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Short communication

Cross amplification of SSR loci in marigold for molecular characterization

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ABSTRACT

The present study was undertaken to develop Simple Sequence Repeats in marigold by evaluating cross amplification from related genera and other taxa as the availability of microsatellite primer is very limited in this crop. A total of 33 primer pairs from Chrysanthemum and carrot were used, of which nine from the former and eight from the latter were selected for generating amplicons. This work confirmed that microsatellite primers developed for a particular species can be used across genera within and between botanical families. The shortlisted primers were utilized to characterize diverse genotypes of marigold to understand the similarities and/or differences between them.

Key words: *Tagetes erecta*, simple sequence repeats, male sterility systems

Marigold (*Tagetes erecta* L) is a multipurpose flowering plant belonging to the *Asteraceae* family. Its habit of free flowering, short duration to produce marketable flowers, wide spectrum of attractive colour, shape, size and good keeping quality attracted the attention of flower growers. In India, it is one of the most commonly grown flowers used in religious and social functions. Besides, there is great demand for marigold in food colouring industry, aromatherapy, therapeutic, cosmetic industry and traditional medicine. Presently, a wide range of neutral genetic markers is available for assessment of molecular characterization in plants. Among different classes of molecular markers, Simple Sequence Repeat (SSR) or microsatellite marker is one of the most effective and widely used marker types for assessment of molecular characterization in crops considering their co dominant inheritance along with their reproducibility, multi-allelic nature, relative abundance and high genome coverage. However, in *Tagetes* sps, only limited SSR markers have been developed and there is an urgent need to identify a set of microsatellite nuclear markers. The genomic SSRs (gSSRs) reported for Chrysanthemum (Li *et al*.,6) was tested as they belong to the same family asteraceae. gSSRs of carrot, which belongs to different taxa, were also used to test cross taxa amplification. The present study is an endeavor to evaluate the cross-amplification of chrysanthemum and carrot gSSRs primers in marigold and to evaluate the utility of selected markers in discriminating diverse marigold germplasm.

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The present study was undertaken in molecular characterization laboratory in the division of Plant Genetic Resources, ICAR-Indian Institute of Horticultural Research, Bengaluru, India during 2014-15. Twelve diverse genotypes viz., homozygous fertile (single and double flower types); apetaloid sterile and petaloid sterile (vegetatively propagated types) were used. Genomic DNA was extracted from 2g of young leaves using CTAB method (Doyle and Doyle, 4) with modifications. The PCR protocol reported for carrot SSRs (Cavagnaro *et al*., 2) and Chrysanthemum SSrs (Li *et al*., 6) was used without any modification. The amplicon data generated by transferable primers were analyzed using using software NTSYS-PC version 2.1 (Rohlf *et al*., 7). The binary data was used to generate Jaccard's similarity coefficient (Jaccard *et al*., 5). These similarity coefficients were used to construct a dendrogram depicting genetic relationships among the genotypes by employing the Unweighted Paired Group Method of Arithmetic Averages (UPGMA) algorithm and SAHN clustering. Gene diversity (H_j), also termed as the polymorphism information content (PIC) and was calculated for all the amplifying SSR. The expected heterozygosity estimated for each individual locus of the 33 genomic SSRs assayed, 17 (9 Chrysanthemum and 8 Carrot) SSRs amplified fragments in all the 12 genotypes of marigold. Eight out of 10 (80%) carrot SSR primer pairs produced amplification in all genotypes and the number of alleles generated ranged from 1-3 (GSSR 3 & GSSR5). All the amplicons generated were monomorphic. The allele size ranged from 100-400 bp. Though transferability of carrot microsatellite markers was high, none of these markers were able to differentiate different genotypes. These cross

taxa amplifiable markers can be used in marigold for characterisation as the number of available SSRs is limited to a few. Greater evolutionary distance between carrot and marigold has greatly decreased chance of successful amplification in terms of polymorphic markers. Studies have indicated that the number of SSRs amplified in a species was positively correlated with the phylogenetic relatedness of that species and the species from which the marker was signed (Saha, 8).

Nine out of 23 Chrysanthemum SSR primer pairs were used to screen 12 genotypes and scoring was considered for the primer pairs that generated amplicons in the expected base pair range (Li *et al*., 6) (Fig. 1). 4 primer pairs out of 9 showed polymorphism (44%) in at least one of the genotypes screened, 14 failed to amplify and 5 were monomorphic. These results clearly indicated that the primers selected in this study cross amplified across genera, and are moderately polymorphic based on fragment size differentiation. High levels of polymorphism associated with microsatellites are expected because of the unique mechanism responsible for generating microsatellite allelic diversity by replication slippage (Tautz and Renz, 9) rather than by simple mutations or insertions/deletions (Datta *et al*., 3).

