

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/326651180>

Cross amplification of SSR loci in marigold for molecular characterization

Article in *Indian Journal of Horticulture* · June 2018

DOI: 10.5958/0974-0112.2018.00059.2

CITATIONS

0

READS

47

4 authors, including:



Tejaswini Prakash

Indian Institute of Horticultural Research

23 PUBLICATIONS 24 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Protection of Plant Varieties - Breeders Rights [View project](#)



Male sterility in marigold [View project](#)

Volume 75, No. 2
June, 2018

Print : ISSN 0972-8538
Online : ISSN 0974-0112

The

Indian Journal of Horticulture



The Horticultural Society of India

F1, National Society's Block, National Agricultural Science Centre
Complex, Todapur, Pusa Campus, New Delhi - 110 012

Website : www.hsi1942.in



Short communication

Cross amplification of SSR loci in marigold for molecular characterization

Anuradha Sane*, Madhuri Ghatke, Archana Gadre**, Tejaswini**

Division of Plant Genetic Resources, ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru 560 089, Karnataka

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.

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

*Corresponding author's E-mail: anuradha@ihr.res.in

**Division of Ornamental Crops, ICAR-IIHR, Hessaraghatta, Bengaluru 560089

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

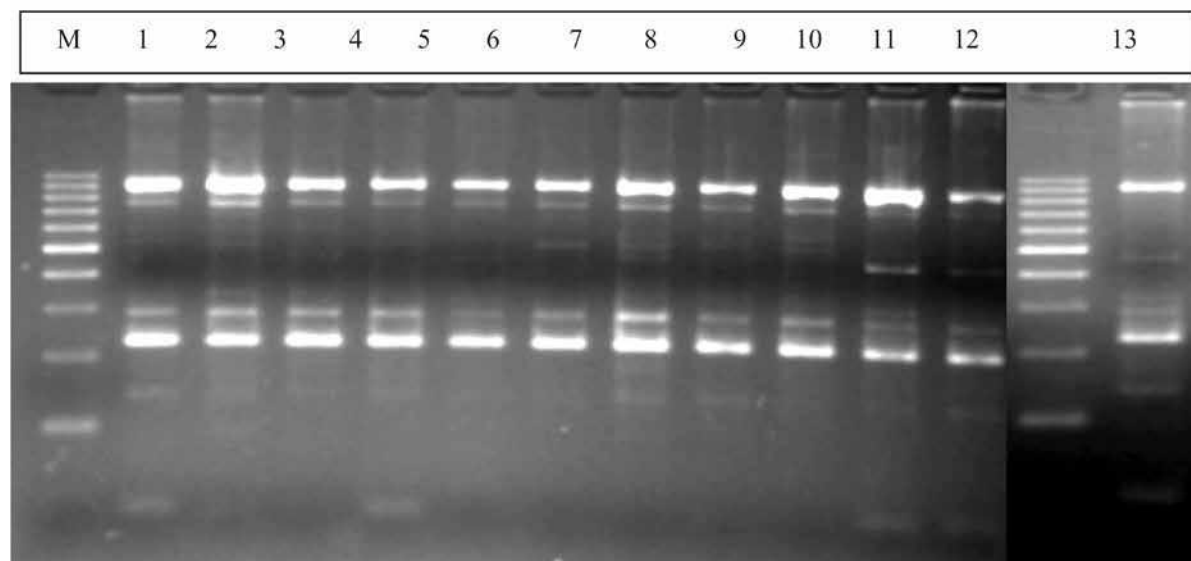


Fig. 1. DNA profile of different male sterility systems using *Chrysanthemum* SSR Primers B05; Lane 1- 100bp Ladder; Lane 2- 9-4(64); Lane 3- 9-3(63); Lane 4-1-2; Lane 5-1-3; Lane 6-R-7; Lane 7-1-1; Lane 8-1-2; Lane 9-2-2; Lane 10-R-7; Lane 11- Arka Bangara; Lane 12- Arka Alankara; Lane 13- Arka Agni.

