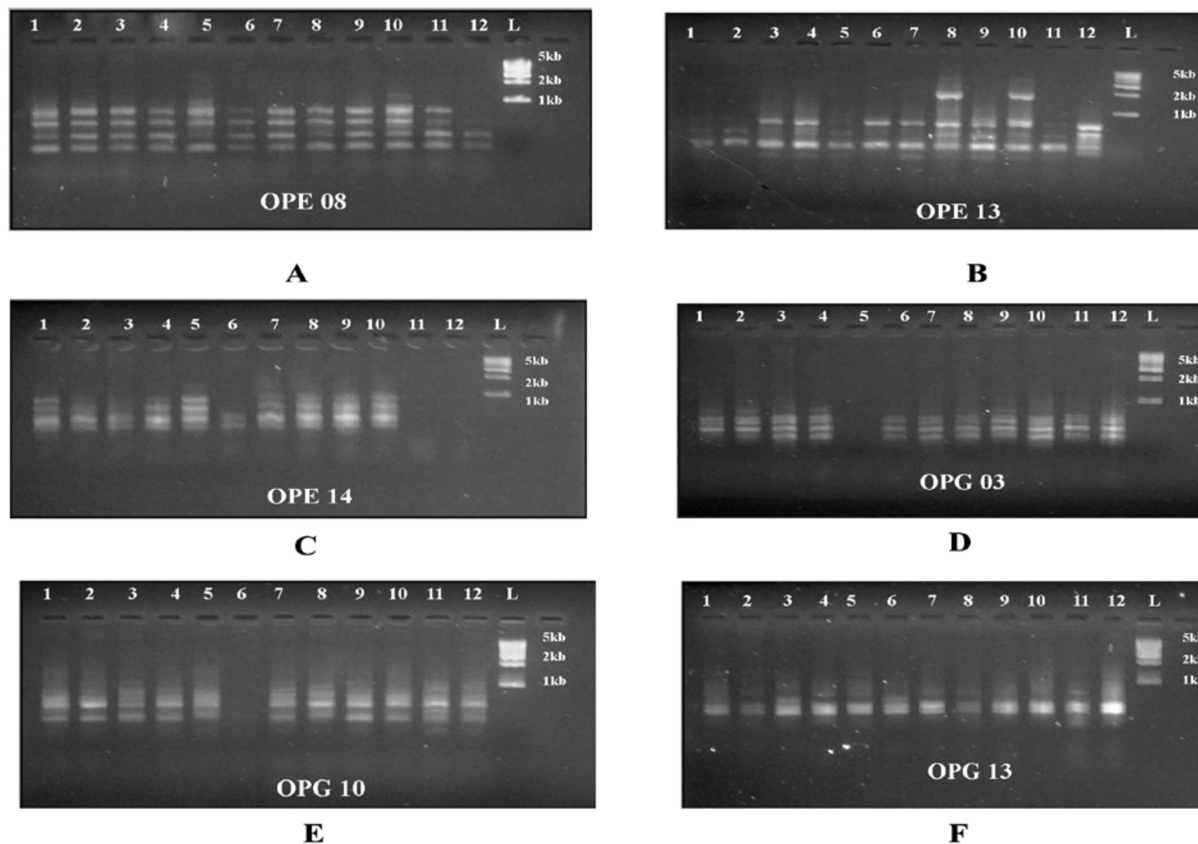

Figure 1. RAPD amplification pattern of 12 different accessions of *Gerbera jamesonii* Bolus using RAPD primers. (Lanes 1-12: 12 accessions of gerbera that is, Stanza, Fana, CF Gold, Diego, Cherany, CF Orange, Lion, Venezia, Torbin, Jaffana, Kento and Ice Queen respectively). A, Amplification pattern with OPE 08; B, amplification pattern with OPE 13; C, amplification pattern with OPE 14; D, amplification pattern with OPG 03; E, amplification pattern with OPG 10; F, amplification pattern with OPG 13.

2 that the lowest genetic similarity coefficient is 0.35 between cluster I and II. Moreover, this major cluster was again divided into two sub-clusters; I (1) and I (2). The sub-cluster I (1) was consisting of nine genotypes (Stanza, Fana, Diego, Venezia, Torbin, Cherany, Lion, CF Gold and Jaffana) while the second sub-cluster I (2) consisting of only two genotypes (Kento and Ice Queen).

The present results show that RAPD can be used for evaluating the molecular variation existing among twelve accessions of gerbera (*G. jamesonii* Bolus). It is evident from the result that some of the primers sequences exhibited 100% polymorphism, which may be more useful to differentiate the gerbera accessions as compared to other primers (Sulan et al., 2002). An UPGMA dendrogram was generated from the Jaccard's similarity values using NTSYS-pc software version 2.1 (Figure 2). Based on this dendrogram, Fana - Diego, Venezia - Torbin and Kento - Ice Queen were found parallel to each other.

DISCUSSION

PCR based markers are gaining much popularity nowadays to assess genetic diversity among horticultural crops. Diversity analysis of any crop species is first step towards crop improvement. The results of present study could assess diversity among 12 accessions of gerbera through RAPD. The absence of genetic variation using RAPD has also been reported in micro-propagated shoots of *Pinus thunbergii* (Goto et al., 1998), *in vitro*-regenerated turmeric (Salvi et al., 2001) and *in vitro*-raised bulblets of Lilium (Varshney et al., 2001). A similarity matrix based on Jaccard's coefficient revealed that the pair-wise varieties have more similarity.

