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Analysis of genetic diversity among *Tagetes patula* L. cultivars based on RAPD markers

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ABSTRACT

Twelve cultivars of *Tagetes patula* with distinct floral characteristics were analysed through RAPD profiles. Twenty two polymorphic primers produced a total of 233 bands out of which 188 bands were polymorphic with an average of 8.1 polymorphic bands per primer. The size of the amplified fragments ranged from 200-6000 bp. The percentage of polymorphic bands ranged from 50 (OPF-01 and OPT-20) to 100% (OPF-04, OPF-14, OPF-15, OPT-17) with an average of 75.8% polymorphism. The Polymorphic Information Content (PIC) values ranged from 0.19 (OPT-20) to 0.44 (OPA-10, OPT-12). A relatively higher genetic variability was detected among the cultivars and had similarity coefficient values ranging from 0.42 to 0.93. Cluster analysis based on Jaccard's similarity coefficient using Unweighted Pair Group Method with Arithmetic Averages (UPGMA) grouped all the 12 cultivars into three major groups at a similarity coefficient of 0.53. The greatest similarity was found between Boy Mix (BM) and Boy Spry (BS) (Jaccard's coefficient = 0.93), while Boy Gold (BG) was found to be most distinct genotype. A total of 8 primers detected in the study produced unique allele in five genotypes. This results showed that the level of genetic variation was high among the *Tagetes* cultivars. It also indicated the higher potential of RAPD markers for identification and maintenance of *Tagetes* germplasm for crop improvement purposes.

Key words: Genetic diversity, polymorphism, RAPD, *Tagetes patula.*

INTRODUCTION

The genus *Tagetes* that includes marigold is a morphologically diverse taxon of the family, Asteraceae. It comprises about 56 species, of which the commercially cultivated species includes *T. erecta* (African marigold), *T. patula* (French marigold), *T. minuta*, (Mexican marigold), and *T. lucida* (Mexican tarragon). *Tagetes patula* is a well known ornamental and medicinal plant which bears yellow to orange coloured flowers. It is commercially cultivated in Mexico, Peru and India. Lutein is the main carotenoid in the marigold flowers (Delgado-Vargas *et al*.,3), which is an antioxidant and possesses pharmacological properties. Lutein suppresses mammary tumor growth and enhances lymphocyte proliferation (Chew *et al*., 2) and reduces the risk of age related degeneration of the human macula (Landrum *et al*., 8). The health benefiting function of the plant is increasingly being recognized and thus its flowers are commercially cultivated, harvested and processed in an industrial scale as a source of carotenoid pigments.

A wide variation is exhibited by *Tagetes patula* cultivars in terms of shape and colour of the flower. The assessment of natural genetic variation is important for its sustainability in agriculture and other industries. Variety identification and estimation of genetic distance are of importance both in plant variety protection and in breeding programmes (Smykal *et al*., 11). The molecular marker techniques have significant utility in plant breeding programme through assisting in plant variety protection as well as distinctness, uniformity and stability testing processes (Heckenberger *et al*., 6). Proper characterization and assessment of genetic relationship among diverse accessions allow breeders to select the desirable genes from different sources and to accumulate them in one cultivar.

So far no report is available on the identification of *Tagetes* cultivars by means of DNA fingerprinting. Thus, the main objective of this study was to use the RAPD markers to analyse genetic relationship *T. patula* cultivars.

MATERIALS AND METHODS

A total of 12 cultivars of *T. patula* namely; Safari Red (SR), Safari Queen (SQ), Safari Scarlet (SC), Little Hero Flame (LH), Hero Spry (HR), Boy Yellow (BY), Sparky Mix (SM), Boy Orange (BO), Boy Gold (BG), Boy Mix (BM), Boy Spry (BS) and one local variety of Jaipur, Jaipur Local (JP) were procured from seed market and investigated in the present study (Table 1, Fig. 1). DNA was extracted from young fresh and healthy leaves collected from 8 week old plants following the cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 4).The extracted DNA was analysed on 0.8% agarose gel

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Table 1. Description of *Tagetes patula* cultivars used in the study.

