DOI : 10.5958/0974-0112.2018.00018.X

Indian J. Hort. 75(1), March 2018: 105-110

DNA fingerprinting in African marigold (*Tagetes erecta* L.) genotypes using ISSR and URP markers

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ABSTRACT

Genetic diversity of 22 marigold (*Tagetes erecta* L.) genotypes was evaluated with Inter Simple Sequence Repeats (ISSR) and Universal Rice Primers (URP) markers. A total of 31 molecular markers comprising of 19 ISSR and 12 URP markers were utilized for the study. A total of 184 amplicons were amplified using 19 ISSR primers, out of which 171 (92.73%) were polymorphic. The polymorphic information content (PIC) ranged from 0.23 to 0.47 with an average of 0.34. The resolving power (RP) ranged from 7.00 to 16.00 with average of 11.20. The marker index (MI) ranged from 0.77 to 4.83 with an average of 2.93. In case of URP markers, a total of 131 amplicons were amplified of which 122 (93.08%) were polymorphic with 12 URP primers. The polymorphic information content (PIC) ranged from 0.17 to 0.39 with an average of 0.32. The resolving power (RP) ranged from 8.82 to 22.45 with average of 13.75. The marker index (MI) ranged from 0.55 to 5.87 with an average of 3.17. The Jaccard's similarity coefficient ranged from 0.33 to 0.81 in ISSR markers and 0.33 to 0.86 for URP markers which suggests a wide range of genetic divergence for marigold genotypes. UPGMA method was used for cluster analysis which categorised 22 genotypes in two main clusters at 0.44 similarity coefficient in both ISSR and URP markers. The two male sterile lines formed a distinct group as confirmed through both the marker systems.

Key words: *Tagetes erecta*, genetic diversity, DNA markers.

INTRODUCTION

Genus *Tagetes* (Asteraceae) is native of South and Central America, especially Mexico. It is commonly known as marigold and comprises of approximately 50 species. Due to its high adaptability to various agro climatic conditions, it is being grown as a major loose flower crop in many parts of India. Moreover, it ranks first among nation's loose flower area and production. Two species, especially *Tagetes erecta* L. (African marigold), *Tagetes patula* L. (French marigold) are most common in cultivation under Indian scenario for loose flower production. Nowadays, it is also being used as landscape plant as well as a cut flower. Marigold is also rich source of various value added compounds *viz*. essential oils, carotenoid pigments etc. which finds applications in many nutraceutical, pharmaceutical and cosmetic industries.

Estimation of genetic diversity is the prerequisite step for any crop improvement programme as genetically diverse genotypes provides wide gene pool for the isolation of favourable gene combinations through various crop improvement techniques. Inter simple sequence repeats (ISSR) which has been proved useful for detecting polymorphisms among accessions in various crop plants offer enormous potential for resolving intra and intergenomic relationships (Zietkiewicz *et al*., 16). ISSR markers are useful for detecting polymorphism and overcome some limitations of other marker system like low reproducibility of RAPD, high cost of AFLP and the need to know the flanking sequences to develop species specific primers for SSR polymorphism. The reason of high reproducibility of ISSR markers may be due to use of long primers (16-25 mers) as compared to RAPD (10 mers) and high annealing temperature (45-60ºC) which leads to high stringency (Reddy *et al*.,11). Namita *et al*. (7) studied genetic diversity of 15 marigold genotypes with 12 ISSR markers and revealed 60.48% polymorphism. Kumar *et al*. (5) reported 86.48% polymorphism with 23 ISSR markers in 75 genotypes of chrysanthemum. Zeng *et al*. (15) reported that ISSR markers serve as a potential tool to assess the genetic diversity of genus *Tagetes*.

Universal Rice Primers (URP) derived from repeat sequences of rice, was first developed by Kang *et al.* (4) and have been proved to be better DNA markers for diversity analysis across genomes. Dikshit *et al*. (1) studied genetic differentiation of 70 *Vigna* genotypes of genus using five universal rice primers (URP) and recorded 71-100% polymorphism. Jhang *et al*. (3) studied genetic variability in 40 elite indigenous breeding lines of subtropical carrots using 10 universal

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rice primers (URP). In marigold, meager research has been carried on assessing genetic diversity with molecular markers. The present study for the first time added a new marker system, *i.e.* URP for molecular analysis of marigold.