Similar results were also obtained by Sreedhar et al. (2007) during the clonal fidelity analysis of long-term micropropagated shoot cultures of vanilla (*Vanilla planifolia* Andrews) by RAPD and inter-simple sequence repeat (ISSR) markers. Earlier, Reynoird et al. (1993) also did not observe any phenotypic variations during vegetative and reproductive phases among the regenerates of gerbera.

Plants regenerated from adventitious buds around axillary buds or from other well-developed meristematic tissues showed the lowest tendency for genetic variation (Joshi and Dhawan, 2007). Even plants derived from organised meristems are not always genetically true to the type in many crops (Devarumath et al., 2002).

Molecular marker study also revealed that the pairs of

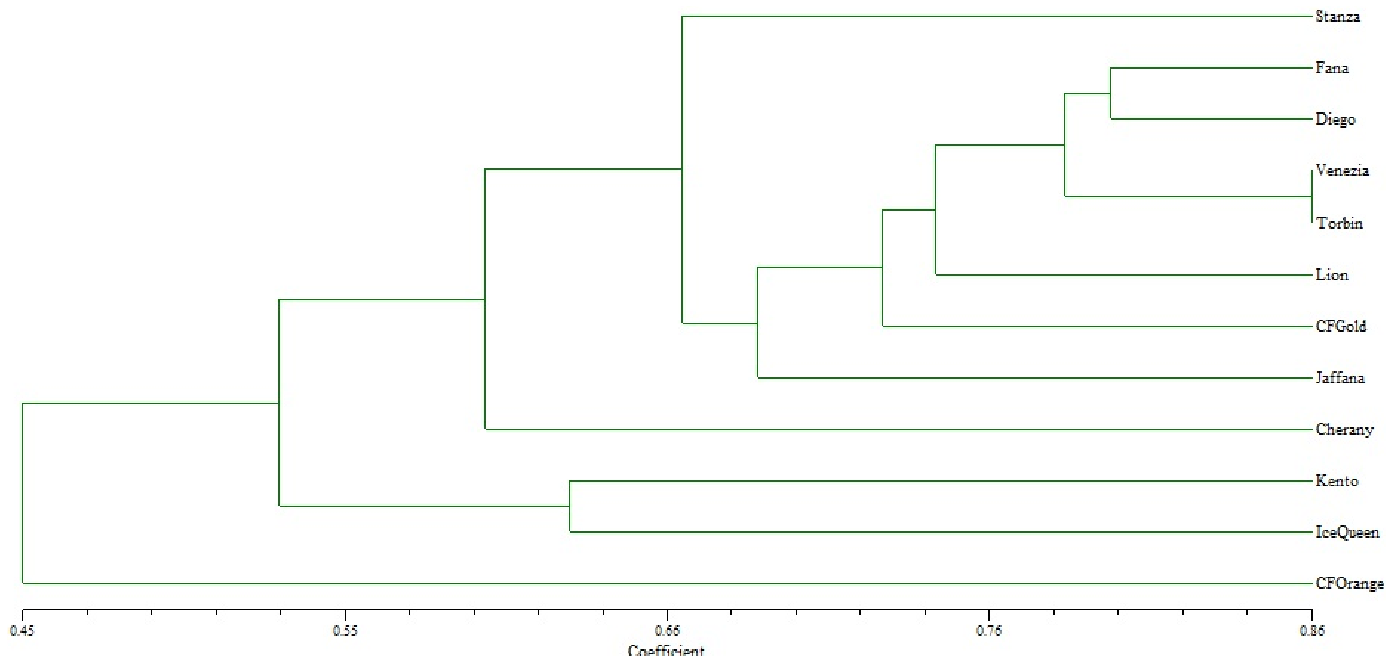

Figure 2. Dendrogram depicting the genetic relationship among different accessions of *Gerbera jamesonii* Bolus based on pooled RAPD data.

the cultivars have very less divergent ability and they are genetically similar to each other. Based on present investigation a good amount of genetic difference was noticed among all the twelve accessions through RAPD molecular marker. Screening of more number of primers is recommended to evaluate the present set of accessions. Moreover, screening of more accessions may also give some divergence. Similar finding was reported by Mo-Suk et al. (1999) in 52 melon lines, Huang and Sun (2000) in *Ipomea* sp., Benedetii et al. (2000) in 5 putative interspecific hybrids of Alstroemeria and Lee et al. (2005) in 55 interspecific hybrids between *Dianthus giganteus* and *Dianthus carthusianorum* and their parents.

In the present study, the average genetic similarity coefficient for the 12 accessions, evaluated by Jaccard index was 0.66 ranging from 0.35 to 0.86. Similar results were reported by Da Mata et al. (2009) for a total of 42 accessions of *G. jamesonii* with 0.55 average genetic similarity coefficients. The banding profiles showed a total of 42 polymorphic and 7 monomorphic bands from twelve accessions of gerbera. The results show that RAPD is a fast, relatively inexpensive and useful technique for genetic divergence characterization between different cultivars of *G. jamesonii* (Rezende et al. 2009).

Conclusion

In summary, significant diversity has been reported among 12 accessions of gerbera through RAPD. Analyses of more number of primers are recommended to evaluate the present set of accessions. Screening of more gerbera accessions with RAPD primers may give further divergence.

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