ASSESSMENT OF GENETIC DIVERSITY IN CHRYSANTHEMUM (*CHRYSANTHEMUM MORIFOLIUM* RAMAT) USING MICROSATELLITE MARKERS

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Abstract: The genetic diversity among 24 chrysanthemum cultivars was investigated by 07 Simple Sequence Repeats (SSRs). A total of 16 bands were produced out of which 15 bands were found polymorphic and 01 band monomorphic. The number of polymorphic fragment varied from 02 (RM1) to 03 (RM433) with an average 2.14 fragment per primer and percent polymorphism varied from 66.75 to 100% with an average of 93.75%. The PIC varied from 0.42 to 0.95 with an average of 0.74. The RP and MI ranged from (0.83 to 0,57) to (4.0 and 2.76) with an average (2.03 and 0.57) respectively. The UPGMA clustering revealed two major groups and found considerable amount of genetic diversity. Among the 24 cultivars, Ravikiran, Selection 44, Kundan, Terri, Sonton and Poncho are divergent and may be used for breeding programme Results suggested that SSRs are highly useful for assessing the genetic diversity analysis among the chrysanthemum germplasm and parental selection studies in chrysanthemum.

Keywords: Chrysanthemum, molecular charecterization, SSR marker, genetic diversity

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflorum* syn. *Chrysanthemum morifolium*) is one of the most important ornamental crops in the world and it has been cultivated more than 2000 years (Martin and Benito, 2005). It is a genus of about 30 species of herbs and shrubs, which are all perennial flowering plants in the family Asteraceae or Compositae. The cultivated chrysanthemum is originally native of Asia (China and Japan) and northeastern Europe. It 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, identification and characterization of cultivars is extremely important in order to protect the plant breeders right (Kumar, *et al*., 2006). It is also interesting particularly in chrysanthemum where many varieties are unknown. 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 and to compliment traditional breeding efforts 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 cultivars.

Among the available molecular markers, microsatellites commonly known as simple sequence repeats (SSRs) have been widely used due to highly polymorphic, heterozygous conserved sequences which can be used as co-dominant markers. (Rallo *et al*., 2000, Cipriani *et al*., 2002 and Rajora and Rahman, 2002). The aim of the study was to assess the genetic diversity among 24 chrysanthemum cultivars grown in Northern India

MATERIAL AND METHOD

Plant material and field experiment

A total, 24 genetically diverse genotypes of chrysanthemum were obtained from NBRI, Lucknow, IARI, New Delhi. (Table 1). Young leaves were collected from the field of Horticultural Research Centre (HRC), put into cultivar named 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 SSR analysis

Genomic DNA extraction and further microsatellite analysis was performed as described earlier (Kumar *et al*., 2009). Details of SSR primers used are provided in Table 3.

Molecular data analysis

Data generated by using 10 microsatellites primers on 24 chrysanthemum genotypes were scored in binary format and further analysed 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

The genetic data generated through SSR profiling among 24 cultivars, a total of 16 bands were detected using 7 SSR primers, out of which only one was monomorphic and 15 were polymorphic (Table 2). Amplification patterns of various SSR primers are shown in Figure 1. SSR DNA bands varied between 2 (RM447, RM284, RM1, RM152 and RM259) and 3 (RM408 and RM433), with an average of 2.29 bands per primer. The maximum numbers of polymorphic bands (3 bands) were obtained using RM408 and RM433 primers (Fig 2a&b). The average number of polymorphic bands was 2.14 per primer. In the present study, 93.75% polymorphism was obtained by SSR assay. PIC value for SSR ranged from 0.42 (RM408) to 0.95 (RM1, thus highly polymorphic), with an average of 0.74. The marker index (MI) ranged from 0.57 (RM 408) to 2.76 (RM433), with average of 1.57. The resolving power (RP) varied between 0.83 (RM1) and 4 (RM408) with an average value of 2.03.

All the 16 bands, generated from 7 SSR primers, were subjected to calculate the genetic similarity index (SSR-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 0.13 to 0.96 (Table 5). Of the 24 pair wise combinations generated by chrysanthemum genotypes, the highest genetic similarity was found between genotypes Gaity and Selection-69; and genotypes Sonoton and Sweeta; while the lowest genetic similarity was observed between genotype Gaity and genotype Ratlam Selection. The UPGMA clustering method for dendrogram construction and cultivar differentiation indicated that application of SSR assay classified all the 24 genotypes of chrysanthemum into two main groups (Group 1 and Group 2) at the coefficient of GS=0.50 (Figure 1). Group 1 and Group 2 further divided in to seven clusters, as described under.

