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Genetic diversity analysis of marigold (*Tagetes* sp) genotypes using RAPD and ISSR markers

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ABSTRACT

The genetic diversity of 15 genotypes of *Tagetes erecta* L. (African marigold; Af/ws-1, Af/ws-2, Af/ws-3, Af/ws-4, Af/ws-5, Af/ws-6 and Af/ws-7) and *Tagetes patula* L. (French marigold; Fr/ws-1, Fr/ws-2, Fr/ws-3, Fr/ws-4, Fr/ws-5, Fr/ws-6, Fr/ws-7 and Fr/ws-8) were assessed using PCR based RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeat) markers. Twenty three RAPD primers were used to generate polymorphism and mean per cent polymorphism was found as 57.72% ranging from 33.33% (OPF-2) to 83.33% (RAPD-07). Five most informative primers (OPA-02, OPA-04, OPO-10, RAPD-07 and RAPD-18) were identified on the basis of level of polymorphism detected by individual primer. Twelve ISSR primers were also used to generate polymorphism and mean per cent polymorphism was 60.48% ranging from 40.00% (ISSR-02, ISSR-17) to 90.00% (ISSR-03). The five most informative primers (ISSR-03, ISSR-06, ISSR-12, ISSR-31 and ISSR-18) were also identified on the basis of polymorphism. The dendrogram obtained from UPGMA cluster analysis of Jaccard's similarity values based on 23 RAPD and 12 ISSR markers grouped 15 genotypes into two clusters species wise (*Tagetes erecta* and *Tagetes patula*) which confirm the reliability of PCR based molecular markers. PCA analysis also confirmed the grouping of genotypes as they were present in same group as revealed by RAPD and ISSR markers.

Key words: Cluster analysis, Genetic diversity, PCA, Polymorphism, RAPD and ISSR markers, *Tagetes* sp

Marigold (*Tagetes* sp) is one of the most important commercial flowers grown world over and are highly valued for their spectacular flowers, brilliant colours, delightful appearance, fragrance and is endowed with large spectrum of commercial potentialities in industrial sector. *Tagetes erecta* L. and *Tagetes patula* L. are more commonly grown for loose flower production and used in landscape architecture due to their variable height and flower colour. It is also an important source of carotenoids which are used in poultry to intensify the colour of yolk (Kaul *et al.* 1997). Marigold, being a cross-pollinated crop, provides an abundant scope for exploitation through heterosis. The success of a breeding programme depends upon the amount of genetic variability in the germplasm and genetic relationships among desirable traits. In this context, molecular markers provide a quick and reliable method for estimating genetic diversity and relationship among genotypes of any organism (Thormann *et al.* 1994). The large number of genotypes for polymorphic loci can be screened using molecular markers.

Among the different DNA based molecular markers, Random amplified polymorphic DNA (RAPD; Williams *et al.* 1990) have been utilized for genetic diversity study (Mor *et al.* 2008), to identify cultivars and hybrids (Sureja *et al.* 2006). Most of the studies to test reliability of RAPD markers in estimating inter varietal and specific relationship found to be as reliable as to RFLPs (Srivastava *et al.* 2007). Another marker, The inter simple sequence repeat (ISSR) is a multilocus marker system and in the analysis involves the polymerase chain reaction amplification of region between two microsatellites, using a single simple sequence repeat (SSR) motif containing primer anchored at the 3 or 5 end by two to four arbitrary often degenerate nucleotides (Zietkiewick *et al.* 1994). ISSR have been extensively used in genetic diversity analysis of rose (Panwar 2009), for identification of accession in cucurbits (Verma *et al.* 2007). Like RAPD, it does not require prior knowledge of the genome, cloning or specific primer design and have high reproducibility than

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RAPDs because of high annealing temperatures. However, there are very few published reports on the analysis of genetic diversity based on RAPD and ISSR markers in marigold. Hence, in the present study, both RAPD and ISSR markers were employed to study the extent of genetic diversity among different genotypes of marigold.

