MOLECULAR CHRACTERIZATION OF CHRYSANTHEMUM (*CHRYSANTHEMUM MORIFOLIUM* RAMAT) GERMPLASM USING RAPD MARKERS

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Abstract: Genetic variation among 24 chrysanthemum cultivars was examined by RAPD markers. A total of 79 fragments was produced with 10 RAPD primers and out of which 64 (81.01%) were found polymorphic and 15 bands (18.99%) monomorphic. The number of polymorphic fragments varied from 4.0 (OPF13) to 15 (OPF06) with an average of 7.9 bands per primer. The PIC was varied from 0.10 to 0.66 with an average .50, MI varied from o.36 to 6.99 with an average 2.92 and RP value was noted in the ranged from 5.17 to 14.50 with an average 9.40. UPGMA clustering revealed two major group (Group1 and Group 2) and these further devided into seven clusters. Among the 24 genotypes, Poncho, Terri, Rangoli, Sweta, Ravikiran and Nanco are divergent and may be useful for breeding programme. Results suggested that RAPDs are highly useful for assessing the genetic diversity analysis among the chrysanthemum germplasm and parental selection studies in chrysanthemum.

Keywords: Chrysanthemum, molecular chracterization, RAPD markers, genetic diversity

INTRODUCTION

Chrysanthemum is the second largest cut flower after rose among the ornamental plants (Kumar *et al.*, 2006). Several species of chrysanthemum are ornamental and grown in gardens for their large, showy, multi colored flowers (Anon 1950). Now a day, ornamental plants market demands new cultivars for different characteristics (Minano *et al*., 2009). However, the information for higher flower yield and yield contributing parameters is limited. Genetic improvement and development of new varieties in chrysanthemum is very difficult due to genome complexity, high level of heterozygocity, occurrence of inbreeding depression, self incompatibility and high rate of failure of many crosses. (Wolff and Peter-van Rijn, 1993) . In newly developed varieties, systematic identification and characterization of cultivars is extremely important in horticultural crops in order to protect the plant breeders right (Kumar, *et al*., 2006). It is also interesting in particularly in chrysanthemum where many varieties are unknown. (Martin et al, 2002) Therefore, it is necessary to estimate the genetic variation and mode of inheritance of different plant parameters in order to select diverse parents for productive breeding programs in chrysanthemum. Morphological characterization is labor intensive and the phenotypic plasticity of plants makes environment variation a major problem. It is a simple technique to assess genetic variation in genotypes under normal growing environment. (Fu *et al.*, 2008 and Condit, 1955). Therefore, molecular markers are considered as useful tools for identification of plant cultivars. The number of molecular markers has been used to detect the variation in ornamental plants (Rout and Mohapatra, 2006). Out of which RAPD is widely used due to easily available markers.

In the present study, our objectives were to assess the genetic diversity in twenty four released varieties of chrysanthemum by using RAPD markers to identified diverse genotype for breeding programme.

MATERIAL AND METHOD

Plant material

A total of 24 genetically diverse genotypes of chrysanthemum were obtained from NBRI, Lucknow, IARI, New Delhi) (Table 1). Young leaves were collected from the field, put into labeled envelopes, and stored in an ice box for transport to the laboratory. In the laboratory the leaves were stored at –20°C in a freezer until their DNA was extracted.

Table. 1: Qualitative traits of 24 genotypes of chrysanthemum

S. No.	Genotypes	Growth habit	Flower colour	Disc colour	Type of Flower	Maturity group
1	Gaity	Upright and Medium	Pink	Yellow	Double	Mid
2	Kundan	Upright and Tall	Yellow	*	Double	Late
3	Santa Dina	Upright and Tall	Dark pink	Orange	Semi Double	Mid
4	Selection-69	Spreading and tall	Pink	Yellow	Double	Late
5	Selection-44	Spreading and Dwarf	Bronze with yellow margin	Yellow	Semi Double	Mid