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:ACTAACCCACCATTCAC R: CAAATCCACCAAACCAAC	2	0	177-208
A31	F: TTGGTGGTAGTGGTGTG R: ACACACTATCTTCCACTTCT '	2	2	145-336
C12	F: GCTCATTCTCACATCT R:ATAAGGCTGAAGACGAG	3	3	115-213
A12	F: 5CTGTCAGTTAGCCGTTTTCG R: CCTCATTTGTAAGGTGTGTG	3	2	191-239
A33	F: ACACAAGTTAGCGGAGATAC R: CACACAGTCCCTAAAATCC	2	1	144-252
B20	F: ATAACGACCAACTCCCTTTC R: GTGTTATGATGGTGAAGTGG	3	2	120-394
C15	F: GCCGAAGAGTAAACAGAG R: CGAACACGACACAAATCC	2	0	200-261

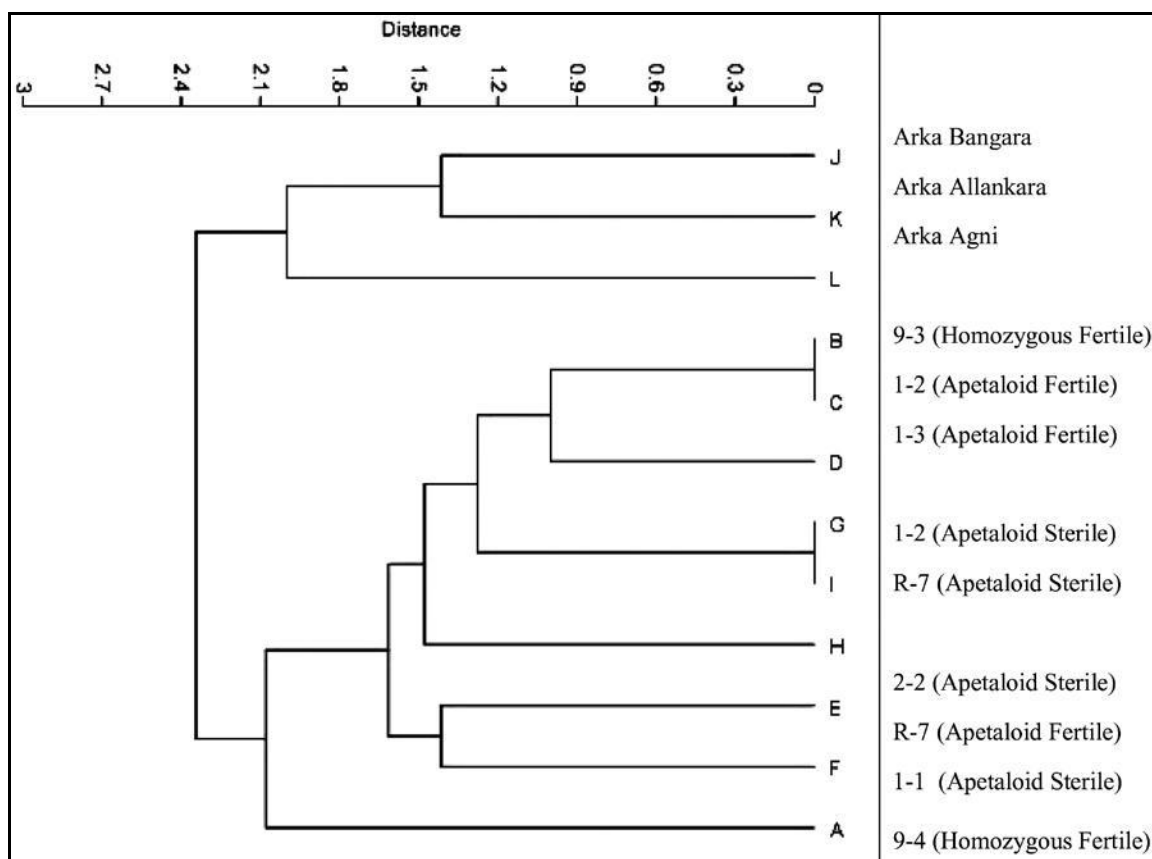


Fig. 2. Dendrogram depicting genetic relationships between genotypes belonging to different male sterility systems.