MATERIALS AND METHODS

Plant material comprised of 22 marigold genotypes, *viz*. three male sterile lines (MS-5, MS-7 and MS-8), two varieties, namely, Pusa Narangi Gainda (PNG) and Pusa Basanti Gainda (PBG) and 17 selections (Af.Sel.1, Af.Sel.2, Af.Sel.3, Af.Sel.4, Af.Sel.5, Af.Sel.6, Af.Sel.7, Af.Sel.8, Af.Sel.9, Af.Sel.10, Af.Sel.11, Af.Sel.12, Af.Sel.13, Af.Sel.14, Af.Sel.15, Af.Sel.16, Af.Sel.17). The genotypes were grown and maintained at the research farm, Division of Floriculture and Landscaping, ICAR-IARI, New Delhi. All the uniform cultural practices were undertaken to raise healthy crop. The laboratory work was undertaken at ICAR- National Research Centre on Plant Biotechnology, New Delhi. Genomic DNA from young healthy leaves of marigold seedlings was extracted with CTAB (Cetyl Trimethyl Ammonium Bromide) method with minor modifications (Murray and Thomson, 6; Saghai-Maroof *et al*., 14). Purification of DNA samples was done and further it was quantified and assessed for quality in 0.8% agarose gel electrophoresis. Final concentration of working samples was made 25 ng/µl.

A total of 19 ISSR (Inter Simple Sequence Repeats) and 12 URP (Universal Rice Primers) markers were used for PCR amplification in 22 marigold genotypes. PCR amplification conditions for ISSR primers were: initial extended step of DNA denaturation at 94°C for 4 minutes followed by 35 cycles of denaturation at 94°C for 1 min. primer annealing at 57°C for 1 min. and elongation at 72°C for 2 min., followed by an elongation step at 72°C for 10 min. The reaction products were mixed with 2 µl of 10 × loading dye. The amplification products were separated on 1.5% agarose gels. Electrophoresis was carried out at 120 V for 2.5 h using horizontal gel electrophoresis system (Bio-Rad). A 1kb DNA ladder (Gene Ruler, Fermentas) was run alongside the amplified products for the determination of approximate band size of PCR product. The resolved amplification products were visualized by using UV-transilluminator and gels was photographed under gel documentation system (Flourchem™ 5500, Alpha Innotech, USA). For URP markers, all the PCR amplification conditions were same as used for ISSR markers except that the annealing temperature was set at 55°C.

For data analysis, the scoring of DNA bands was done manually and all amplifications were repeated twice and only reproducible bands were considered for analysis.The presence of bands were recorded (1) and absence were recorded (0) and missing data was denoted by 9 in order to construct a binary matrix. The discriminatory power of markers was calculated by three parameters, *viz*. polymorphic information content (PIC), resolving power (Rp) and marker index (MI). The polymorphic information content (PIC) was calculated as proposed by Roldan-Ruiz *et al*. (13). The resolving power (Rp) of primers was calculated according to Prevost and Wilkinson (10). The marker index (MI) was calculated as proposed by Powell *et al*. (9). Similarity index values for ISSR and URP markers were calculated for all the possible pair wise comparisons using Jaccard's similarity coefficient (Jaccard, 2). The similarity matrix was subjected to cluster analysis by unweighted pair group method for arithmetic average (UPGMA) and a dendrogram was generated. Computation for multivariate analysis was done using NTSYS-pc Version 2.1 (Numerical Taxonomic System) software (Rohlf, 12). The reliability of the node of UPGMA tree was tested by bootstrap analysis using 1000 permutations.

