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Estimation of Genetic Diversity of Four *Chrysanthemum* Mini Cultivars Using RAPD

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Abstract: The objective of the present study was to estimate the genetic relationship among the different *Chrysanthemum* cultivars with relation to their morphological and biochemical characteristics and geographical distribution. DNA fingerprinting using RAPD is very easy and inexpensive way to study the genetic diversity. Genetic distance between four mini *Chrysanthemum* cultivars was studied through RAPD analysis. Total of 40 primers have been screened from which four have been trailed for all the four genotypes. Similarity among the cultivars was very high showing low genetic diversity, which is quite expected. One of these primers can differentiate cultivars from each other. So RAPD can be used successfully to estimate the genetic distance and also for the species identification.

Key words: Genetic diversity, *Chrysanthemum* miniature cultivars, RAPD

INTRODUCTION

Chrysantemum morifolium Ramat. has a tremendous demand in the floriculture for its different types of flowers. Though it is mainly of large flower and small flower different type of new varieties have been developed through spontaneous and induced mutation. Mini cultivars gain maximum popularity because they don't need pinching or stacking. There is profuse divergence of colour also, starting from different shades of yellow to pink, red, mauve, white, terracotta. One of the selected species, shizuka is collected from Japan. So there is an environmental difference also as well as morphological diversities.

Basic chromosome number of *Chrysanthemum* is X= 9. Hexaploid species are most common but heptaploid, aneuploid are not rare. Though chromosome number does not differ in the five species, RAPD proved to be a good technique to estimate how closely the genotypes are related. RAPD have been widely used for:

1. Determining the genetic relationships between different related species[1]
2. For the identification of cultivars[2] and
3. For estimating genetic relationships and diversity among crop germplasms[3-5]

Marker-Assisted Selection (MAS) is a very useful tool to guide the introgression of target genes from related species by Restriction Fragment Length Polymorphism (RFLP) in the past several years[6].

Main objective of the experiment was to characterize the selected genotypes at the molecular level and to establish a reliable method for identification of closely related species independent of any environmental influence.

MATERIALS AND METHODS

All the mini cultivars of *Chrysanthemum morifolium* Ramat. are collected from the germplasms maintained in National Botanical Research Institute, Lucknow. Name of the cultivars used for RAPD analysis are as follows:

1. Pancho- Single whorl, floret flat, disc prominent, disc floret tubular
2. Little darling-Orange with small red stripes
3. Satbhawana- white anemone type
4. Shizuka-one to seven whorls, floret tip bifid, disc floret tubular, honey comb type

Chlorophyll content of the four genotypes was determined by the method of Schroeder *et al.*[7].

DNA extraction and PCR analysis: Genomic DNA was extracted from the very young leaves of *Chrysanthemum* by using DNA extraction procedure of Sahgai Maroof *et al.*[8] with two times washing in Chloroform: isoamyl alcohol (24:1) and two washes in 76% ethanol/0.2 M Sodium acetate prior to the wash in 76% ethanol/10 mM NH_4Oac. Finally DNA was dissolved in TE Buffer (pH 8.0). Average yield was 50 ng from 0.2 g of dry leaf tissue.

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PCR condition applied for RAPD analysis: After preheating at 94°C for 4 min, 45 cycles at 94°C for 15 sec, 36°C for 45 sec, 72°C for 1 min 30 sec, followed by one final extension at 72°C for 4 min. Total reaction mixture was consist of 20 μL which consist of 5 ng of template, Taq-1 unit, dNTP 100 μM, Primer 0.2 μM, $MgCl_2$ 2.5 mM, 10 mM Tris-HCl (pH-9.0), 50 mM KCl, 0.01% gelatin. After amplification PCR product was resolved by electrophoresis in 1% agarose gel with 1X TAE buffer. Bands were visualized by staining with ethidium bromide (0.5 μg mL^{-1}) under UV light. Experiment was repeated several times to get a reliable and reproducible data, only those bands were counted for data analysis, which were reproducible.

RESULTS AND DISCUSSION

Length of ray and disc floret was measured starting from periphery to the centre of the flower (Table 1). As all the cultivars are grouped in the miniature group of *Chrysanthemum*, so the length does not vary greatly for the cultivars. Total chlorophyll content of each cultivar is also measured (Table 2) which vary in each cultivar.