6	White Prolific	Upright and Tall	White	Light yellow	Double	Mid
7	Terry	Upright and Medium	Bronze	Yellow	Semi Double	Early
8	Nanaco	Upright and Tall	Yellow	*	Double	Early
9	Rangoli	Upright and Dwarf	Dark pink	Yellow	Semi Double	Mid
10	Kirti	Spreading and Medium	White	Yellow	Semi Double	Early
11	Ravi Kiran	Upright and Tall	Red with pink margin	Dark red	Semi Double	Mid
12	Sonoton	Upright and Tall	Dark pink	Yellow	Single	Early
13	Sweeta	Upright and Tall	Light pink	*	Double	Mid
14	Poncho	Spreading and Medium	Orange	Orange	Single	Mid
15	Basmati Yellow	Upright and Tall	Dark Yellow	*	Double	Mid
16	Kamaudi	Upright and Tall	Mauve	*	Double	Late
17	Delilah	Upright and Medium	Pink	Yellow	Semi Double	Mid
18	Ratlam Selection	Upright and Tall	White with cremish center	*	Double	Mid
19	SKC-83	Upright and Dwarf	Pink	Yellow	Single	Early
20	White Bouquet	Upright and Medium	White with cremish center	*	Double	Early
21	Reagan Yellow	Upright and Tall	Pink	Yellow	Double	Mid
22	Mother Teresa	Upright and Dwarf	White with cremish center	Yellow	Double	Early
23	Birbal Sahni	Upright and Tall	White	*	Double	Mid
24	Fish Tail	Upright and Medium	White	*	Double	Mid

DNA extraction and RAPD analysis

Total genomic DNA was extracted from fresh and young leaf tissues following CTAB method (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.

Molecular data analysis

Data generated by using 10 RAPD primers on 24 chrysanthemum genotypes were scored in binary format and further analyzed as described previously (Kumar *et al*., 2009). Besides this, PIC (polymorphism information content) Botstein et al. (1980), marker index (MI) (Milbourne *et al*., 1997) and Resolving Power (Rp) (Prevost and Wilkinson's, 1999) were also calculated.

RESULT

RAPD Analysis

Diversity analysis based on RAPD fingerprinting showed that the total number of polymorphic bands, number of monomorphic bands, PIC, marker index (MI) and resolving power (Rp) obtained for each primer are shown in the Table 3 and comparative list is presented in the Table 1&3. A total of 79 bands were detected using 10 RAPD primers on the basis of the presence (1) or absence (0) of the bands, out of these 15 were monomorphic and 64 were polymorphic thus generating 80.01% polymorphism (Table 2) among the 24 genotypes included in the investigation. Amplification patterns of various RAPD primers are shown in Figure 2a&b. RAPD DNA bands varied between 4 (OPF-13) and 15 (OPF-06) with an average of 7.9 bands per primer. The maximum number of polymorphic bands (13 bands) was obtained using OPF-06 primer. The average number of polymorphic bands was 6.4 per primer. The molecular size of the bands amplified using ten primers were in the range of 100-2100 bp (Table 2).

Polymorphic Information Content (PIC) *i.e.* the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency were calculated for individual primers. PIC values for RAPD primers ranged from 0.10 for OPD-08 to 0.66 for OPF-17, with an average of 0.50. Thus the study indicated that the RAPD primer OPF-17 used in the study was most polymorphic. Primers OPF-06, OPJ-08, OPC-15 and OPF-14 had PIC values more than average and therefore, may well be considered to be well spread over the entire genome randomly. The marker index (MI) varied between 0.36 (OPD-08) and 6.99 (OPF-06) with average marker index of 2.92.The resolving power (RP) varies between 5.17 (for OPF-17) and 14.50 (OPF-06) with an average value of 9.40.

All the 79 bands, generated from 10 RAPD primers, were subjected to calculate the genetic similarity index (RAPD-GS) among the 24 genotypes. Genetic similarities were calculated using the Nei-Li similarity co-efficient. Significant genetic variation was found among all chrysanthemum genotypes with the GS value ranging from 0.59 to 0.94 (Table 5). Of the 24 pair wise combinations generated by chrysanthemum genotypes, the highest genetic similarity was found between genotype Terry and genotype Nanaco, and genotype Reagan Yellow and genotype Birbal Sahni; while the lowest genetic similarity 0.59 was observed between genotype Selection-69 and genotype Mother Teresa. UPGMA clustering method for dendrogram construction and cultivar differentiation indicated that all 24 genotypes of chrysanthemum were discriminated successfully by RAPD markers. All the 24 genotypes of chrysanthemum were classified into two main groups (Group 1 and Group 2) at the coefficient of GS=0.70 (Figure 1). Group 1 and Group 2 divided further to give a total of Seven clusters, as described under. Group 1 divided into two clusters (Cluster I and Cluster II) at the coefficient of GS=0.73. Cluster I further subdivided into two sub clusters (Cluster Ia and Cluster Ib) at the coefficient of GS=0.83. Cluster Ia included 4 genotypes namely, Ratlam Selection, Reagan Yellow, Delilah and Kamaudi. Cluster Ib comprised of 3 genotypes viz., SKC-83, Birbal Sahni and Basanti Yellow. Cluster II subdivided into two sub clusters (Cluster IIa and Cluster IIb) at the coefficient of GS=0.75. Cluster IIa included only a single genotype Poncho; while Cluster IIb included 5 genotypes, viz., Sweeta, Sonoton, Fish Tail, Ravi Kiran and Nanaco. Group 2 further divided into two clusters (Cluster III and Cluster IV) at the coefficient of GS=0.81. Cluster III contained only two genotypes, Kirti and Mother Teresa. Cluster IV divided into two sub clusters (Cluster IVa and Cluster IVb) at the coefficient of GS=0.82. Cluster IVa included just one genotype, Terry. Cluster IVb included most of the genotypes (eight) namely, Rangoli, Selection-44, Kundan, Selection-69, Santa Dina, White Prolific, White Bouquet and Gaity.