RESULTS AND DISCUSSION

Total 19 ISSR primers generated 184 reproducible amplicons, out of which 171 were found polymorphic (Table 1). The number of amplicons per primer ranged from 6 (ISSR-24) to 13 (ISSR-1, ISSR-5, ISSR-12 and ISSR-16), with an average of 9.68 amplicons per primer. The number of polymorphic amplicons was observed as 9 amplicons per primer. This was in close confirmation with many researchers viz., as 10.4 amplicons per primer in rose (Panwar, 8), 6.75 amplicons per primer by Namita *et al*. (7) in marigold. The percentage of polymorphism in our study ranged from 63 to 100% with average polymorphism of 92.73%, which was higher than obtained by Kumar *et al.* (5) in chrysanthemum (86.48%) and Namita *et al.* (7) in marigold (60.48%). The PIC value ranged from 0.23 (ISSR-27) to 0.47 (ISSR-32) with average of 0.34. High PIC values were also observed for the primers, *viz.* ISSR-9, ISSR-21, ISSR-11, ISSR-17 and ISSR-20. These results are in confirmation with Namita *et al.* (7) in marigold. The RP ranged from 7.00 (ISSR-22) to 16.00 (ISSR-16) with average of 11.20. Other primers having higher RP values were ISSR-15, ISSR-17, ISSR-20, ISSR-5 and ISSR-12. The marker index ranged from 0.77 (ISSR-7) to 4.83 (ISSR-17) with an average of 2.93. The other primers recorded higher marker index values were ISSR-20, ISSR-12 and ISSR-21. These results are in confirmation with Namita *et al.* (7) in marigold. The Jaccard's similarity coefficient for ISSR markers ranged from 0.33 to 0.81.

Table 1. Details of banding pattern and discriminative statistics of ISSR markers.

Primer	Sequence (5'-3')	TB	PB	P(%)	PIC (SD)	RP	MI
ISSR-1	CAC ACA CAC ACA CAC ARG	13	13	100	0.27 (±0.11)	7.18	3.55
ISSR-2	CAC ACA CAC ACA CAC ARC	8	6	75	0.26 (±0.21)	9.91	1.19
ISSR-5	GAG AGA GAG AGA GAG AYG	13	12	92	0.32 (±0.17)	13.55	3.56
ISSR-7	AGA GAG AGA GAG AGA GYC	8	5	63	0.24 (±0.22)	10.73	0.77
ISSR-9	ACA CAC ACA CAC ACA CYG	9	9	100	0.41 (±0.09)	10.45	3.67
ISSR-10	GTG TGT GTG TGT GTG TYC	7	7	100	0.35 (±0.13)	7.82	2.48
ISSR-11	GTG TGT GTG TGT GTG TYG	7	7	100	0.40 (±0.14)	8.55	2.83
ISSR-12	AGA GAG AGA GAG AGA GYG	13	13	100	0.36 (±0.13)	13.45	4.74
ISSR-15	AGA GAG AGA GAG AGA GC	11	9	82	0.32 (±0.19)	15.45	2.32
ISSR-16	AGA GAG AGA GAG AGA GG	13	12	92	0.34 (±0.18)	16.00	3.80
ISSR-17	GAG AGA GAG AGA GAG AT	12	12	100	0.40 (±0.10)	14.09	4.83
ISSR-20	GTG TGT GTG TGT GTG TC	12	12	100	0.40 (±0.14)	13.91	4.76
ISSR-21	TCT CTC TCT CTC TCT CG	10	10	100	0.41 (±0.10)	12.82	4.08
ISSR-22	TCT CTC TCT CTC TCT CC	7	7	100	0.33 (±0.15)	7.00	2.33
ISSR-24	CTC TCT CTC TCT CTC TRG	6	6	100	0.35 (±0.16)	8.73	2.10
ISSR-27	BDB CAC ACA CAC ACA CA	10	8	80	0.23 (±0.21)	10.45	1.49
ISSR-30	HVH TGT GTG TGT GTG TG	9	8	89	0.24 (±0.14)	12.73	1.70
ISSR-31	AGA GAG AGA GAG AGA GVC	9	8	89	0.32 (±0.16)	12.73	2.27
ISSR-32	CCC GTG TGT GTG TGT GT	7	7	100	0.47 (±0.04)	7.27	3.26
Total	19 primers	184	171	92.73	0.34 (±0.16)	11.20	2.93