MATERIALS AND METHODS

The planting material utilized for the present study consisted 15 elite breeding lines of *Tagetes erecta* (Af/ws-1, Af/ws-2, Af/ws-3, Af/ws-4, Af/ws-5, Af/ws-6 and Af/ws-7) and *Tagetes patula* (Fr/ws-1, Fr/ws-2, Fr/ws-3, Fr/ws-4, Fr/ws-5, Fr/ws-6, Fr/ws-7 and Fr/ws-8). These were grown during the crop season of 2009-10 at Research Farm of Division of Floriculture and Landscaping, Indian Agricultural Research Institute and diversity analysis using molecular markers was done in NRC for Plant Biotechnology, New Delhi, India. The origin and salient features of the marigold genotypes used in this study are given in Table 1.

Total genomic DNA was extracted from five gram of young and healthy leaves of 15 days old seedlings of all the genotypes using CTAB (Cetyl trimethyl ammonium bromide) method (Murray and Thompson 1980) with minor modifications. The extracted DNA solution was purified and concentration of DNA was estimated by electrophoresis through 0.8% (w/w) agarose gels.

RAPD analysis

A random set of decamer oligonucleotides (operon Technologies Inc., USA) consisting of 54 primers were screened for the RAPD analysis (Williams *et al.* 1990) using 15 genotypes. Out of these, 23 which gave sufficient polymorphism were used for assessing genetic diversity among genotypes. The amplification reactions were carried out in a 25 µl volume containing 10X PCR buffer without $MgCl_2$, 25mM $MgCl_2$, 10mM dNTPs (dATP, dCTP, dGTP and dTTP), 10 p mol/µl primer, 3 U/µl *Taq* DNA Polymerase (Bangalore Genei, India) and 12.5 ng/µl genomic DNA template. DNA amplifications were performed in duplicate in a DNA thermal cycler (Perkins-Elmer, Boston, MA, USA) programmed for a preliminary step of RAPD amplification. DNA was denatured at 94°C for 5 minutes followed by 40 amplification cycles (94°C for 1 minute; 35°C for 1 minute; 72°C for 2 minutes) and a final extension step at 72°C for 10 minutes. The amplified products were resolved by electrophoresis in 2% agarose gel run in 1X TBE buffer and amplified fragments were visualized by ethidium bromide staining. The resolved product was photographed under UV light using gel documentation Flourchem^{TM} 5500 (Alpha InfoTech, USA.).

A random set of 32 primers were used for the ISSR analysis (Zietkiewick *et al.* 1994). Out of these, 12 primers which gave sufficient polymorphism were used for assessing plant to plant variation using 15 genotypes. The PCR amplification reactions were carried out in a 25 µl reaction volume containing 10X PCR buffer without $MgCl_2$, 25mM $MgCl_2$, 10mM dNTPs (dATP, dCTP, dGTP and dTTP), 10 p mol/µl primer, 3 U/µl *Taq* DNA Polymerase (Bangalore Genei, India) and 12.5 ng/µl genomic DNA template. DNA amplifications were performed in duplicate in a DNA thermal cycler (Perkins-Elmer, Boston, MA, USA) programmed for a preliminary step of RAPD amplification. Denaturation was done at 94°C for 5 minutes; followed by 45 cycles at 94°C for 1 minute; 57°C for 1 minute; 72°C for 2 minutes and a final extension at 72°C for 7 minutes. Further the amplified PCR products were resolved in a similar manner as in RAPD