Table 2: Analysis of RAPD markers.

Components	**RAPD**
Total number of Primers used	10
Polymorphic markers	all
Total number of bands amplified	79
Average number of bands per primer	7.9
Maximum number of bands amplified by a single primer	15
Number of polymorphic bands	64
Percentage of polymorphic bands (%)	81.01
Average number of polymorphic bands per primer	6.4
Maximum number of polymorphic bands amplified by a primer	13
PIC	
maximum	0.66
minimum	0.10
average	0.50
Marker Index (MI)	
maximum	6.99
minimum	0.36
average	2.92
Resolving power (Rp)	
maximum	14.50
minimum	5.17
average	9.40
Size of PCR product	0.1-2.1kbp

Taable 3: RAPD Primer code, no. of polymorphic alleles, no. of monomorphic alleles & PIC, MI and Rp value of 24 chrysanthemum genotypes

S.No.	Primer code	Polymorphic bands	Monomorphic bands	Diversity index (PIC)	Marker Index (MI)	Resolving Power (Rp)
1	OPF-06	13	2	0.62	6.99	14.5
2	OPC-07	7	0	0.50	3.49	8.42
3	OPJ-08	11	2	0.62	5.81	13.17
4	OPD-08	5	2	0.10	0.36	13.25
5	OPK-11	4	2	0.33	0.88	8.75
6	OPF-13	2	2	0.37	0.37	5.92
7	OPJ-13	5	2	0.52	1.84	8.25
8	OPF-14	7	1	0.65	4.01	9
9	OPC-15	5	2	0.62	2.21	7.58
10	OPF-17	5	0	0.66	3.29	5.17