(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.

ACKNOWLEDGEMENT

Authors thank ICAR, New Delhi for funding Flagship programme on male sterility systems in horticultural crops

REFERENCES

1. Barbara, H. Palma-Silva, C. Paggi, G.M. 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. *Mol. Ecol.* **16**, 3759–67.
2. Cavagnaro, P.F., Chung, S., Manin, S., Yildiz, M., Ali, A., Alessandro, M.S., Iorizzo, M., Senalik, D.A., Simon, P.W. 2011. Microsatellite isolation and marker development in carrot - genomic distribution, linkage mapping, genetic diversity analysis and marker transferability across Apiaceae. *BMC Genomics*, **12**, (online publication 1-20).
3. Datta, S., Mahfooz, S., Singh, P., Choudhary, A.K., Singh, F., Kumar, S. 2010. Cross-genera amplification of informative microsatellite markers from common bean and lentil for the assessment of genetic diversity in pigeonpea. *Physiol Mol Biol Plants*, **16**: 123-34.
4. Doyle, J.J., Doyle, J.L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochem Bull.* **19**: 11-15.
5. Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. *Bull Soc Vaudoise Sci.* **44**: 223-70.
6. Li, Y.H., Luo, C., Wu, Z.Y., Zhang, X.H., Cheng, X., Dong, R, and Huang, C.L. 2013. Microsatellite Enrichment by Magnetic Beads in Chrysanthemum. *Acta Hort.* 977.
7. Rohlf, F. 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. *Syst Biol.* **47**: 147-58.
8. Saha, M.C., Mian, M.A.R., Eujayl, I., Zwonitzer, J.C., Wang, L. and May, G.D. 2004. Tall fescue EST-SSR markers with transferability across several grass species *Theor Appl Genet.* **109**: 783-91.
9. Tautz, D. and Renz, M. 1984. Simple sequences are ubiquitous repetitive components of eukaryote genomes. *Nucleic Acids Res.* **12**: 4127-83.
10. Zhang, D. X. and Hewitt, G. M. 2003. Nuclear DNA analyses in genetic studies of population: Practice problems and prospects. *Mol Ecol.* **12**: 563-84.

