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Short communication

Genetic diversity analysis in snapdragon (*Antirrhinum majus*) through RAPD markers

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ABSTRACT

Snapdragon (*Antirrhinum majus*) is an annual ornamental with various attractive flower genotyoes colours. RAPD markers were used to study the genetic structure of 10 different *A. majus* genotypes with 10 random primers. According to Jaccard's coefficient from RAPD data, similarity coefficient ranged from 0.43 to 1.00. The results obtained confirm the usefulness and suitability of RAPD markers for identification of snapdragon varieties. Out of 10 primers OPO-01 showed the highest polymorphism (80.77%). Vilmorin and AG-5 were closely related as they showed high value of similarity coefficient (0.735), followed by SA-1 with 0.696 of similarity coefficient. AG-2 showed least similarity (0.475) with other genotypes. The study revealed presence of enough variability at genetic level among studied genotypes suggesting that heterosis breeding may be suitable to harness their potential.

Keywords: Snapdragon, morphological, polymorphic, heterosis, RAPD.

The diversity level of *Antirrhinum majus* was evaluated using various systems such as morphological, chemical and biochemical markers. Morphological markers are highly influenced by environment. In contrast to this the biochemical markers are proteins produced by gene expression. However, the environment influences the protein formation. RAPD technique is reliable and the most frequently used one for measuring genetic diversity in crops (Williams *et al*., 6). This technique has proved to be a useful tool for the genetic characterization of varieties as it requires no prior sequence specific information and only small quantity of plant tissues. The amplified fragments provide a large number of potentially polymorphic loci, which make RAPD appropriate for distinguishing between related genotypes (Huen *et al*., 1). The genus *Antirrhinum* is a classical model system for floral development, for which the genetic control of organ morphogenesis has been extensively studied (Theissen and Saedler, 3). Several workers carried out studies to find out variability present in the cultivable as well as wild and important genotypes of *Antirrhinum* spp. existing in nature (Jimenez *et al*., 2; Torres *et al*., 4; Torres *et al*., 5). Therefore, the aim of present work was to verify the usefulness of the RAPD method for identification and genetic diversity studies in representation of 10 snapdragon varieties.

The experiment was carried out with 10 snapdragon genotypes, *viz*., AG-1, AG-2, AG-3, AG-4, AG-5, AG-6, Vilmorin, Sant-11, Sant-22 and SA-1. DNA was extracted as per procedure reported by Torres *et al*. (4) with some modifications. About 2 g newly appeared leaves were weighed for each genotypes and then washed with distilled water followed by 70% ethanol. The samples were ground to a fine powder in presence of liquid nitrogen in a pre-chilled mortar and pastel. The powder was transformed to a 50 ml centrifuge tube containing 10 ml DNA extraction buffer and incubated at 65°C for 45 min. The tubes were cooled at room temperature. Equal amount of chloroform:iso amyl alcohol (24:1 volume) was added and mixed thoroughly by genetic inversion and centrifuged at 8,500 rpm for 15 min. at 20°C. The upper layer was transferred to a centrifuge and 2/3rd part of chilled isopropanol was added to it.

The tubes were mixed thoroughly by quick gentle inversion and kept overnight at 20°C. Then the tubes were spun at 5,000 rpm for 30 min at 4°C and pellet obtained was washed with 70% ethanol and dried by inverting the tube on towel paper for 10 min. Finally pallet was redissolved in TE buffer and kept at -20°C. Purification and quantification of the DNA was done. RAPD reactions were performed in 25 µl, containing 10 mM Tris-HCl (pH 8.8, 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dATP, dCTP, dGTP and dTTP, 15 ng primer, approximately 25 ng genomic DNA and 1.5 unit of *Taq* polymerase. One control, containing all components except genomic DNA, was included in each set of reactions to prove that no contamination occurred. Amplifications were carried out in an thermocycler (Eppendorf) under the

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following conditions an initial cycle at 94°C for 2 min., 39 cycles of 30 sec at 94°C, 30 sec at 36°C, 1 min. at 72°C. A final cycle at 72°C for 10 min. was included (Jimenez *et al*., 2). Ten RAPD primers suggested by Theissen and Saedler (3) and Jimenez *et al*. (2) were used in the present study (Table 1). Amplifications were conducted twice to ensure reproducibility and minimize the errors. Amplification products were separated on 1.5% agarose with ethidium bromide staining. After destaining in distilled water, the gel image was viewed and stored on a gel documentation system. Photographs from ethidium bromide stained gels were used to score the data manually and independently for RAPD analysis. The presence or absence of a band was considered as single trait, and values 1 and 0 were assigned, respectively. These data matrices were entered into NTSYS-PC.