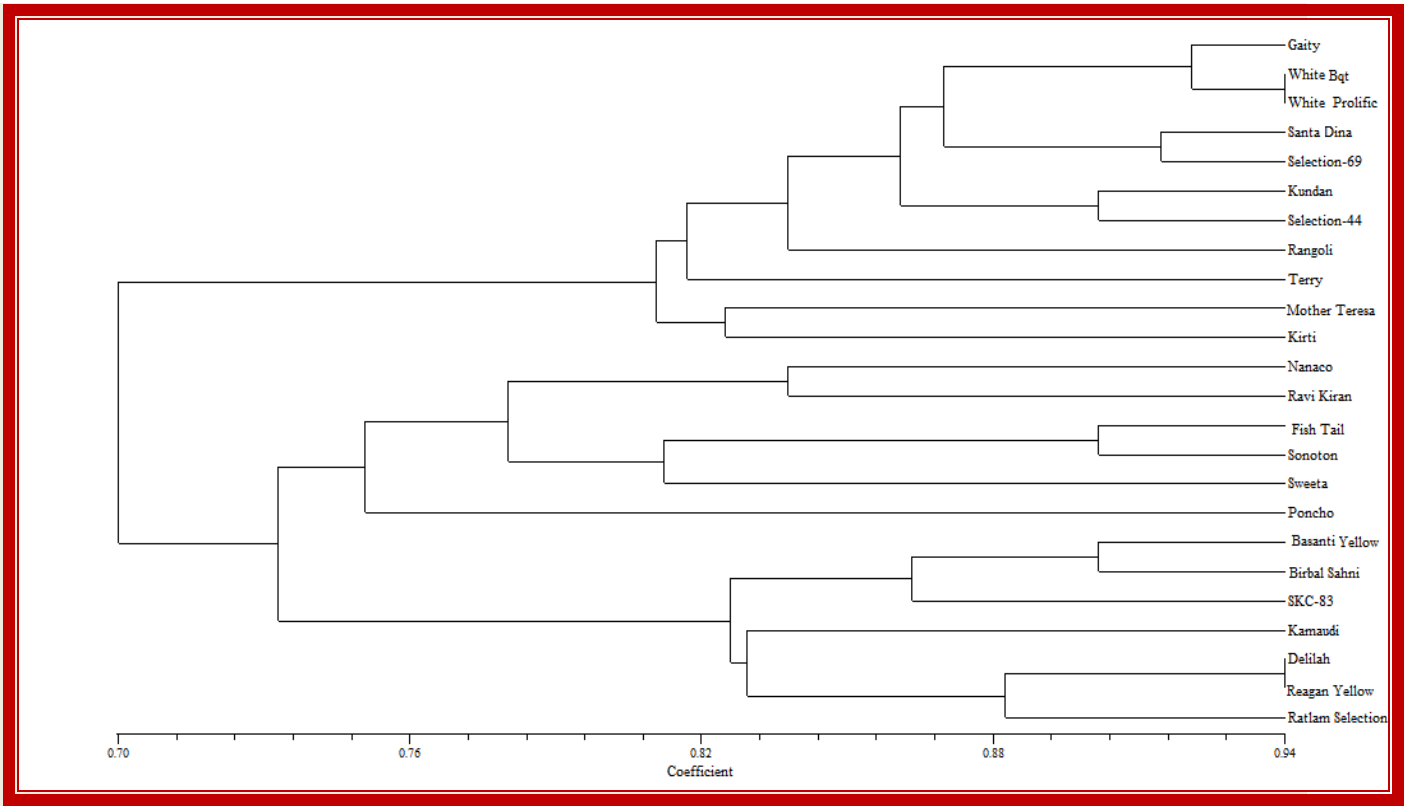

Fig. 1: Dendrogram showing clustering of 24 chrysanthemum genotypes constructed using UPGMA based on Jacquard's similarity coefficient obtained from RAPD analysis.

Table 4: List of RAPD primers

S.No.	Primer Code	Sequence	Make
1.	OPF-06	5'GGGAATTCGG3'	IDT
2.	OPC -07	5'GTCCCGACGA3'	IDT
3.	OPJ-08	5'CATACCGTGG3'	IDT
4.	OPD-08	5'GTGTGCCCCA3;	IDT
5.	OPK-11	5'AATGCCCCAG3'	IDT
6.	OPF-13	5'GGCTGCAGAA3'	IDT
7.	OPJ13	5'CCACACTACC3'	IDT
8.	OPF14	5'TGCTGCAGGT3'	IDT
9.	OPC-15	5'GACGGATCAG3'	IDT
10.	OPF17	5'AACCCGGGAA3'	IDT