Table 1 Salient characteristics of marigold (*Tagetes* sp) genotypes

Genotype (Inbred line)	Species	Flower form	Flower diameter*	Flower colour	Source
Af/ws-1	*Tagetes erecta*	Double	Medium	Orange	IARI, New Delhi
Af/ws-2	*Tagetes erecta*	Double	Medium	Light orange	IARI, New Delhi
Af/ws-3	*Tagetes erecta*	Single	Small	Yellow	IARI, New Delhi
Af/ws-4	*Tagetes erecta*	Single	Small	Maroon	IARI, New Delhi
Af/ws-5	*Tagetes erecta*	Double	Medium	Yellow	IARI, New Delhi
Af/ws-6	*Tagetes erecta*	Semi double	Medium	Orange	IARI, New Delhi
Af/ws-7	*Tagetes erecta*	Single	Small	Golden yellow	IARI, New Delhi
Fr/ws-1	*Tagetes patula*	Single	Small	Golden yellow	IARI, New Delhi
Fr/ws-2	*Tagetes patula*	Single	Small	Maroon petal with yellow margin	IARI, New Delhi
Fr/ws-3	*Tagetes patula*	Single	Small	Dark maroon petal with yellow margin	IARI, New Delhi
Fr/ws-4	*Tagetes patula*	Single	Small	Golden yellow	IARI, New Delhi
Fr/ws-5	*Tagetes patula*	double	Medium	Yellow petal with maroon margin	IARI, New Delhi
Fr/ws-6	*Tagetes patula*	Semi double	Medium	Golden yellow	IARI, New Delhi
Fr/ws-7	*Tagetes patula*	Semi double	Medium	Yellow	IARI, New Delhi
Fr/ws-8	*Tagetes patula*	Double	Medium	Maroon	IARI, New Delhi

*Flower diameter (Small: 0-5.00 cm; medium: 6.00-9.00 cm; large: >9.00 cm)

analysis.

The scoring of reproducible DNA bands (bands present in both repetitions of an individual sample) was done manually. Weak bands with negligible intensity were excluded from the final data analysis. The scoring of band profiles for each parent was carried out in a binary mode (1 indicating its presence: 0 indicating its absence). Similarity index values for RAPD and ISSR patterns were calculated for all the possible pair wise comparisons, using Jaccard's (genetic) similarity coefficient (GS): a/(n-d), where 'a' is the number of positive matches; 'd' is the number of negative matches and 'n' is the total sample size (Jaccard 1908). The resolving power for each primer was calculated using formula, $Rp = \sum I_b$, where $I_b = 1 - \{2x\ (0.5 - p)\}$, where 'p' is the proportion of the 15 genotypes containing the bands (Prevost and Wilkinson 1999).

The basic information that determines their application in genetic mapping both the marker systems RAPD and ISSR were calculated by using the polymorphism information content (PIC) (Lynch and Walsh 1998). It was calculated by using formula, $PIC = 1 - \sum pi^2$, where pi is the frequency of the ith alleles. The marker index (MI) as proposed by Powell *et al.* (1996) and used by Milbourne *et al.* (1997) was also calculated. Computation for multivariate analysis was done using NTSYS-pc Version 2.1 (Numerical Taxonomic System) software (Rohlf 2000) and similarity matrix was subjected to the cluster analysis of Unweighted Paired Group method using Arithmetic Averages (UPGMA) and dendrogram was constructed. The Jaccard's similarity matrix was subjected to principal coordinate analysis (PCA). This coordination method makes use of multidimensional solution of the observed relationships. PCA resolves complex relationships into interactions of fewer and simpler factors.

RESULTS AND DISCUSSION

RAPD polymorphism

All 23 RAPD primers efficiently amplified DNA fragments from the marigold samples to generated polymorphism. Darokar *et al.* (2000) used 20 and Mor *et al.* (2008) 25 decamer random primers for studying polymorphism in *Tagetes* species of which only 15 primers amplified template DNA and generated polymorphism. The number of bands per primer ranged from 8 (RAPD-05, RAPD-18, RAPD-22 and OPO-10) to 15 (OPA-04) with an average of 10.69 bands per primer (Table 2). The variation in number

Table 2 Nucleotide sequences of RAPD primers, TNB and NPB per primer, polymorphism, PIC, Rp, MI and size of bands