Name	Cultivar code	Characters
Safari Red	SR	Capitulum small, petals 5, attractive maroon, centre yellowish red
Safari Queen	SQ	Capitulum medium, petals 5, deep yellow with maroon streaks
Safari Scarlet	SC	Capitulum medium sized, petals 5, deep yellow with maroon streaks
Little Hero Flame	LH	Capitulum large, petals rosette with mixed yellow and red color
Hero Spry	HR	Capitulum large, petals rosette with maroon colour margined with yellow colour
Boy Yellow	BY	Capitulum small, petals rosette with single lemon yellow colour
Sparky Mix	SM	Capitulum medium, petals rosette with mixed maroon and yellow colour
Boy Orange	BO	Capitulum medium, petals rosette with single orange yellow colour
Boy Gold	BG	Capitulum medium, petals rosette with single golden yellow colour
Boy Mix	BM	Capitulum large, petals rosette with mixed yellow and red colour
Boy Spry	BS	Capitulum large, petals rosette with mixed yellow and red colour
Jaipur Local	JP	Capitulum large, petals rosette with mixed yellow and red colour

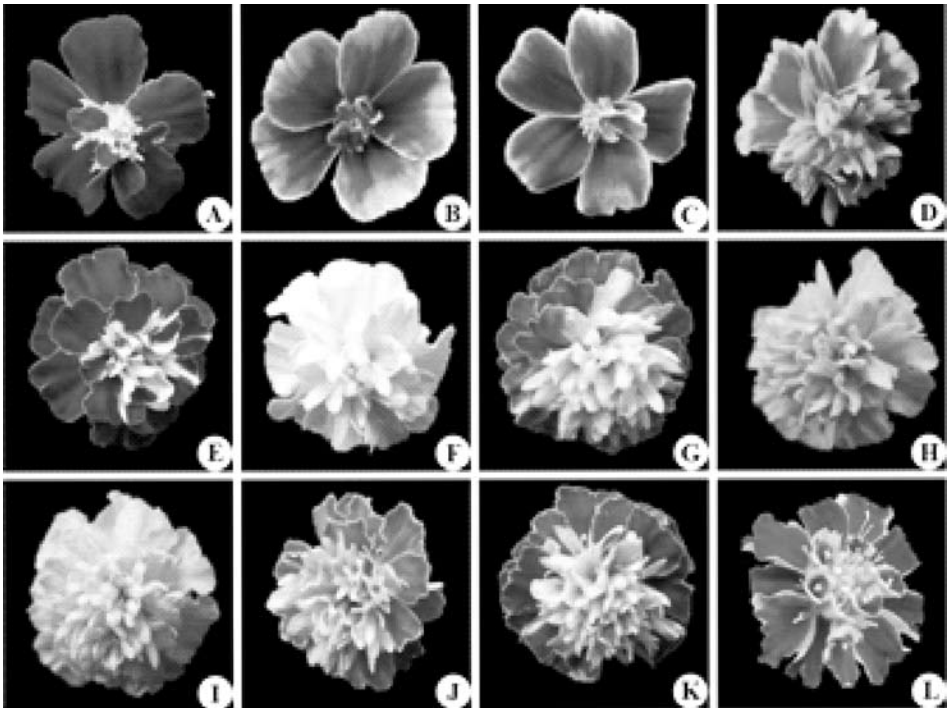

Fig. 1. Flower morphology of 12 *T. patula* cultivars. A = Safari Red (SR), B = Safari Queen (SQ), C = Safari Scarlet (SC), D = Little Hero Flame (LH), E = Hero Spry (HR), F = Boy Yellow (BY), G = Sparky Mix (SM), H = Boy Orange (BO), I = Boy Gold (BG), J = Boy Mix (BM), K = Boy Spry (BS), L = Jaipur Local (JP).

and was diluted in TE to a concentration of 25 ng/µl for polymerase chain reaction (PCR).