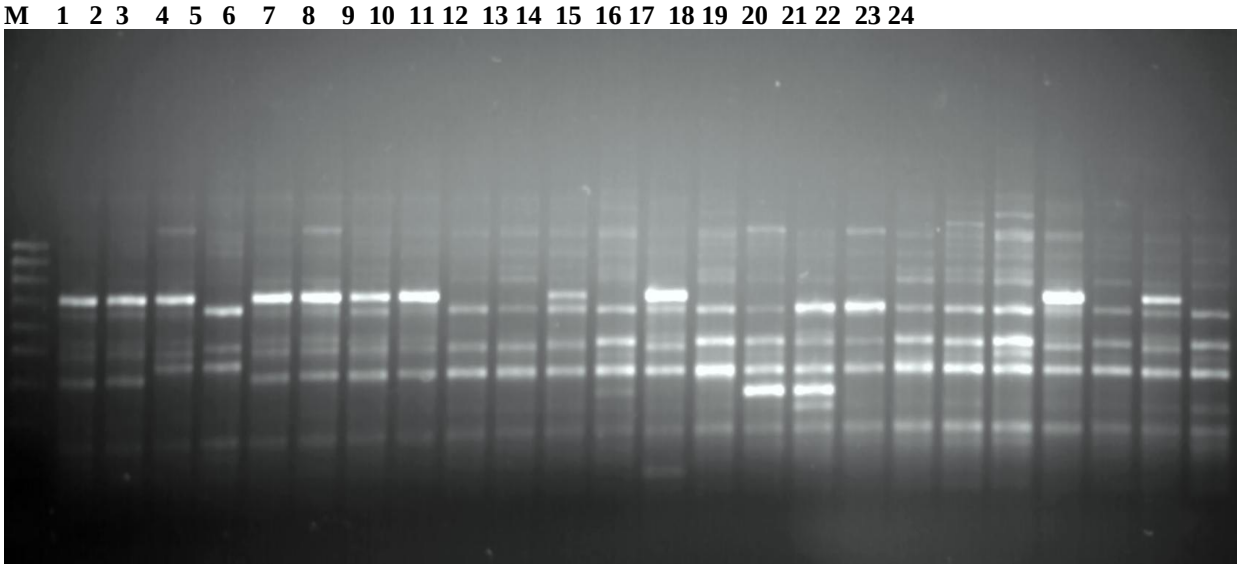

Fig. 2a: RAPD profiling pattern of 24 chrysanthemum genotypes with OPF-06 primer

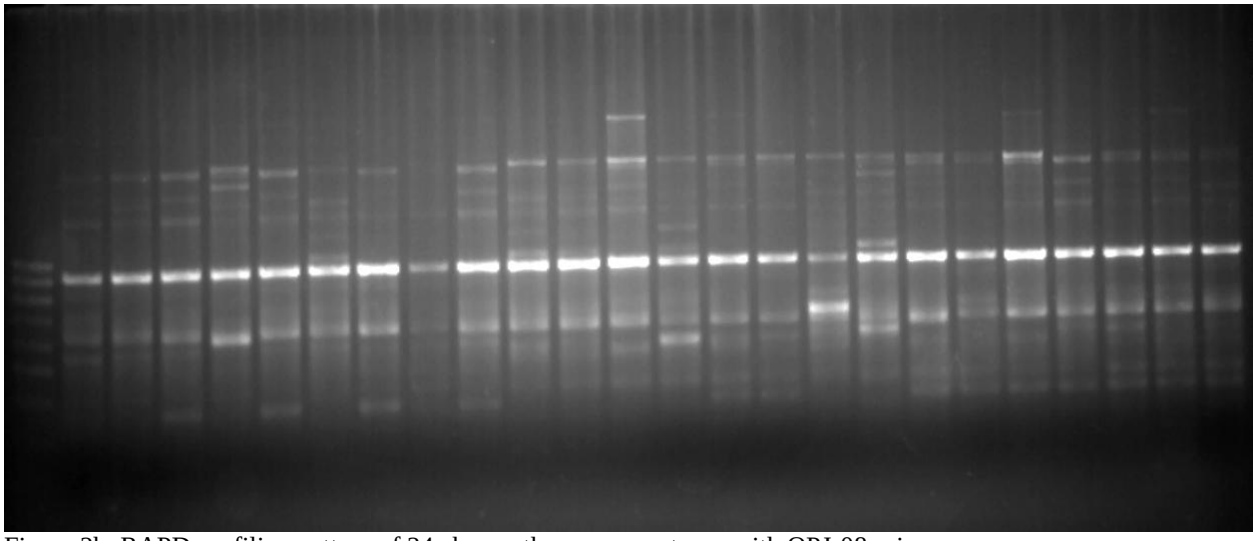

Figure 2b. RAPD profiling pattern of 24 chrysanthemum genotypes with OPJ-08 primer

Table 5: Similarity matrix generated by Jaccard's similarity coefficient for 24 genotypes of chrysanthemum obtained from RAPD analysis.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
C1	1																							
C2	0.91	1																						
C3	0.85	0.86	1																					
C4	0.85	0.81	0.82	1																				
C5	0.89	0.87	0.91	0.81	1																			
C6	0.89	0.9	0.86	0.78	0.87	1																		
C7	0.91	0.85	0.86	0.81	0.9	0.8	1																	
C8	0.92	0.86	0.85	0.82	0.86	0.81	**0.94**	1																
C9	0.82	0.81	0.82	0.8	0.86	0.84	0.81	0.77	1															
C10	0.8	0.81	0.75	0.75	0.76	0.86	0.76	0.75	0.82	1														
C11	0.86	0.8	0.81	0.78	0.82	0.85	0.85	0.86	0.78	0.78	1													
C12	0.82	0.81	0.8	0.82	0.81	0.78	0.81	0.82	0.77	0.72	0.83	1												
C13	0.76	0.8	0.73	0.68	0.77	0.85	0.72	0.71	0.73	0.84	0.77	0.71	1											
C14	0.76	0.75	0.68	0.68	0.72	0.72	0.77	0.73	0.76	0.78	0.77	0.76	0.8	1										
C15	0.68	0.72	0.66	0.61	0.67	0.7	0.7	0.66	0.71	0.76	0.67	0.71	0.8	0.9	1									
C16	0.7	0.73	0.65	0.62	0.66	0.76	0.63	0.62	0.67	0.75	0.66	0.64	0.78	0.76	0.86	1								