The percentage of polymorphic loci and expected heterozygosity among individuals at each locus/ population were calculated (Table 1). The total number of bands amplified from 10 primers was 538. This gave an average of 53.8 bands/primer. Out of 538 bands, 87.65% polymorphism was shown for one or more genotypes. The size of amplified product ranged from 100 to 4000 bp. All the 10 primers used were informative and gave polymorphic bands with most of the genotypes (Figs. 1 & 2). However, genotype SA-1 did not give any band with primer UBC-557 and UBC-514. Primer UBC-543 did not give any band with Vilmorin, Sant-22 did not show any banding pattern with primers UBC-557, UBC-507 and UBC-536, primer UBC-507 did not give any band with AG-1, AG-3 and Sant-11 genotypes, and primer UBC-514 did not provide any band with Sant-11, however, AG-2 did not exhibit the result with primer UBC-511. Monomorphic bands were obtained with primers UBC-523, OPO-20, OPO-01 and UBC-540. Variety SA-1 can be easily identified with primer UBC-523. Primers UBC-523, UBC-540 and OPO-20 were separately sufficient to identify all materials studied. These results indicated that a small number of RAPD primers is sufficient to prepare genotype-specific banding patterns for variety identification. Primers UBC-557, UBC-507, UBC-536, UBC-511, UBC-514 and UBC-543 gave maximum number of polymorphic bands (100% polymorphism,), whereas, primer UBC-540 showed least polymorphism (56%). The results obtained confirm the usefulness and suitability of RAPD markers for identification of snapdragon varieties and breeder's rights protection.

Association among the 10 genotypes, revealed by unweighed pair group method with arithmetic mean cluster analysis (UPGMA) is presented in Table 2 and the Fig. 3 showed the pair-wise similarities among 10 genotypes. The similarity coefficients ranged from 0.43 to 1.00. The results of pairwise combinations indicated that two genotypes, *i.e.* Vilmorin and AG-5 were highly related which resulted from high value of similarity coefficient (0.735). Genotype AG-2 showed least similarity (0.475) with other genotypes. In other words, it was the most diverse among other studied genotypes. It was followed by AG-4, which showed 0.494 of similarity coefficient with Node 7 (consisting of all genotype except AG-2). There were 0.533 and 0.575 of similarity coefficients recorded in between, Node 6 and Sant-11 and Node 5 and Node 4, respectively. Although the genotypes AG-4

Table 1. Total number of RAPD loci detected using 10 RAPD primers.

S. No.	Primer code	Electrophoresis data		
		Total bands	Polymorphic bands	Polymorphism (%)
1	UBC-557	46	46	100.00
2	UBC-507	17	17	100.00
3	UBC-523	69	49	71.01
4	UBC-536	76	76	100.00
5	OPO-20	64	44	68.75
6	OPO-01	52	41	80.77
7	UBC-511	28	28	100.00
8	UBC-514	23	23	100.00
9	UBC-540	91	51	56.00
10	UBC-543	72	72	100.00
	Total	538	447	876.53
	Average	53.8	44.70	87.65

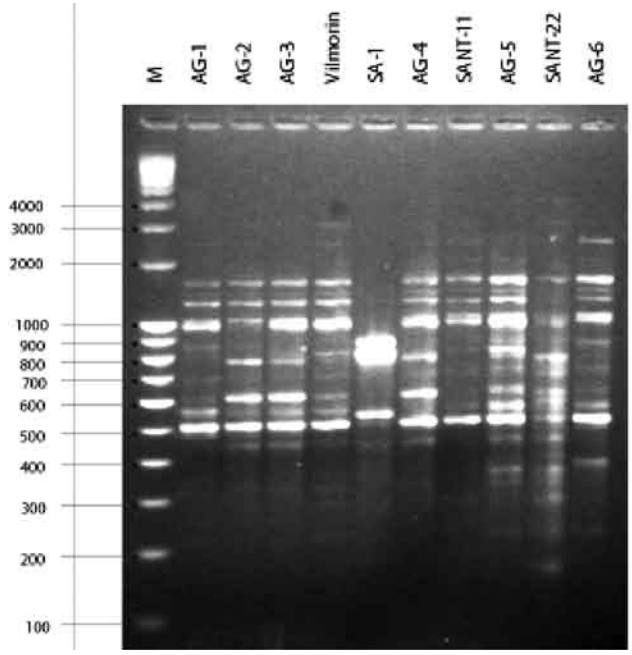

Fig. 1. RAPD of snapdragon genotypes with primer UBC-523.