Group 1 divided into 2 main clusters (Cluster I and Cluster II) at the coefficient of GS=0.68. Cluster I further divided into two sub clusters (Cluster Ia and Cluster Ib) at the coefficient of GS=0.85. Cluster Ia included just two genotypes namely, Ratlam Selection and SKC-83. Cluster Ib included six genotypes namely, Kamaudi, Delilah, Reagan Yellow, Mother Teresa, Basanti Yellow and Poncho. Cluster II divided into two sub clusters (Cluster IIa and Cluster IIb) at the similarity coefficient of GS=0.73. Cluster IIa contained only one genotype, Ravi Kiran. Cluster IIb comprised of six genotypes, viz., Sonoton, Sweeta, Fish Tail, Kirti, Rangoli and Nanaco. Group 2 divided into 2 main clusters (Cluster III and Cluster IV) at the coefficient of GS=0.62. Cluster III included two genotypes, Selection-44 and Kundan. Cluster IV divided into 2 sub clusters (Cluster IVa and Cluster IVb) at the coefficient of GS=0.76. Cluster IVa comprised only a single genotype, Terry. Cluster IVb included six genotypes, viz., White Bouquet, Santa Dina, White Prolific, Birbal Sahni, Selection-69 and Gaity.

Table 2: Analysis of SSR markers.

Components		**SSR**
Total number of Primers used		7
Polymorphic markers		all
Total number of bands amplified		16
Average number of bands per primer		2.29
Maximum number of bands amplified by a single primer		3
Number of polymorphic bands		15
Percentage of polymorphic bands (%)		93.75
Average number of polymorphic bands per primer		2.14
Maximum number of polymorphic bands amplified by a primer		3
PIC		
maximum		0.95
minimum		0.42
average		0.74
Marker Index (MI)		
maximum		2.76
minimum		0.57
average		1.57
Resolving power (Rp)		
maximum		4
minimum		0.83
average		2.03
Size of PCR product		0.4-1.0kbp