C17	0.7	0.73	0.7	0.7	0.68	0.76	0.63	0.62	0.75	0.7	0.68	0.7	0.73	0.73	0.78	0.8	1							
C18	0.73	0.72	0.71	0.66	0.72	0.7	0.75	0.71	0.71	0.73	0.72	0.71	0.77	0.85	0.87	0.78	0.78	1						
C19	0.73	0.7	0.68	0.63	0.7	0.67	0.72	0.71	0.66	0.68	0.72	0.71	0.75	0.82	0.85	0.76	0.68	0.9	1					
C20	0.67	0.66	0.67	0.62	0.68	0.63	0.66	0.62	0.65	0.64	0.66	0.64	0.66	0.76	0.76	0.67	0.7	0.81	0.83	1				
C21	0.71	0.72	0.76	0.63	0.77	0.7	0.7	0.68	0.71	0.63	0.72	0.68	0.67	0.7	0.75	0.68	0.78	0.8	0.77	0.81	1			
C22	0.67	0.68	0.67	**0.59**	0.68	0.66	0.63	0.62	0.67	0.7	0.63	0.62	0.68	0.76	0.81	0.72	0.75	0.83	0.81	0.85	0.89	1		
C23	0.75	0.73	0.75	0.65	0.76	0.71	0.71	0.7	0.7	0.64	0.76	0.7	0.68	0.73	0.76	0.7	0.77	0.83	0.81	0.82	**0.94**	0.87	1	
C24	0.68	0.65	0.66	0.66	0.65	0.62	0.65	0.63	0.63	0.66	0.67	0.63	0.67	0.75	0.77	0.71	0.71	0.85	0.87	0.83	0.82	0.86	0.86	1

DISCUSSION

Molecular traits based diversity

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. A total of 79 bands were detected using 10 RAPD primers in the present study, out of these 15 were monomorphic and 64 were polymorphic (Table 2), thus generating 81.01% polymorphism. The results of polymorphism generated by RAPD in chrysanthemum genotypes were in close conformity with those of earlier investigators (Wolff. and Peters, 1993; Wolff, 1996; Martin, *et al.,* 2002, Kumar, *et al.,* 2006. The maximum number of polymorphic bands (13 bands) was obtained using OPF-06 primer. The average PIC, MI and Rp values for RAPD primers were 0.50, 2.92 and 9.40 respectively. RAPD primers except OPF-13 are significantly efficient in analysis considering the values of PIC and Rp.

In the present study, a dendrogram was constructed based on the basis of RAPD markers which showed 81.01 per cent of the bands observed were polymorphic between the 24 chrysanthemum genotypes. This seems to be relatively high when compared to the reports of other RAPD studies, e.g. in Brassica spp (Demeke *et.al.,* 1992), Alternaria spp (Wilkie *et.al*., 1993) Sorghum (Tao *et al*.1993), Alfalfa (Yu and Nguyen, 1994), celery (Yang and Quiros, 1993) and Sweet Potato (Connolly *et.al.,* 1994). One of the reasons for this high level of polymorphism could be that the intra-specific variation in chrysanthemum is extensive. The other reason could be that we have used primers with 60 to 70% GC content, whereas some other workers, including Yamamoto *et al*. (1994) have included primers with less GC content also in their studies. Fukuoka *et al.* (1992) observed an increase in the number of bands with increasing GC content of the primers. They got an average of 0.8 bands per primer with 40 per cent, 6.1 bands with 50 per cent and 8.6 bands with 60 per cent GC content. The explanation for this correlation between the GC content of the primer and the number of bands is that the stability of base complementation is high when G is pairing with C by three hydrogen bonds than the complementation of A with T by two hydrogen bonds.

CONCLUSION

In the present study, RAPD analysis provided good insight of genetic diversity. UPGMA analysis clearly separated the genotypes into distinct groups. Therefore, the present study suggested that molecular markers could be used to achieve a reliable evaluation and robust characterization of the species diversity. Present study showed that some genotypes like Poncho, Terri, Rangoli, Sweta, Ravikiran and Nanco were more diverse than others and these genotypes could be a good alternative source for fruitful chrysanthemum breeding program.

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