Primer	Sequence	TNB	NPB	P (%)	PIC	Rp	MI	Band size range (bp)
RAPD-04	5´- TGGTGACTGA-3´	12	5	41.66	0.212	4.18	2.54	400-1500
RAPD-05	5´- TGCCGAGCTG-3´	8	6	75.00	0.159	9.21	1.27	300-1100
RAPD-07	5´- GGTGCACGTT-3´	12	10	83.33	0.427	1.63	5.12	550-3300
RAPD-09	5´- AGGTGACCGT-3´	11	8	72.72	0.177	1.81	1.94	700-3000
RAPD-10	5´- GGGTAAAGCC-3´	9	5	55.55	0.143	2.54	1.28	450-1400
RAPD-11	5´- CCATGCGGAG-3´	12	7	58.33	0.201	4.06	2.41	250-1550
RAPD-13	5´- TTTGGGCCCC-3´	9	4	44.44	0.150	3.09	1.35	450-1500
RAPD-15	5´- CTGTCTGTGG-3´	13	8	61.53	0.193	2.16	2.50	300-1250
RAPD-18	5´- CTGCCACGAG-3´	8	6	75.00	0.099	5.18	0.79	250-1100
RAPD-19	5´- GAGCAAGGCA-3´	12	6	50.00	0.203	3.27	2.43	300-1550
RAPD-20	5´- CTACGATGCC-3´	11	6	54.54	0.204	1.72	2.24	450-1200
RAPD-21	5´- CCAGTCCCAA-3´	11	4	36.36	0.201	4.00	2.21	350-1500
RAPD-22	5´- TTACCCCGCT-3´	8	5	62.50	0.135	2.54	1.08	400-1300
OPA-02	5´- TGCCGAGCTG-3´	9	7	77.77	0.195	2.90	1.75	350-1350
OPA-04	5´- AATCGGGCTG-3´	15	12	80.00	0.172	4.80	2.58	500-1350
OPA-10	5´- GTGATCGGAC-3´	14	7	50.00	0.144	1.82	2.01	350-1250
OPB-08	5´-GTCCACGCAG -3´	12	5	41.66	0.168	3.18	2.11	350-1650
OPF-12	5´-ACGGTACCAG -3´	9	3	33.33	0.177	3.09	1.59	400-1450
OPG-09	5´-CGATCCGCGC -3´	9	4	44.44	0.247	2.72	2.22	550-1100
OPO-10	5´-TCAGAGGCGCC-3´	8	6	75.00	0.213	4.36	1.70	300-1150
OPP-10	5´-TCCCGCCTAC -3´	11	7	63.63	0.245	4.12	2.69	400-1200
OPS-08	5´- TTCAGGGTGG -3´	12	6	50.00	0.136	5.62	1.63	300-1450
OPZ-03	5´-CAGCACCGCA -3´	11	5	45.45	0.196	2.10	2.15	250-1650

TNB, Total number of bands; NPB, number of polymorphic bands; P, polymorphism; PIC, polymorphism information content; Rp, resolving power; MI-Marker index.

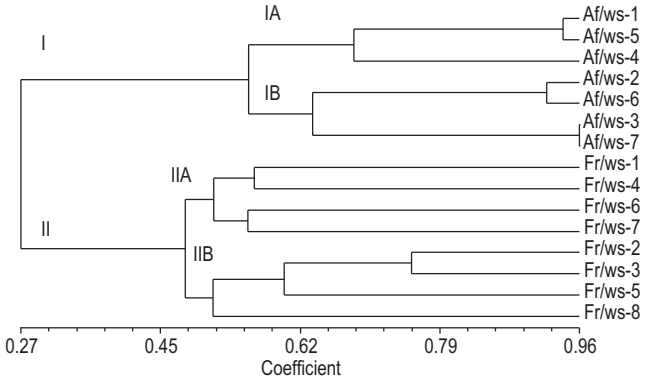

Fig 1 Dendrogram depicting genetic relationships among all genotypes of marigold based on RAPD markers, using Jaccard's coefficient of similarity