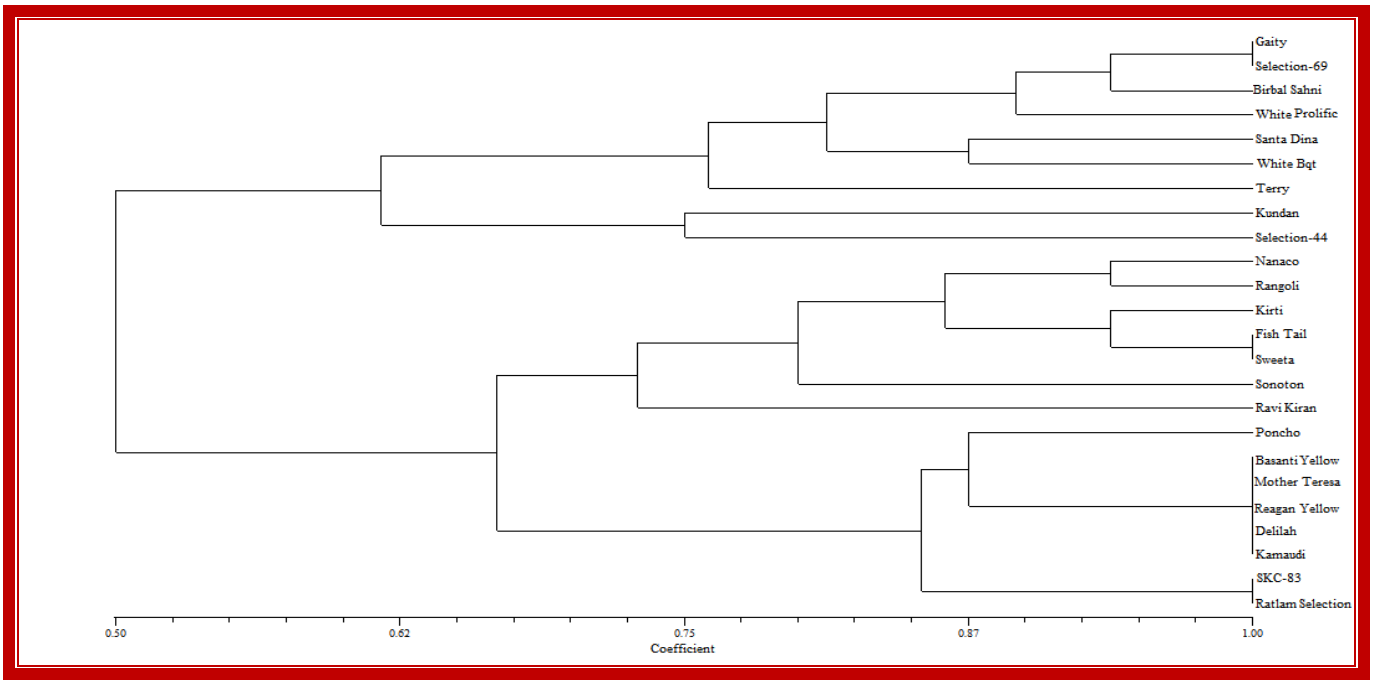

Fig. 1: Dendrogram showing clustering of 24 chrysanthemum genotypes constructed using UPGMA based on Jacquard's similarity coefficient obtained from SSR analysis.