A total of 60 arbitrary decamer primers (Operon Tech. Inc., USA) OPA (1-20), OPF (1-20) and OPT (1-20) were initially screened, out of which 22 primers showed clear scorable and highly polymorphic bands. PCR amplification reaction (25 µl) contained 25 ng of genomic DNA, 2.5 mM $MgCl_{2,}$ 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 200 mM dNTPs mix, 0.5 µM primer and 0.25 units of *Taq* DNA polymerase performed in a programmable thermocycler DNA Engine (Biorad, Germany). After a pre-denaturation step of 3 min. at 94ºC, amplification reactions were optimized with 40 cycles of 94ºC for 45 sec, 37ºC for 45 sec and 72ºC for 150 sec. A final extension was allowed for 10 min. at 72ºC.

Upon completion of the reaction, the amplified products were separated on 1.5% agarose gel in 1x TBE buffer using ethidium bromide staining dye. The size of the amplified fragments was determined using 1 Kb and 100 bp DNA ladders (Bangalore Genie, India) as standard markers. DNA fragments were visualized under UV- transilluminator and photographed using gel documentation system (Biorad, Germany).

The amplicons obtained from different RAPD markers were scored based on the presence (taken as 1) or absence (taken as 0) of bands for each primer. Banding pattern for each primer was scored by visual observations, where only clear and unambiguous bands were scored. The position of PCR bands were compared with molecular weight standards (DNA ladders). Accordingly, a rectangular binary matrix is obtained and statistical analysis was performed using the NTSYS-pc version 2.02e (RohIf, 10). A pair wise similarity matrix was generated using simple matching co-efficient (by means of SIMQUAL procedure of NTSYS-pc) and the cluster analysis was performed (by means of SAHN procedure of NTSYS-pc) *via* Unweighted Pair Group Method with Arithmetic averages (UPGMA) to develop a dendrogram. A two dimensional and three dimensional principal component analysis (PCA) was constructed to provide another means of testing the relationship among the cultivars using the EIGEN programme (NYSTS-pc). To estimate the strength of the grouping, generated by cluster analysis, bootstrap analysis was performed with 1000 replication using the WINBOOT programme (Yap and Nelson, 14).

RESULTS AND DISCUSSION

In the present study, RAPD technique has been successfully used to detect genetic variation among the 12 *Tagetes patula* cultivars, which differ in shape and colour of the inflorescence. Amid the 60 RAPD primers used for initial screening, 22 markers produced polymorphic, reproducible and scorable bands. A total of 233 bands were produced out of which 188 (75.8%) were polymorphic. Only one primer (OPT-11) did not produce any polymorphic amplification fragments for any of the 12 *T. patula* varieties. The remaining 22 primers ranged in their polymorphic amplified fragments between 3 (OPT-20) to 15 (OPA-10) with an average of 8.1 polymorphic bands per primer. The size of the amplified fragments ranged from 200-6000 bp. The polymorphism ranged from 50% (OPF-01 and OPT-20) to 100% (OPF-04, OPF-14, OPF-15, OPT-17) (Table 2). Figure 2 shows the amplification pattern obtained from primer OPF-04 that produced 10 polymorphic bands. The Polymorphic Information Content (PIC) values ranged from 0.19 (OPT-20) to 0.44 (OPA-10, OPT-12). A total of 8 primers detected in the study produced unique allele in five genotypes (Table 3). These primers can be utilized to distinguish variety with one flower colour from other varieties having different flower colours.

The pairwise comparison of RAPD profiles based on both shared and unique amplification products was made to generate a similarity matrix. Similarity indices established on the basis of 22 primers ranged from 0.42 to 0.93. The dendrogram (Fig. 3) is a close representation of the values obtained in the similarity matrix. From the data obtained in the dendrogram the 22 polymorphic primers discriminated all varieties and divided them into three major clusters at 53% similarity. Cluster I was the largest and dominated with 9 varieties (SR, SQ, HR, BY, SM, BO, BM, BS, JP) and was divided into two sub-clusters at a similarity value of (0.67). Among these varieties BO and BY are single colour, while rest 7 are mixed colour varieties. The highest value of similarity coefficient (0.93) was detected between two mixed coloured varieties BM and BS. The second cluster had only one single coloured variety BG. The third cluster contains two varieties SC and LH. Boot strap values are highest for the RAPD dendrogram showing that cluster obtained by RAPD are more robust and reliable for phenetic studies.