of bands for each primer may be due to the dependence of efficiency of the primer on the primary sites available. The size of the amplified products varied from approximately 250 bp to 3300 bp. In parallel to this experiment in marigold, Mor *et al.* (2008) obtained the band size from 235 to 2027 bp (base pair). These RAPD primers provided 246 reproducible bands. Of these 142 (57.72%) were polymorphic and the rest were monomorphic. The mean polymorphism level (i e per cent polymorphism) calculated as number of polymorphic bands/total bands per primer was 57.92% ranging from 33.33% (OPF-12) to 83.33% (RAPD-07) among 23 primers. The maximum number of polymorphic bands (12) was obtained with primer OPA-04. The average number of polymorphic bands was 6.17 per primer. Sixteen primers (69.56% of primers used) showed a relatively high polymorphism (>50%) and seven (30.43% of the primers used) were less informative (<50% polymorphism). Five most informative primers (OPA-02, OPA-04, OPO-10, RAPD-07 and RAPD-18) were identified on the basis of level of polymorphism detected by individual primer. These markers together amplified a unique fingerprint for all genotypes and therefore, were effective in distinguishing them from each other. The PIC was calculated for each RAPD marker and it ranged from 0.099 (RAPD-18) to 0.427 (RAPD-07) with an average of 0.191. The resolving power (Rp) of all the primers ranged from 1.63 (RAPD-07) to 9.21 (RAPD-05) with an average of 3.48. The marker index ranged from 0.79 (RAPD-18) to 5.12 (RAPD-07) with an average of 2.36 (Table 2).

The degree of RAPD polymorphism expressed as percentage of polymorphic fragments in marigold was higher than that reported in *Tagetes minuta* (7%; Daroker *et al.* 2000) and lower than *Tagetes patula* (70%; Daroker *et al.* 2000) and marigold (84%; Mor *et al.* 2008). The high level of polymorphism obtained in marigold genotypes was because of presence of mating system of strict out crossing which resulted in high level of genetic variability in the gene pool. PCR amplification profile of 15 genotypes employing random primer OPA -04 is shown in Fig 5.

ISSR polymorphism

The 12 ISSR primers were used for diversity analysis in 15 genotypes of marigold. The number of bands per primer ranged from 10 (ISSR-03, ISSR-07, ISSR-17, ISSR-29 and ISSR-31) to 15 (ISSR-02 and ISSR-12) with an average of 11.58 bands per primer. In contrast to this experiment, Zeng *et al.* (2010) obtained less number of bands per primer ranged from 5 to 11 with an average of 6.8 bands per primer. These ISSR primers provided 139 reproducible bands. Of these 81 (58.27%) were polymorphic which were less than 138 polymorphic loci detected with 10 ISSR primers in *Tagetes patula* and *T. erecta* lines (Qi *et al.* 2007). The size of amplified bands varied from approximately 250 bp to 1750 bp (Table 3). The mean per cent polymorphism was 58.57% ranging from 40.00% (ISSR-02, ISSR-17) to 90.00% (ISSR-03) among 12 ISSR primers. The maximum number of polymorphic bands (10) was obtained with ISSR-06

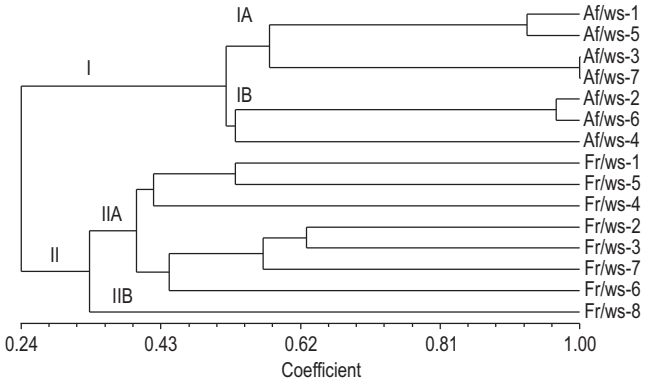

Fig 2 Dendrogram depicting genetic relationships among all genotypes of marigold based on ISSR markers, using Jaccard's coefficient of similarity