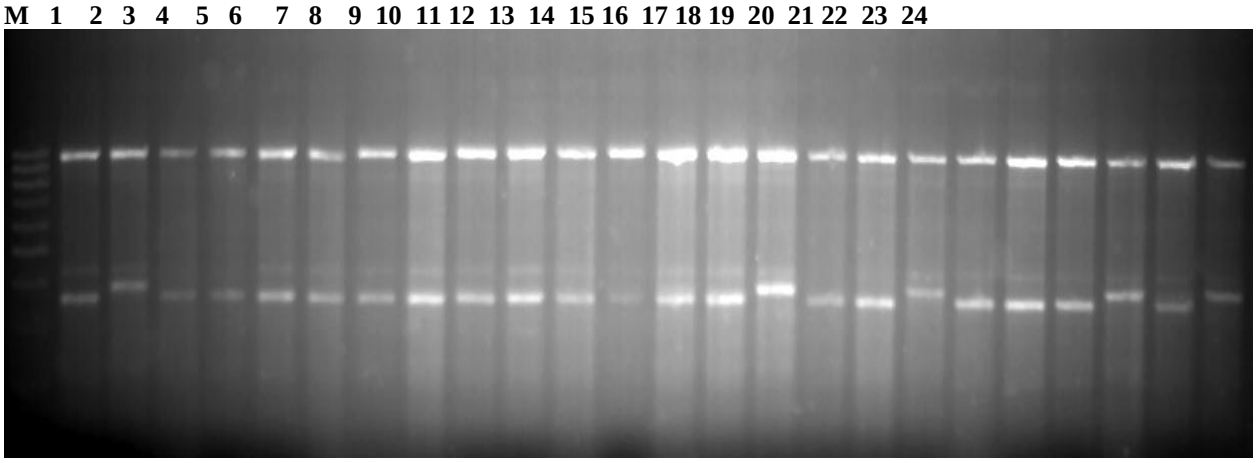

Fig. 2a: SSR profiling pattern of 24 chrysanthemum genotypes with RM408 primer

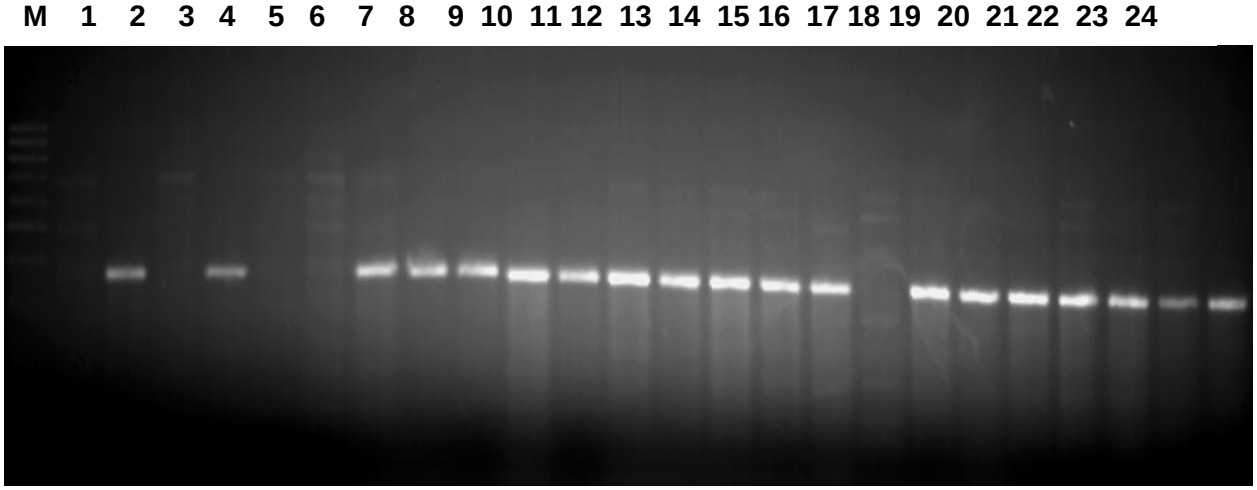

Fig. 2b: SSR profiling pattern of 24 chrysanthemum genotypes with RM433 primer

DISCUSSION

The term SSR (simple sequence repeat markers) microsatellite was coined by Litt and Luty (1989). These markers essentially belong to the repetitive DNA family. Fingerprints generated by the corresponding primers are also known as oligonucleotide fingerprints. Microsatellites or short tandem repeats/simple sequence repeats (STRs/SSRs) consist of 1 to 6 bp long monomer sequences which are repeated several times. In the present study, a total of 16 bands were detected using 07 SSR primers, out of which only one was monomorphic and 15 were polymorphic (Table 2). SSR bands varied between 2 (RM447, RM284, RM1, RM152 and RM259) and 3 (RM408 and RM433), with an average of 2.29 bands per primer. The maximum numbers of polymorphic bands (3 bands) were obtained using RM408 and RM433 primers. The average number of polymorphic bands was 2.14 per primer thus generated 93.75% polymorphism . This seems to be relatively high when compared to the reports of other SSR studies, e.g. in Gerbera hybrida (Gong and Deng, 2012), Pelargonium (Becher *et.al*., 2000). In our study, SSR markers gave more polymorphism then RAPD markers which has been earlier used by some workers in chrysanthemum (Kumar *et al*., 2006, Baliyan et al, 2014). It might be due to that microsatellite markers are more informative than RAPD markers. Therefore, it is highly polymorphic, consistant and co-dominant markers which provide excellent markers for clone and cultivar identification in poplars. (Rehman and Rajora, 2002), (Dayanadan *et al.* 1998; Rehman *et al.* 2000; Rajora and Rehman 2002), as well as in a number of agricultural and horticultural plants (Becher *et al.* 2000; Li *et al.*, 2000). . The primer RM1, was observed to be highly polymorphic (PIC value of 0.95). The average PIC, MI and Rp values for SSR primers were 0.74, 1.57 and 2.03 respectively. Depending upon the value of PIC, MI and Rp, it may be concluded that most of the SSR primers except RM1 were highly significant in analysis. Through SSR assay (Fig 1), the 24 chrysanthemum genotypes were divided into seven clusters. The information on SSR analysis in chrysanthemum in literature is not available.

Table 3. SSR 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	RM447	2	0	0.73	1.47	2
2	RM284	2	0	0.82	1.64	1.67
3	RM408	2	1	0.42	0.57	4
4	RM433	3	0	0.92	2.76	1.67
5	RM1	2	0	0.95	1.90	0.83
6	RM152	2	0	0.57	1.14	2.08
7	RM259	2	0	0.75	1.50	2

Table 4: List of SSR primers

S.No.	Primer Code	Forward Sequence	Reverse Sequence	Make
1.	RM 447	CCC TTG TGC TGT CTC CTC TC	AGC GGC TTC TTC TCC TCC TC	IDT
2.	RM 284	ATC TCT GAT ACT CCA TCC AT	CCT GTA CGT TGA TCC GAA GC	IDT
3.	RM 408	CAA CGA GCT AAC TTC CGT CC	ACT GCT ACT TGG GTA GCT GA	IDT
4.	RM 433	TGC GCT GAA CTA AAC ACA GC	AGA CAA ACC TGG CCA TTC AC	IDT