Genetic relationship among *T. patula* accessions were also visualized by performing PCA based on RAPD data. The results of PCA were comparable to the cluster analysis with minor differences (Fig. 4). Genotypes grouped within the same cluster in the dendrogram were also occupying the same position in two dimensional and three dimensional scaling, except the two varieties SQ and SR, which formed a separate group in principle component analysis confirming its genetic distinctness from other *T. patula* varieties included in this study. The advent of molecular markers have facilitated the detection of important genes and permit breeding programmes from elite cultivar backgrounds to be completed in a shorter time and in a more cost-effective manner(Tanksley *et al*., 12). The successful use of RAPD marker in genetic diversity analysis of various ornamental plants of asteraceae is well reported in gerbera (Mata *et al*., 9), chrysanthemum (Wolff and Peters-van Rijn, 13) and *Helianthus* (Isaacs *et al*., 7).

In the present study, a fingerprint key based on the banding pattern of RAPD markers that can be used to identify *T. patula* cultivars, has been generated. It is observed that *T. patula* cultivars exhibit a wide range of genetic diversity, thus serve as a wide reservoir of useful gene that could be utilized for interspecific and inter-varietal crosses to generate novel ornamental commercially important elite cultivar. Estimation of

Table 2. Amplified DNA bands and polymorphism generated in *Tagetes patula* cultivars using RAPD markers.

Locus code	Total bands	No. of polymorphic bands	Per cent polymorphism	Polymorphism Information Content (PIC)	Range of molecular size (bp)
OPA-01	13	12	92.3	0.36	250-4000
OPA-02	9	6	66.6	0.23	200-1500
OPA-03	11	10	90.9	0.26	200-1500
OPA-05	12	10	83.3	0.28	350-4000
OPA-10	16	15	93.7	0.44	200-2500
OPF-01	12	6	50.0	0.34	300-5000
OPF-02	13	10	76.9	0.35	200-4500
OPF-03	8	5	62.5	0.28	400-3000
OPF-04	10	10	100.0	0.30	600-5000
OPF-09	10	8	80.0	0.31	300-3000
OPF-11	6	5	83.3	0.26	350-5000
OPF-12	10	7	70.0	0.33	400-6000
OPF-13	7	4	57.1	0.32	400-4000
OPF-14	14	14	100.0	0.42	350-2500
OPF-15	9	9	100.0	0.34	250-2100
OPT-07	15	11	73.3	0.40	200-3500
OPT-08	13	10	76.9	0.29	500-5000
OPT-11	1	0	0.0	0.0	1000
OPT-12	8	6	75.0	0.44	450-1500
OPT-13	7	5	71.4	0.39	400-1500
OPT-15	11	10	90.9	0.37	200-3000
OPT-17	12	12	100.0	0.37	200-2000
OPT-20	6	3	50.0	0.19	400-2000
Total	233	188			
Mean	10.1	8.1	75.8	0.33	

Table 3. Primers capable of amplifying unique alleles from different *T.patula*genotypes.

Primer	Total bands	No. of unique alleles	Allele size (bp)	Cultivar	Phenotype (Flower colour)
OPF-09	10	1	500	Safari Queen (SQ)	Mixed colour
OPF-12	10	1	600	Safari Queen (SQ)	Mixed colour
OPF-13	7	1	1000	Sparky Mix (SM)	Mixed colour
OPF-15	9	1	2500	Boy Orange (BO)	Orange yellow
OPA-02	9	1	250	Little Hero Flame(LH)	Mixed colour
OPA-03	11	1	250	Boy Gold (BG)	Golden yellow
OPT-08	13	1	2500	Little Hero Flame(LH)	Mixed colour
OPT-15	11	1	600	Boy Gold (BG)	Golden yellow

genetic relationship using molecular technique is a useful tool in plant breeding, allowing breeders to make decision regarding the selection of germplasm to be used in breeding programmes. Molecular markers are more reliable than morphological traits for accessing genetic diversity since they are not subjected to environmental effects and are independent of the developmental stages of the plants. The RAPD