Nine chrysanthemum SSR primers amplified scorable bands in the expected size range in all the marigold genotypes. A total of 21 alleles were amplified by the 5 microsatellite markers (Table 1). The number of alleles per locus varied from 1 (A33) to 3 (C12).PIC values ranged from 0.269 to 0.325. PIC values higher than 0.5 will be highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of the marker at specific locus. The expected heterozygosity ranged from 0.337-1.0. These results confirm that cross amplification across the genera is possible in Asteraceae family, where chrysanthemum primers can be successfully used in Marigold. High amplification success was observed between genera indicating a great potential to use microsatellites and their flanking regions as a source of single- or low-copy nuclear sequences (Zhang & Hewitt,10). Hence, screening of SSR primers from different genera can lead to the development of SSR loci in crops where SSRs are not available.

Since carrot gSSRs resulted in monomorphic amplicons, the scored data of amplicons generated by chrysanthemum primers was utilized for estimating genetic distance using Jaccard coefficient and UPGMA algorithm. Based on genetic distance 12 genotypes were clustered into two major groups (Fig. 2). Arka Bangara, Arka Agni and Arka Alankara, all three are petaloid male sterile samples were found in one cluster as expected with close proximity. The rest which include apetaloid fertile, apetaloid sterile and homozygous fertile grouped together in the second cluster. In the second cluster, 9-3 (Fertile double types) and 1-2 (Apetaloid Fertile) shared 100% similarity. Similar is the case with 1-2 (Apetaloid Sterile) and R-7 (Apetaloid Sterile) also. R-7 (Apetaloid Fertile) and (Apetaloid Sterile) formed a sub cluster. Two homozygous fertile types viz., 9-4

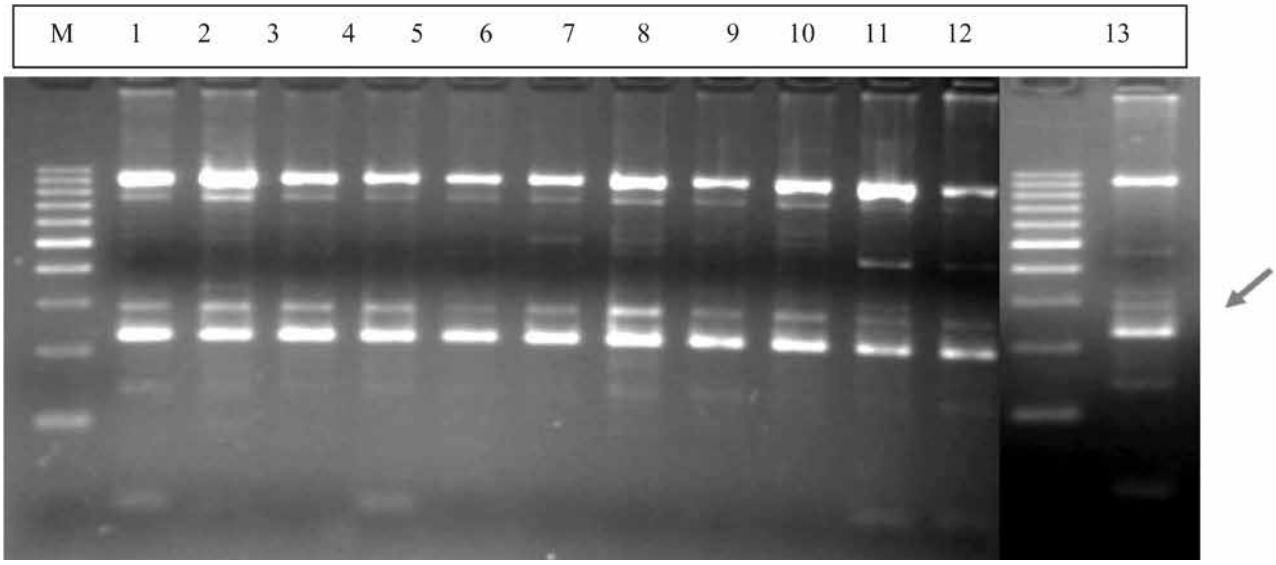

Fig. 1. DNA profile of different male sterility systems using Chrysanthemum SSR Primers B05; Lane 1- 100bp Ladder; Lane 2- 9-4(64); Lane 3- 9-3(63); Lane 4-1-2; Lane 5-1-3; Lane 6-R-7; Lane 7-1-1; Lane 8-1-2; Lane 9-2-2; Lane 10-R-7; Lane 11- Arka Bangara; Lane 12- Arka Alankara; Lane 13- Arka Agni.

Table 1. Properties of polymorphic Chrysanthemum SSR used.