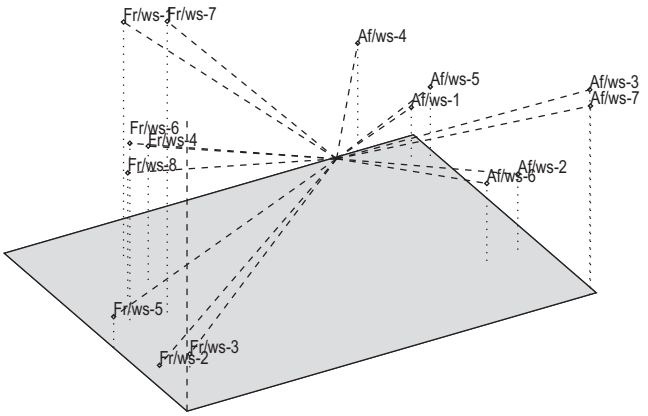

Fig 3 PCA depicting genetic relationships among all genotypes of marigold based on RAPD markers

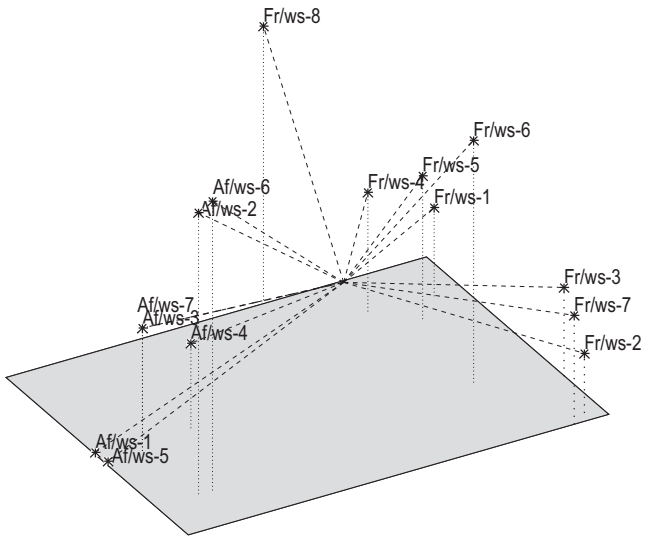

Fig 4 PCA depicting genetic relationships among all genotypes of marigold based on ISSR markers

(Table 3). The average number of polymorphic bands per primer was 6.75. Ten primers (83.33% of primers used) showed a relatively high polymorphism (>50%) and two (16.67% of the primers) used were less informative (<50% polymorphism).The five most informative primers (ISSR-03, ISSR-06, ISSR-12, ISSR-18 and ISSR-23) were identified on the basis of polymorphism detected by individual primers. These ISSR markers together amplified a unique fingerprint for all the genotypes and therefore, were effective in distinguishing them from each other. The PIC was calculated for each ISSR marker and it ranged from 0.171 (ISSR-04) to 0.267 (ISSR-03) with an average of 0.207 (Table 3). The resolving power ranged from 2.54 (ISSR-04) to 7.82 (ISSR-03) with an average of 4.53 and marker index was calculated for each primer and it ranged from 1.75 (ISSR-07) to 3.39 (ISSR-12) with an average of 2.39 (Table 3).

The ISSRs exhibited more number of bands per primer and polymorphism than RAPD markers in several crop species and these primers were found to be very informative for studying polymorphism in marigold genotypes (Qi *et al.* 2007, Zeng *et al.* 2010) and in wild sunflower (Garayalde *et al.* 2011). PCR amplification profile of 15 genotypes employing random primer OPA-04 is shown in Fig 6.