5.	RM 1	'GCA AAA ACA CAA TGA AAA AA'	GCG TTG GTT GAC CTG AC'	IDT
6.	RM 152	GAA ACC ACC ACA CCT CAC CG	CCG TAT ACC TTC TTG AAG TA	IDT
7.	RM 259	TGG AGT TTG AGA GGA GGG	CTT GTT GCA TGG TGC CAT GT	IDT

Table 5: Similarity matrix generated by Jaccard's similarity coefficient for 24 genotypes of chrysanthemum obtained from SSR 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	0.56	1																						
C3	0.88	0.69	1																					
C4	**0.96**	0.63	0.81	1																				
C5	1	0.56	0.88	0.84	1																			
C6	0.69	0.75	0.81	0.63	0.69	1																		
C7	0.88	0.56	0.75	0.84	0.88	0.56	1																	
C8	0.75	0.69	0.88	0.81	0.75	0.69	0.88	1																
C9	0.75	0.44	0.63	0.81	0.75	0.44	0.88	0.75	1															
C10	0.63	0.19	0.5	0.56	0.63	0.44	0.5	0.38	0.62	1														
C11	0.56	0.43	0.44	0.5	0.56	0.38	0.56	0.44	0.69	0.91	1													
C12	0.63	0.19	0.5	0.56	0.63	0.31	0.63	0.5	0.75	0.88	0.84	1												
C13	0.5	0.44	0.63	0.44	0.5	0.56	0.5	0.63	0.5	0.62	0.69	**0.96**	1											
C14	0.69	0.25	0.56	0.63	0.69	0.38	0.69	0.56	0.69	0.81	0.88	0.84	0.81	1										
C15	0.56	0.38	0.44	0.5	0.56	0.25	0.56	0.44	0.56	0.69	0.75	0.81	0.69	0.88	1									
C16	0.69	0.25	0.56	0.63	0.69	0.38	0.69	0.56	0.69	0.81	0.88	0.93	0.81	1	0.88	1								
C17	0.63	0.19	0.5	0.56	0.63	0.31	0.62	0.5	0.75	0.75	0.81	0.88	0.62	0.81	0.69	0.81	1							
C18	**0.13**	0.44	0.5	0.31	0.38	0.31	0.38	0.5	0.5	0.5	0.56	0.62	0.62	0.56	0.69	0.56	0.75	1						
C19	0.16	0.31	0.63	0.44	0.5	0.44	0.5	0.62	0.62	0.62	0.69	0.75	0.75	0.69	0.56	0.69	0.88	0.88	1					
C20	0.5	0.31	0.63	0.44	0.5	0.44	0.5	0.62	0.62	0.62	0.69	0.75	0.75	0.69	0.56	0.69	0.88	0.88	1	1				
C21	0.5	0.31	0.63	0.44	0.5	0.44	0.5	0.62	0.62	0.62	0.69	0.75	0.75	0.69	0.56	0.69	0.88	0.88	1	1	1			
C22	0.38	0.44	0.5	0.31	0.38	0.31	0.38	0.5	0.5	0.5	0.56	0.62	0.62	0.56	0.69	0.56	0.75	1	0.88	0.88	0.88	1		
C23	0.5	0.31	0.63	0.44	0.5	0.44	0.5	0.62	0.62	0.62	0.69	0.75	0.75	0.69	0.56	0.69	0.88	0.88	1	1	1	0.88	1	
C24	0.5	0.31	0.63	0.44	0.5	0.44	0.5	0.62	0.62	0.62	0.69	0.75	0.75	0.69	0.56	0.69	0.88	0.88	1	1	1	0.88	1	1

CONCLUSION

In the present study, SSR-based analysis provided good insight of genetic diversity. On the other hand, SSR data, UPGMA analysis clearly separated the genotypes into distinct groups. Therefore, the present study suggested that microsatellite markers should be used in order to achieve a reliable evaluation and robust characterization of chrysanthemum germplasm. Present study showed that some genotypes like SKC-83, Ratlam Selection, Gaity, and Selection-69 were more diverse than others and these genotypes could be a good alternative source for fruitful chrysanthemum breeding program.

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