TB = Total bands, PB = Polymorphic bands, P(%) = Per cent polymorphism, PIC = Polymorphism Information Content, RP = Resolving Power, MI = Marker Index, SD = Standard Deviation

Total 12 URP primers generated 131 reproducible amplicons, out of which 122 were polymorphic (Table 2). The number of amplicons per primer ranged from 5 (URP-25 F) to 16 (URP-9F), with an average of 10.91 amplicons per primer. The average number of polymorphic amplicons per primer was 10.16. The percentage of polymorphism ranged from 73.00 to 100.00% with an average of 93.08%, which was similar to those obtained by Jhang *et al*. (3) in carrot (94.0%) and Dikshit *et al*. (1) in *Vigna* (94.2%). The average PIC value was 0.32 and ranged from 0.17 (URP-25F) to 0.39 (URP-2R). Other primers having higher PIC values were URP-30F, URP-4R, URP-13R and URP-32F. The resolving power ranged from 8.82 (URP-30F) to 22.45 (URP-9F) with average of 13.75. Other primers having higher RP values were URP-6R, URP-1F, URP-17R, URP-2F and URP-2R. Jhang *et al*. (3) reported Rp (6.91) in carrot for URP markers.The marker index ranged from 0.55 (URP-25F) to 5.87 (URP-2R) with an average of 3.17. The other primers recorded higher marker index values were URP-30F, URP-32F, URP-6R and URP-9F. The Jaccard's similarity coefficient for URP markers ranged from 0.33 to 0.86.

The dendrogram generated from the Jaccard's similarity values using NTSYS software based on genotyping data generated by 19 ISSR and 12 URP primers. For ISSR markers at similarity coefficient 0.44 the genotypes were grouped in two main clusters (Fig. 1). The cluster I represent maximum number of genotypes (20), whereas only two genotypes were grouped in cluster II. Further cluster I was divided into two sub-clusters consequently named as cluster IA and cluster IB. The sub-cluster IA consisted of 4 genotypes, namely, Af.Sel.1, Af.Sel.10, Af.Sel.17 and Af.Sel.7, whereas sub-cluster IB consisted of 16 genotypes, namely, Af.Sel.4, Af.Sel.9, Af.Sel.2, Af.Sel.5, Af.Sel.11, Af.Sel.15, Af.Sel.12, Af.Sel.8, Af.Sel.16, Af.Sel.13, Af.Sel.6, Af.Sel.3, PNG, PBG, Af.Sel.14 and MS-5. Cluster II grouped genotypes MS-7 and MS-8.

For URP markers also the genotypes were grouped in two main clusters at similarity coefficient 0.44 (Fig. 2). The cluster I represented maximum number of genotypes (20), whereas only two were represented by cluster II. Further Cluster I was grouped into two sub-clusters, namely, sub-cluster IA and sub-cluster IB. The sub-cluster IA consisted

Table 2. Details of banding pattern and discriminative statistics of URP markers.

Primer	Sequence (5'-3')	TB	PB	P(%)	PIC (SD)	RP	MI
URP-1F	ATCCAAGGTCCGAGACAAC	13	12	92	0.25 (±0.13)	15.45	2.75
URP-2F	GTGTGCGATCAGTTGCTGGG	10	10	100	0.35 (±0.14)	14.64	3.48
URP-2R	CCCAGCAACTGATCGCACAC	15	15	100	0.39 (±0.12)	14.27	5.87
URP-4R	AGGACTCGATAACAGGCTCC	9	9	100	0.37 (±0.10)	12.00	3.32
URP-6R	GGCAAGCTGGTGGGAGGTAC	12	12	100	0.30 (±0.18)	17.36	3.65
URP-9F	ATGTGTGCGATCAGTTGCTG	16	13	81	0.34 (±0.20)	22.45	3.57
URP-13R	TACATCGCAAGTGACACAGG	9	9	100	0.37 (±0.15)	10.45	3.29
URP-17R	AATGTGGGCAAGCTGGTGGT	11	10	91	0.32 (±0.15)	15.18	2.95
URP-25F	GATGTGTTCTTGGAGCCTGT	5	4	80	0.17 (±0.12)	9.00	0.55
URP-30F	GGACAAGAAGAGGATGTGGA	10	10	100	0.38 (±0.13)	8.82	3.79
URP-32F	TACACGTCTCGATCTACAGG	10	10	100	0.37 (±0.11)	12.82	3.67
URP-38F	AAGAAGCATTCTACCACCAC	11	8	73	0.19 (±0.18)	12.55	1.09
Total	12 Primers	131	122	93.08	0.32 (±0.16)	13.75	3.17