Data analysis: Four miniature cultivars of *Chrysanthemum morifolium* Ramat. were scanned by 5 selected primers which gave better profile among 40 random primers. In the Table 3 similarity coefficient was calculated for each of the cultivars using similarity coefficient of Nei and Li[9]. The formula for estimating similarity coefficient (S) is:

$$S = \frac{2 N_A N_B}{N_A + N_B}$$

D = 1-S (D = distance)

When:

N_AN_B = Number of bands shared by individual A and B

N_A = Number of bands present in individual A and

N_B = Number of bands present in individual B

Table 1: Showing length of ray and disc floret of the selected cultivars

Cultivars	Ray floret length (cm)	Disc floret length (cm)
Pancho	1.1-1.3	0.3-0.40
Little Darling	1.1-1.4	0.3-0.55
Satbhawana	1.8-2.3	0.3-0.40
Shizuka	1.4-1.8	0.3-0.40

Table 2: Estimation of chlorophyll content of selected four mini cultivars

	Chlorophyll a (μg g^{-1} of fresh wt.)	Chlorophyll b (μg g^{-1} of fresh wt.)	Chlor. a+b (μg g^{-1} of fresh wt.)
Pancho	215.83872	108.0864	323.92512
Little Darling	184.05120	84.1200	268.17120
Satbhawana	231.60480	126.4992	358.10400
Shizuka	336.61632	169.6944	506.31072

Table 3: Similarity matrix for Nei's and Li's coefficients of four *Chrysanthemum* mini cultivars obtained from RAPD markers the four *Chrysanthemum* cultivars

	1	2	3	4
Pancho	1.00	0.883	0.782	0.7272
Little Darling		1.000	0.808	0.8440
Satbhawana			1.000	0.8330
Shizuka				1.000

Total of 40 primers were screened from which 5 primers were selected for RAPD analysis. Sequences of the selected primers are as follows:

P1- 5' CAAACGTCGG 3'
P2- 5' AGCGTGTCTG 3'
P3- 5' TGCCGAGCTG 3'
P4- 5' GAGCCCTCCA 3'
P5- 5'CAGCTCACGA 3'

Primer P-1 is most effective to differentiate between the cultivars (Fig. 1). Band of 1750 and 1500 bp is present in Satbhawana, which is not present in Pancho and Little Darling, beside that; band of 1500 bp is not present also in Shizuka while other bands are present. One band of 750 bp is present only in Pancho and Little Darling. Shizuka has an extra band at the position of 750 bp using primer-4. Amplification product ranges from 400-1900 bp.

Similarity matrix shows a high similarity coefficient between the cultivars ranges from 72-88%. Shizuka is more closely related to Little Darling than Pancho (Table 2). Cluster analysis of the similarity matrix was done using unweighed pair group method of Sneath and Sokal[10].

On the basis of chlorophyll content it can be said that, pancho is more closely related to Satbhawana and Shizuka, which is imported from Japan, has a higher amount of chlorophyll content. RAPD analysis also supports the observation. Satbhawana is more closely related to Shizuka while it varies distinctly in its morphological and geographical distribution. Shizuka does not show any great divergence from the rest species which is supported by the study which states that interpopulation genetic distance showed no association with geographical distance between population sites of origin, negating a simple isolation by distance model. Data shows an estimation of