RAPD based genetic diversity and clustering pattern of marigold genotypes

Jaccard's pairwise genetic similarity coefficients were computed for all the 105 combinations of the 15 genotypes based on 23 RAPD markers. These similarity coefficients among 15 genotypes ranged from 0.17 (Fr/ws-5 *versus* Af/ws-5) to 0.96 (Af/ws-3 *versus* Af/ws-7). The lowest similarity coefficient showed maximum divergence among genotypes. All informative primers differentiated the genotypes from each other and distinct clusters or sub clusters were obtained in cluster analysis (Fig 1). The dendrogram obtained from UPGMA cluster analysis of Jaccard's similarity values based on 23 RAPD markers is presented in Fig 1. The cluster analysis resolved 15 genotypes into two major clusters. The genotypes (Af/ws-1, Af/ws-2, Af/ws-3, Af/ws-4, Af/ws-5, Af/ws-6 and Af/ws-7) of African marigold (*Tagetes erecta*) formed one cohesive cluster I and all genotypes of French marigold (*Tagetes patula*) (Fr/ws-1, Fr/ws-2, Fr/ws-3, Fr/ws-4, Fr/ws-5, Fr/ws-6, Fr/ws-7 and Fr/ws-8) were resolved in another cluster II. The cluster I was again sub-grouped into two smaller clusters containing Af/ws-1, Af/ws-5 and Af/ws-4 in sub-cluster IA and Af/ws-2, Af/ws-6, Af/ws-3 and Af/ws-7 in sub-cluster IB. Similarly, cluster II was sub-

Table 3 Nucleotide sequences of ISSR primers, TNB and NPB per primer, polymorphism, PIC, Rp, MI and size of bands

Primer	Sequence	TNB	NPB	P (%)	PIC	Rp	MI	Band size range (bp)
ISSR-02	5´-CACACACACACACACARC-3´	15	6	40.00	0.193	4.90	2.89	300-1350
ISSR-03	5´-GAGAGAGAGAGAGAGAYT-3´	10	9	90.00	0.267	7.82	2.67	500-4100
ISSR-04	5´-GAGAGAGAGAGAGAGAYC-3´	11	6	54.54	0.171	2.54	1.88	250-1200
ISSR-06	5´-AGAGAGAGAGAGAGAGYT-3´	13	10	76.92	0.228	4.36	2.96	450-3500
ISSR-07	5´-AGAGAGAGAGAGAGAGYC-3´	10	5	50.00	0.175	2.72	1.75	250-1800
ISSR-12	5´-AGAGAGAGAGAGAGAGC-3´	15	9	60.00	0.226	3.09	3.39	300-1100
ISSR-17	5´-GAGAGAGAGAGAGAGAT-3´	10	4	40.00	0.181	7.18	1.81	400-1450
ISSR-18	5´-GAGAGAGAGAGAGAGAC-3´	11	8	72.72	0.216	3.27	2.37	350-1500
ISSR-23	5´-CTCTCTCTCTCTCTC TRC-3´	13	7	53.84	0.223	5.54	2.89	250-1150
ISSR-26	5´-CTCTCTCTCTCT-3´	11	6	54.84	0.180	6.27	1.98	300-1250
ISSR-29	5´-VHVGTGTGTGTGTGTGT-3´	10	5	50.00	0.204	2.86	2.04	450-1300
ISSR-31	5´-AGAGAGAGAGAGAGAGVC-3´	10	6	60.00	0.214	3.86	2.14	500-1750

TNB, Total number of bands; NPB, number of polymorphic bands; P, polymorphism; PIC, polymorphism information content; Rp, resolving power; MI, marker index.

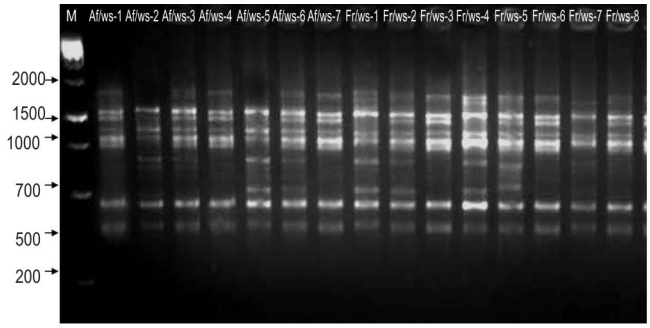

Fig 5 PCR amplification products obtained with OPA 04 primer. Lane M represents1kb marker, all lanes represent marigold genotypes