TB : Total bands, PB: Polymorphic bands, P(%): Per cent polymorphism, PIC: Polymorphism Information Content, RP: Resolving Power, MI: Marker Index, SD: Standard Deviation

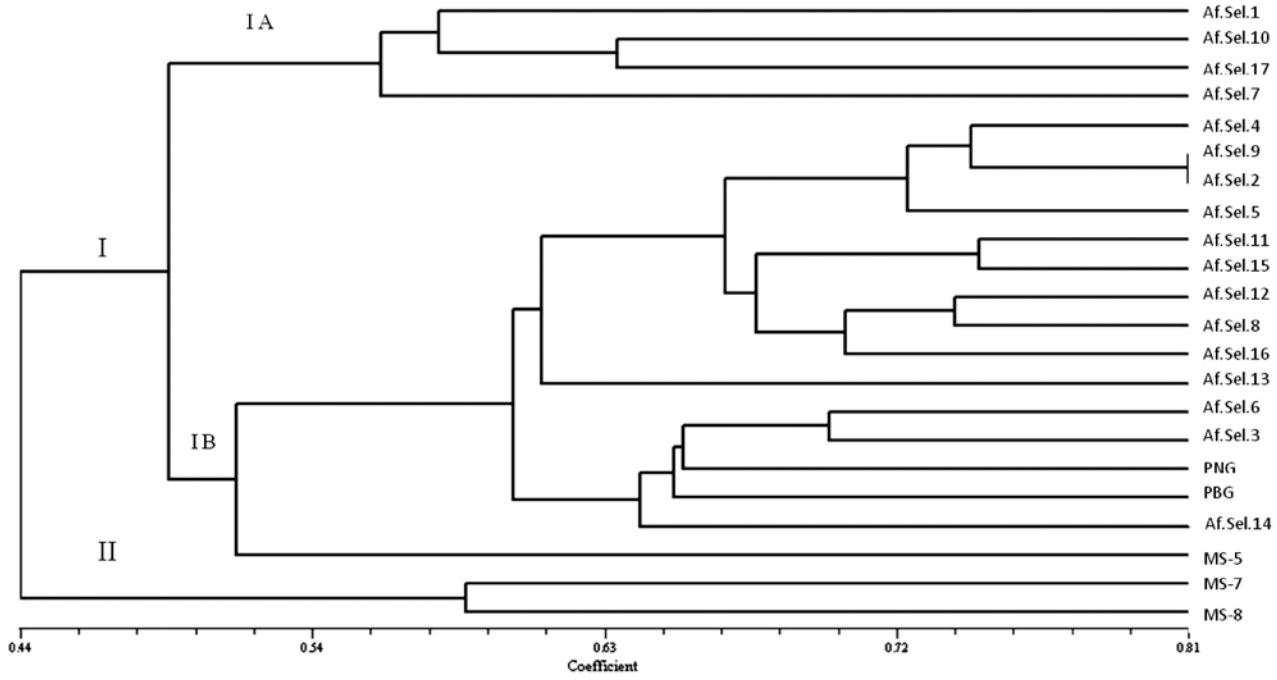

Fig. 1. Dendrogram based on the similarity index values of 22 marigold genotypes using ISSR markers.

of 19 genotypes, namely, Af.Sel.1, Af.Sel.7, Af.Sel.6, PBG, Af.Sel.4, Af.Sel.9, Af.Sel.2, Af.Sel.3, Af.Sel.5, Af.Sel.11, Af.Sel.12, Af.Sel.15, Af.Sel.16, PNG, Af.Sel.8, Af.Sel.14, Af.Sel.13, Af.Sel.10 and Af.Sel.17, whereas sub-cluster IB consisted of one genotype, *i.e.* MS-5. Cluster II consisted of 2 genotypes (MS-7 and MS-8). In both the cases the two male sterile lines MS-7 and MS-8 are represented by separate cluster, which showed their genetic similarity between themselves.