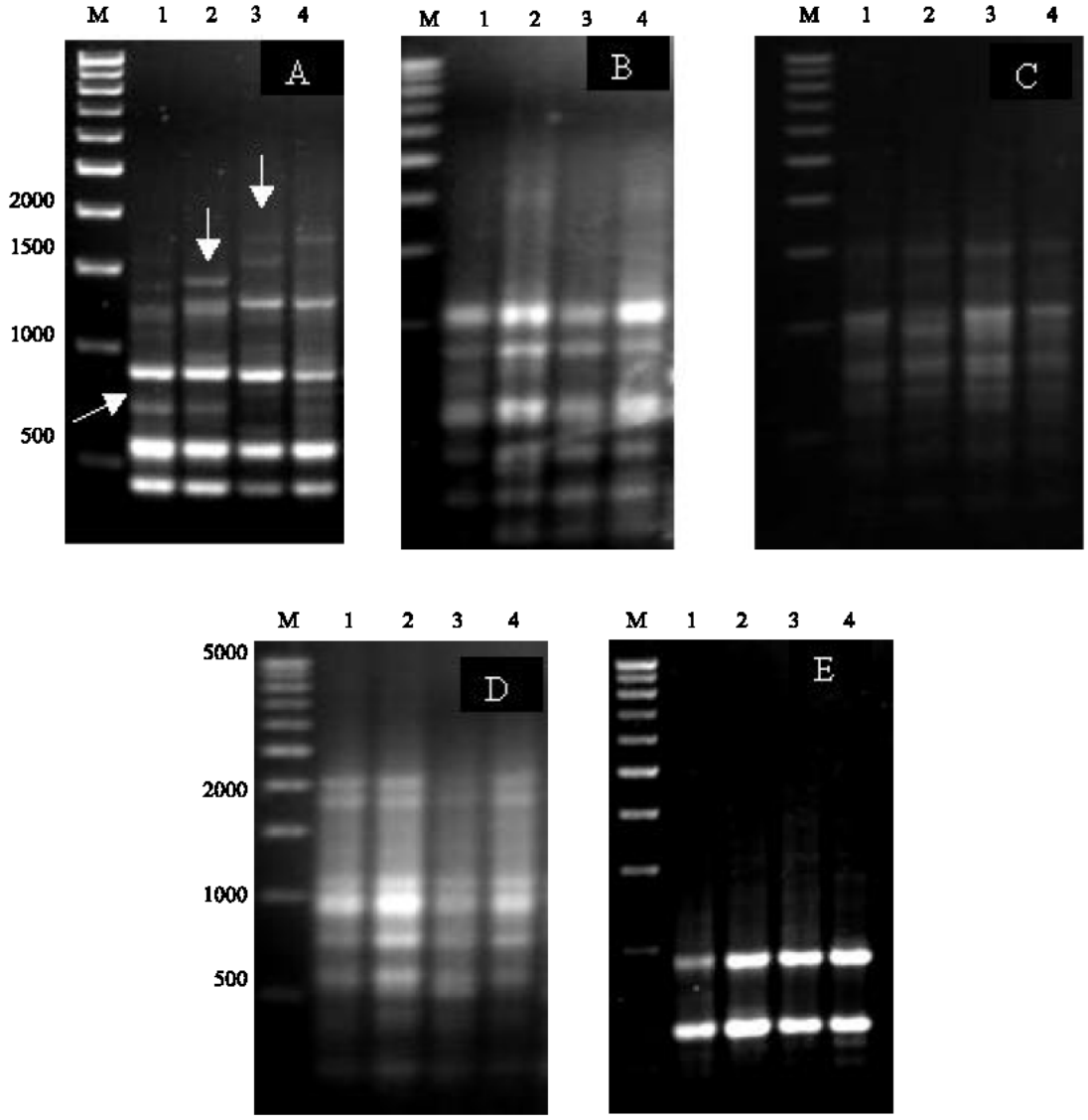

Fig. 1: RAPD fragment of four mini *Chrysanthemum* cultivars obtained on 1% agarose gels with the primers P1(A), P2(B), P3(C), P4(D) and P5(E). M= molecular marker of 500 bp ladder, lane 1=Pancho, lane 2= Little Darling, lane 3= Satbhawanw, lane 4= Shizuka, → Shows specific bands for different cultivars

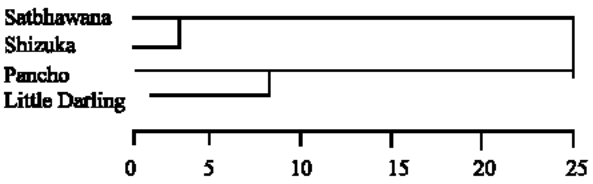

Fig. 2: Dendrogram of 4 *Chrysanthemum* mini cultivars generated by RAPD data using UPGMA method

genetic diversity which can not be simply interpreted by classical morphological, cytological studies. This study proves to be a very useful data for the farmers to improve the flower quality of *Chrysanthemum* and marker assisted selection for some disease resistant loci.

Low percentage of genetic diversity may be due to release of superior variety after mutation breeding or crossing with exotic parents, thus narrowing the genetic pool of the species[11]. There is a certain need to broaden the genetic base of germplasm for crop improvement for better field performances of the cultivars. Present experiment of estimation of genetic diversity is further useful for the farmers for the breeding purpose to increase the heterosis of hybrids and introgress the new genes in the gene pool. These studies can be very effectively proof to be a technique for the development of mapping population for tagging of agronomically importance traits. Difference in morphology is totally independent of geographical distance, negating a simple isolation by

distance model. As all the selected cultivars are morphologically very similar it is very difficult to identify them until the blooming season. DNA fingerprinting is the only solution to solve this problem. This RAPD profile serve as a way for identification of species specific marker and also produces a reliable data to construct the phylogenetic relationship of the genus *Chrysanthemum.*

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