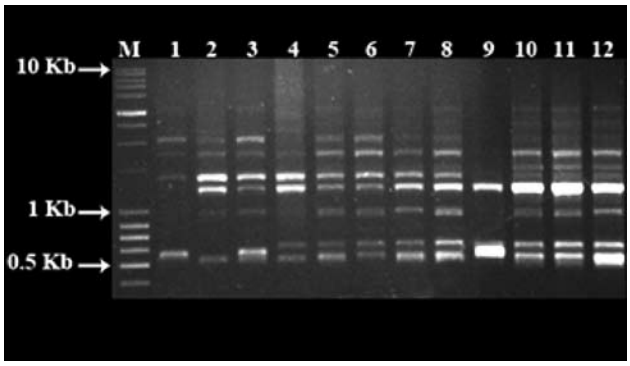

Fig. 2. RAPD profile of 12 *T. patula* cultivars produced with primer OPF-04. Lane M represent DNA ladder, lane 1 to 12 represent different cultivars.

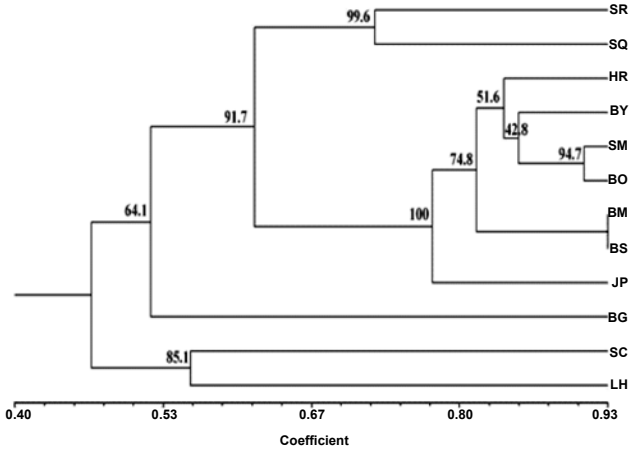

Fig. 3. Dendrogram based on the shared polymorphic amplification products resultant from the use of 22 random primer on 12 *T. patula* cultivars.

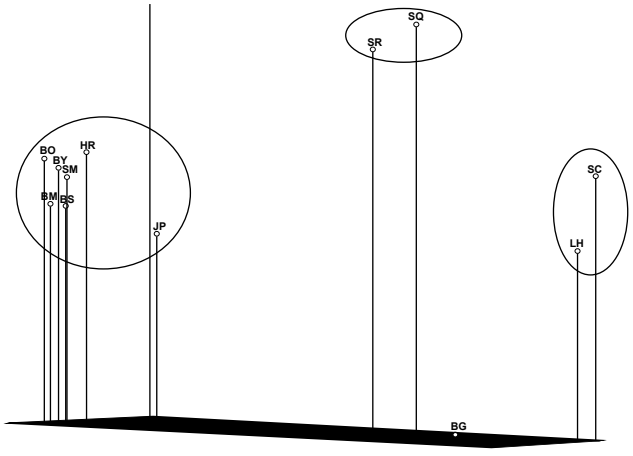

Fig. 4. Three dimensional PCA scaling of 12 *T. patula* cultivars using RAPD markers.

technique reveals an extensive amount of variation leading to clear cultivar identification (Ayana *et al*., 1). The RAPD assay also identified cultivar specific markers that have the potential of being used as diagnostic tools for cultivar identification or that could be developed into cultivar specific Sequence Characterised Amplified Region (SCAR) markers. Identification of a SCAR marker linked to a recessive male sterile gene (Tems) and its application in breeding of marigold was developed (He *et al*., 5). Conversion of the amplified fragments into stable markers makes them more convenient for use, and the sequence characterized amplified region (SCAR) markers, which is commonly exploited by breeders in selected breeding.

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