The assessment of different marker systems (ISSR, URP) presented here is useful to evaluate suitability of various marker systems in crop plants. The study provides an additional marker resource by demonstrating use of URP for genotyping of marigold genotypes. Moreover, the genetic diversity analysed

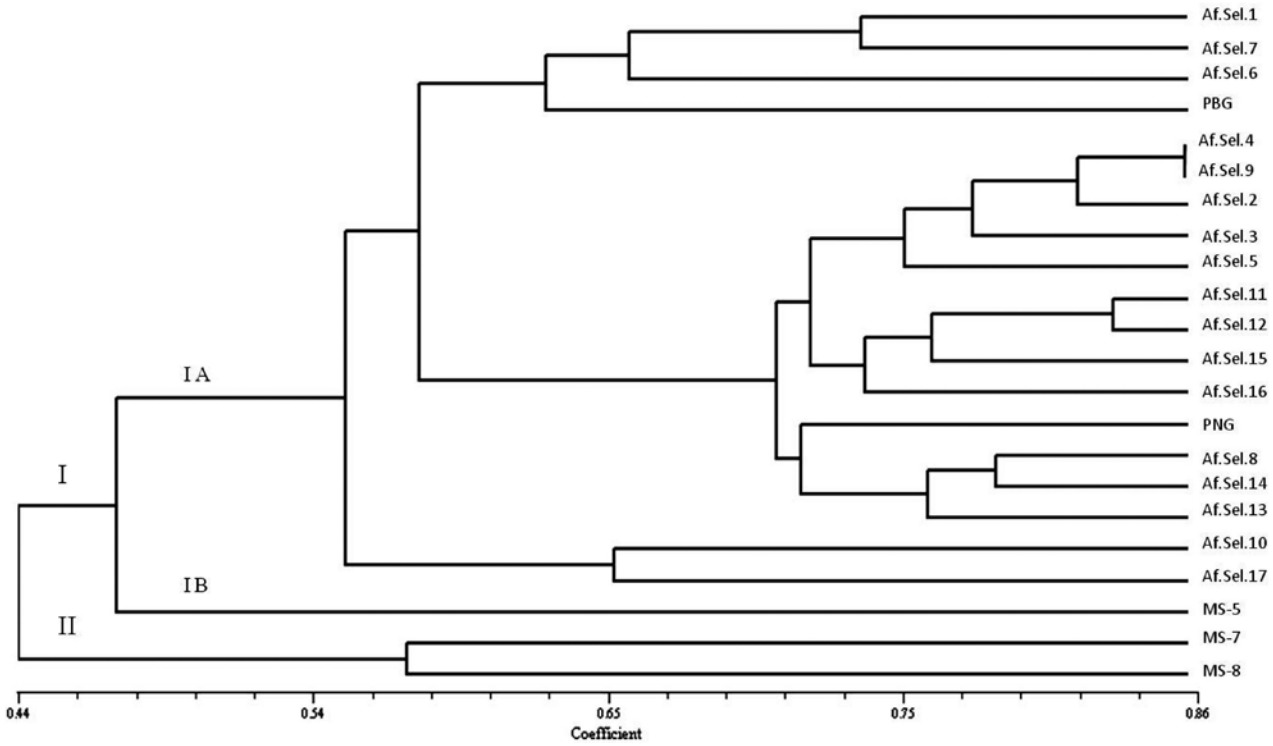

Fig. 2. Dendrogram based on the similarity index values of 22 marigold genotypes using URP markers.

among marigold genotypes will be useful to exploit genetic resources in more effective manner. The high level of genetic variability among the cultivars would be useful for selecting parents in the development of elite marigold varieties which will be further utilized for genome mapping and breeding.

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Received : December, 2016; Revised : November, 2017; Accepted : January, 2018