Received : May, 2016; Revised : April, 2018;
Accepted : May, 2018

CONTENTS

Genetic diversity in qualitative and quantitative traits of papaya – Kaluram, Jai Prakash, S. K. Singh, A. K. Goswami, Preeti Singh, Zakir Hussain and A. K. Singh	
Morphological characterization of walnut genotypes of diverse origin – J.I. Mir, N. Ahmed, D.B. Singh, Megna Rashid, S.R. Singh, O.C. Sharma, S. Lal and Anil Sharma	
Existence of genetically diverse ecotypes of <i>Ziziphus nummularia</i> : a wild species of <i>ber</i> from western India – Palaiyur N Sivalingam, Karun Gurjar, Dhurendra Singh, Sarita Chauhan and Chander Bhan	
Quality characteristics and antioxidant activity of passion fruit (<i>Passiflora edulis</i> Sims.) accessions – S.M. Charan, Saji Gomez, K.B. Sheela, P. Meagle Joseph and C.V. Sruthi	
Response of prohexadione calcium and paclobutrazol on growth and physio-chemical characteristics of pear cv. Clapp's Favorite – Manmohan Lal, M.M. Mir, Umar Iqbal and Amit Kumar	
Effect of fertigation on growth, yield and quality of almond under Kashmir conditions – Dinesh Kumar and D.B. Singh	
Nutritional status of Santa Rosa Japanese plum as affected by nitrogen and boron under rainfed conditions of Kashmir – Gowhar A. Dar, Amit Kumar, F.A. Misgar and Kounser Javeed	
Mapping of spatial variability in soil properties for site-specific nutrient management of Nagpur Mandarin in Central India – S.S. Sawant, M.S.S. Nagaraju, Rajeev Srivastava, Jagdish Prasad, R.A. Nasre and D.S. Mohekar	
Characterization of cultivated and wild species of <i>Capsicum</i> using microsatellite markers – Arpita Srivastava, Manisha Mangal, Gokul Gosavi and Pritam Kalia	
Characterization and association of phenotypic and biochemical traits in onion under short day tropical conditions – Ravindra Dangi, Anil Khar, Sabina Islam and Amrender Kumar	
Development of pollen germination medium to test pollen viability of eggplant and its wild species – P. Jayaprakash, Sheeba D, Vikas, V.K., Sivasamy. M, and T. Sabesan	
Comparative evaluation of hybrid seed production of bitter melon in rainy and spring-summer season – Nagamani Sandra, Sudipta Basu and T.K. Behera	
Influence of fertigation and training systems on yield and other horticultural traits in greenhouse cucumber – Sanjeev Kumar, N.B. Patel and S.N. Saravaiya	
Performance of soilless cucumbers under partially controlled greenhouse environment in relation to deficit fertigation – M.C. Singh, K.G. Singh and J.P. Singh	
Morphological diversity of trichomes and phytochemicals in wild and cultivated eggplant species – P.D. Kamala Jayanthi, M.A. Ravindra, Vivek Kempuraj, T.K. Roy, K.S. Shivashankara and T.H. Singh	265
Circumventing phenolic exudation and poor survival in micropropagation of marigold – K. Ravindra Kumar, Kanwar Pal Singh, D.V.S. Raju, Prabhat Kumar, Sapna Panwar and Reeta Bhatia	273
Productivity and economic advantages of flower crops in coconut based intercropping system – S. Rani, D. Rajakumar, N. Shoba and H.P. Maheswarappa	279
Effect of pre-treatment and packaging on quality of β -carotene rich mango powder – V.R. Sagar	283
Development and evaluation of vitamin C enriched low calorie <i>Aloe vera</i> - aonla blended functional squash using stevioside – Rakesh Sharma, Ranjana Sharma and Abhishek Thakur	289
Value addition and economics of Arecanut processing plant – A study from North-Eastern India – G. Mula, S.C. Sarker and A. Sarkar	295
Effect of different packaging films and pre washing on the shelf life of button mushrooms – Narges Hassani and Orang Khademi	306
Post-harvest losses in different varieties of onion – Kalyani Gorrepati, A.A. Murkute, Yogesh Bhagat and Jai Gopal	314
Storage behaviour of apple cultivars under ambient conditions – Arun Kishor, Raj Narayan, Manoj Brijwal, Brij Lal Attri, Anil Kumar and Sovan Debnath	319
Short communications	
Budding and grafting time and height as determining factors for bud take and successive plant growth in some temperate fruits – Biswajit Das, Arun Kishor and N. Ahmed	326
Estimates of heterosis for yield and its contributing traits in cucumber – T.L. Bhutia, A.D. Munshi, T.K. Behera, A.K. Sureja, S.K. Lal and Azeze Seyie	332
Stability of yield and its components in vegetable amaranth – R.S. Pan and A.K. Singh	337
Projected climate changes and environment suitability of foot yam in major growing areas of India – G. Byju, Sabitha Soman and M. Vani	341
Cross amplification of SSR loci in marigold for molecular characterization – Anuradha Sane, Madhuri Ghatke, Archana Gadre, Tejaswini	345
Characterization of phenolic compounds in petal extracts of rose – Poonam Kumari, D.V.S. Raju, Kanwar Pal Singh, K.V. Prasad and Sapna Panwar	349
Effect of planting dates on growth, flowering and seed production of snapdragon – Priyanka Sharma, Y.C. Gupta, S.R. Dhiman and Puja Sharma	352

Published by Dr K.L. Chadha and edited by Dr Pritam Kalia for the Horticultural Society of India, F1, National Society's Block, National Agricultural Science Centre Complex, Todapur, Pusa Campus, New Delhi 110 012, India and printed at Malhotra Publishing House, B-6, DSIDC Packaging Complex, Kirti Nagar, New Delhi 110 015, India.