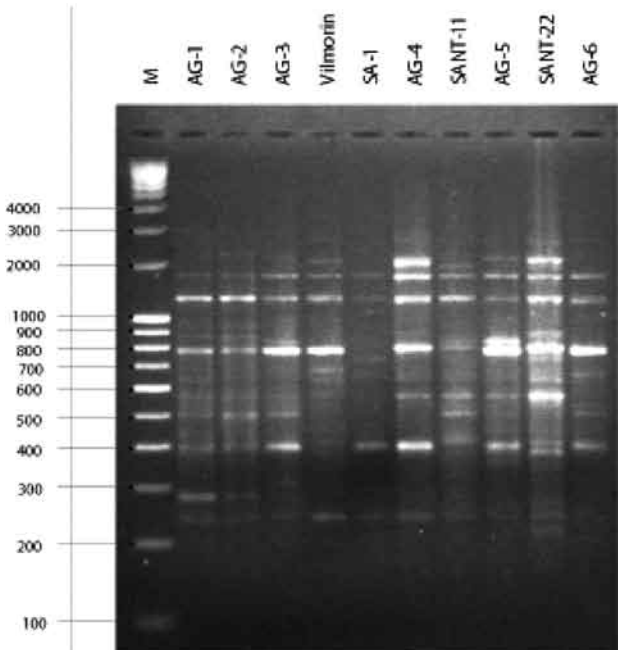

Fig. 2. RAPD of snapdragon genotypes with primer OP-O20.

Table 2. Similarity matrix for Jaccard's coefficient: range of values from 0.43 to 1.0 indicating increasing similarity.

Genotype	AG-1	AG-2	AG-3	Vilmorin	SA-1	AG-4	Sant-11	AG-5	Sant-22	AG-6
AG-1	1									
AG-2	0.437	1								
AG-3	0.594	0.574	1							
Vilmorin	0.606	0.471	0.521	1						
SA-1	0.549	0.43	0.592	0.712	1					
AG-4	0.529	0.475	0.485	0.545	0.513	1				
Sant-11	0.486	0.452	0.441	0.594	0.494	0.438	1			
AG-5	0.622	0.536	0.583	0.735	0.679	0.543	0.588	1		
Sant-22	0.569	0.435	0.597	0.609	0.59	0.485	0.603	0.721	1	
AG-6	0.588	0.469	0.522	0.631	0.506	0.455	0.524	0.697	0.694	1
	AG-1	AG-2	AG-3	Vilmorin	SA-1	AG-4	Sant-11	AG-5	Sant-22	AG-6

and SA-1 looked very much similar in morphology, but genetically it was proved that they were enough diverse with each other. In contrast to this, AG-6 and Sant-22 were genetically similar and they were much closer by genealogy but had different morphology.

All the varieties banding pattern with primers UBC-523, OPO-20, OPO-01 and UBC-540 and out of this OPO-01 showed highest polymorphism (80.77%). A moderate (*Antirrhinum subbaeticum* 41.9%) to high (*Antirrhinum microphyllum*, 90%) proportions of polymorphic amplified products were detected by Jimenez *et al*. (2) and Theissen and Saedler (3), respectively. Torres *et al*. (4) observed that every studied plant had a different RAPD phenotype and the high percentage of polymorphic bands (88.41%) available whereas a measure of variability, resulted into high level of diversity in *Antirrhinum microphyllum*. Since, their objective was to conserve these species, and detection of less genetic diversity alone cannot prove the cause of concern for its viability in wild. In the present study, enough variability was present, among various snapdragon genotypes, they might be exploited for further hybridization for producing F_1 hybrids.

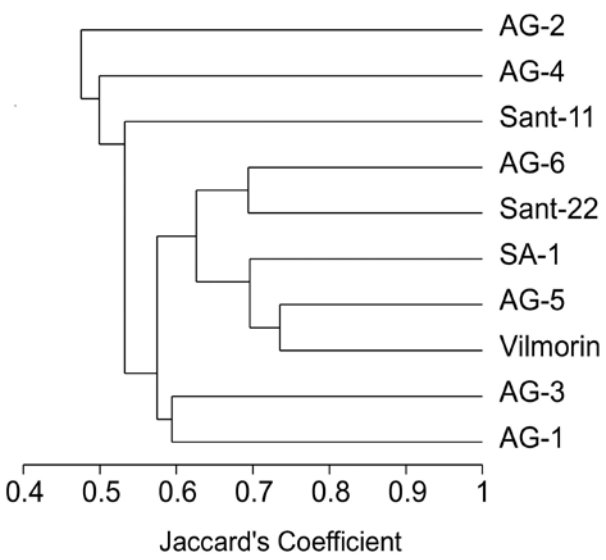

Fig. 3. Dendrogram of snapdragon genotypes constructed using UPGMA based on Jaccard's similarity coefficient.

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