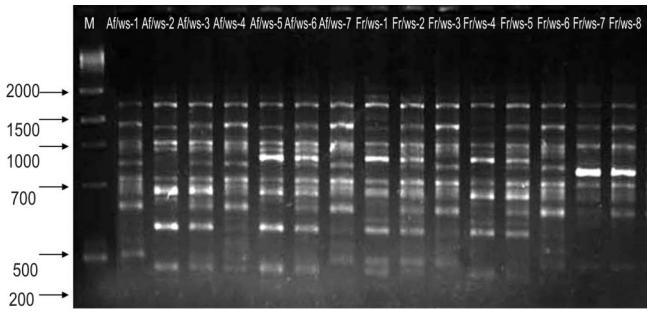

Fig 6 PCR amplification products obtained with ISSR 03 primer. Lane M represents1kb marker, all lanes represent marigold genotypes

grouped into two smaller clusters containing Fr/ws-1, Fr/ws-4, Fr/ws-6 and Fr/ws-7 in sub-cluster II A and Fr/ws-2, Fr/ws-3, Fr/ws-5 and Fr/ws-8 in sub-cluster II B. It can be observed from the dendrogram that the variation between genotypes Af/ws-3 and Af/ws-7 was the least in cluster I.

ISSR based genetic diversity and clustering pattern of marigold genotypes

Jaccard's pairwise genetic similarity coefficients were computed for all the 105 combinations of the 15 genotypes based on 12 ISSR markers. These similarity coefficients among 15 genotypes ranged from 0.14 (Fr/ws-3 *versus* Af/ws-1 and Fr/ws-3 *versus* Af/ws-5) to 1.00 (Af/ws-3 *versus* Af/ws-7). The lowest similarity coefficient showed maximum divergence among genotypes. The dendrogram obtained from UPGMA cluster analysis of Jaccard's similarity values based on 12 ISSR markers is presented in Fig 2. The cluster analysis resolved 15 genotypes into two major clusters. The genotypes (Af/ws-1, Af/ws-2, Af/ws-3, Af/ws-4, Af/ws-5, Af/ws-6 and Af/ws-7) of African marigold formed one cohesive cluster I and all genotypes of French marigold (Fr/ws-1, Fr/ws-2, Fr/ws-3, Fr/ws-4, Fr/ws-5, Fr/ws-6, Fr/ws-7 and Fr/ws-8) were resolved in another cluster II. The cluster I was again divided into two small clusters containing Af/ws-1, Af/ws-5, Af/ws-3 and Af/ws-7 in sub-cluster IA and Af/ws-2, Af/ws-6, and Af/ws-4 in sub-cluster IB. Similarly, cluster II was sub-grouped into two smaller clusters containing Fr/ws-1, Fr/ws-2, Fr/ws-3, Fr/ws-4, Fr/ws-5, Fr/ws-6 and Fr/ws-7 in sub-cluster II A and Fr/ws-8 in sub-cluster II B. It can be further observed from the dendrogram that the genotype Fr/ws-8 was the most diverse genotype within the cluster.

Clustering of genotypes based on RAPD derived dendrogram was similar when compared to ISSR derived dendrogram. In both the cases, African and French genotypes were separated from each other. The clustering of genotypes with in sub clusters was not similar when ISSR and RAPD derived dendrograms were compared. This might be due to marker sampling error and/or to the level of polymorphism detected, reinforcing the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among genotypes (Loarce *et al.* 1996). Putatively alike bands obtaining from RAPDs in different individuals are not essentially homologous, which might account for the mismatch between the RAPD and ISSR results (Fernandez *et al.* 2002). PCA analysis also confirmed the grouping of genotypes as they were present in same group as revealed by RAPD (Fig 3) and ISSR (Fig 4).

The foregoing results and discussion indicated that the RAPD and ISSR profiling offered an effective means of assessing genetic variation and thus would be useful for differentiation of elite breeding lines and varieties. Conversion of specific ISSR segments into SCAR (Sequenced characterized amplified region) markers could enhance the value of these markers for the identification of marigold genotypes. The genotypes were found to be diverse based on RAPD and ISSR markers and can be further utilized in crop improvement programmes. The high level of genetic variability among the genotypes would be useful for selecting parents in the development of new varieties. Moreover, the present study can be a major input into conservation biology and this information is helpful in many breeding programmes.

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