Primer ID	Sequence (5'-3')	No. of alleles	No. of polymorphic alleles	Size range of alleles (bp)
B05	F: CTCCTGCTTCCCTCTCCTCC R: CCATCTTGGGTCCATTTAG	2	0	231-283
B12	F:GATGCGAGCAAATGAGCC R: CGAACGACTGGACACGAC	2	0	156-229
B10	F:ACTAACCCACCATTCCAC R: CAAATCCACCAAACCAAC	2	0	177-208
A31	F: TTGGTGGTAGTGGTGTG R: ACACACTATCTTCCACTTCT '	2	2	145-336
C12	F: GCTCATTCCTCACATCT R:ATAAGGCTGAAGACGAG	3	3	115-213
A12	F: 5CTGTCAGTTAGCCGTTTTCG R: CCTCATTTGTAAGGTGTGTG	3	2	191-239
A33	F: ACACAAGTTAGGCGAGATAC R: CACACAGTCCCTAAAATCC	2	1	144-252
B20	F: ATAACGACCAACTCCCTTTC R: GTGTTATGATGGTGAAGTGG	3	2	120-394
C15	F: GCCGAAGAGTAAACAGAG R: CGAACACGACACAAATCC	2	0	200-261

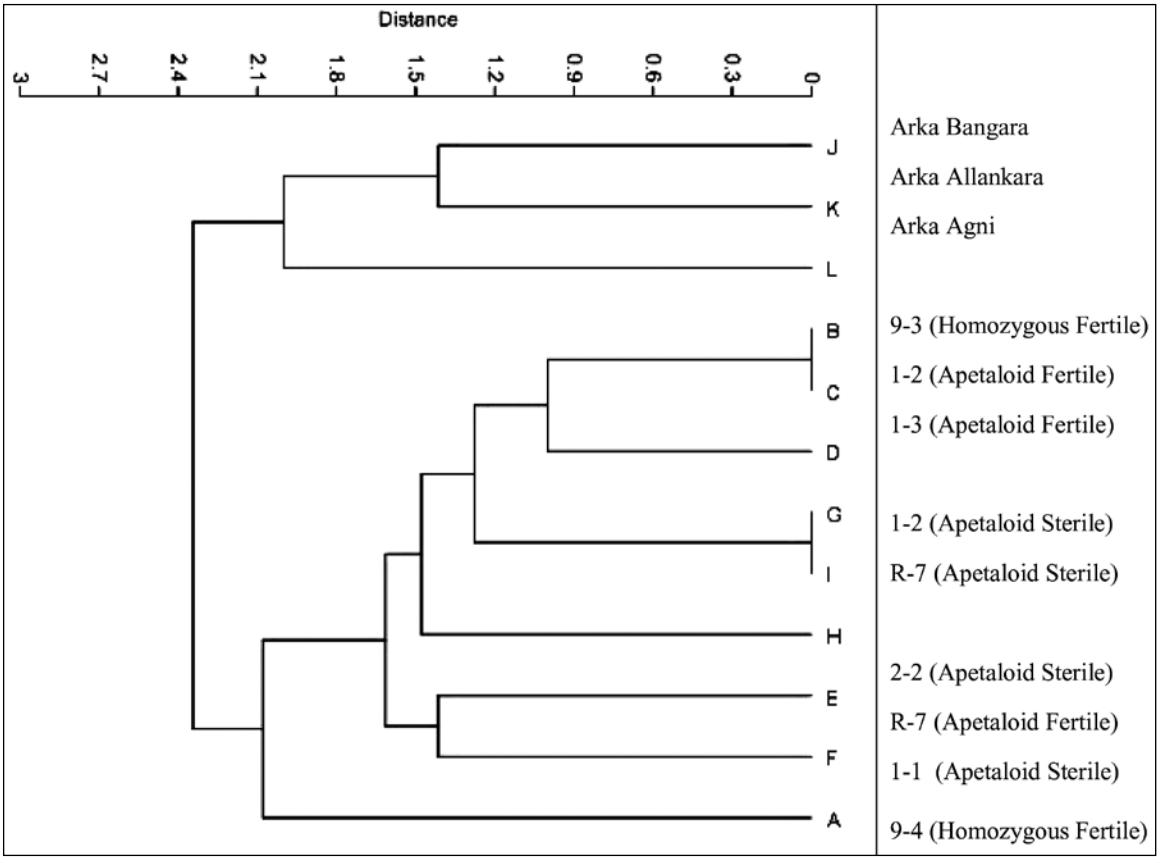

Fig. 2. Dendrogram depicting genetic relationships between genotypes belonging to different male sterility systems.

(Fertile single types) and 9-3 (Fertile double types) were found in two different sub clusters indicating greater distance between the two.

It is, however, important to bear in mind that when using SSR markers across distantly-related species the amplification of a PCR product does not necessarily imply locus conservation, since size homoplasy, i.e. convergence in size of non-homologous fragments, may occur. Considering the possibility of this source of confusion, verification of the PCR product identity by sequencing has been suggested previously, particularly when working across genera and if there is uncertainty regarding the size range of the amplicons obtained (Barbara *et al*.,1). However, verification through sequencing may not be necessary if working within the same genus as the species from which the SSRs markers were developed.

Chrysanthemum primers generated information in different genotypes of marigold that can be used to categorize them based on alleles shared and genetic distance. Since very limited SSRs are available in this crop, this kind of cross amplification within a family can save a lot of time and capital. Although the number of loci tested generally remains small, there appears to be moderate cross species transferability.

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