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Short Communication

Molecular markers for working out genetic relationship among genotypes of carnation (*Dianthus caryophyllus* L.)

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Accurate identification of genotypes and their interrelationships are essential for breeding, and for claiming protection under plant breeder's rights. Carnation (*D. caryophyllus* L.) an important flower crop has several varieties coming into the market every year. In our germplasm collection of carnation, one of the genetic stock material CG-109 was observed to give rise to frequent mosaic mutants bearing chimeric flowers. This group of unastable and mosaic mutants for flower pigmentation were separated and maintained with a different identity as IIHRS-1. From IIHRS-1, a stable mutant with entire flower bearing uniform pigmentation of red colour was isolated, purified and stabilized through tissue culture and established as a separate stable line, IIHRP-1. Three carnation lines *viz*., CG-109 (parental genotype), IIHRP-1 (purified stable line) and IIHRS-1 (red and white striped chimeric flowering line) were used in this study. Young leaves were selected and genomic DNA was extracted adopting the cetyltrimethyl-ammonium bromide (CTAB) method [1].

A total of five 10-mer oligonucleotides with arbitrary sequence from Operon Technologies, Alameda, USA (kit E) were used in RAPD analysis (Table 1) and 20 primers based on dinucleotide, tetranucleotide or pentanucleotide repeats were used in ISSR analysis (Table 2). The PCR reaction mixture consisted of 20-50ng genomic DNA, 1X PCR buffer, 18mM $MgCl_2$, 1 µmol of each dNTP, 0.3µmol primer and 0.5 unit *Taq* polymerase in a 25µl volume. The amplification protocol was at 94ºC for 4 minutes to pre-denature, followed by 45 cycles of 94°C for 1 min, 36°C (for RAPD analysis) or 50°C (for ISSR analysis) for 1 min and 72°C for 1 min, with a final extension at 72°C for 10 min. Amplification products were separated on 1.2% (for RAPD analysis) and 2% (for ISSR analysis) agarose gel.

RAPD and ISSR data were scored for presence (1) or absence (0) of a band for each genotype and for each primer. Each band was considered as a locus. The genetic similarities (GS) were calculated using both RAPD and ISSR markers, and were estimated using Jaccard's coefficient [2] as well as Nei and Li coefficients [3].

In RAPD analyses, a total of 105 bands were detected, with polymorphic information content of 85.71% (Table 1). Highest polymorphic information content (PIC) of 100% was recorded in case of OPE-5. In ISSR analysis, a total of 96 bands were observed, of which fifty-one (53.12%) were polymorphic. Two ISSR primers (i.e., ISSR15 and ISSR17) had the highest PIC values (100%). Polymorphic information content generated by RAPD was higher than that realized from ISSR indicating the superiority of RAPD for discriminating genotypes and for analyzing diversity.

RAPD and ISSR primers together generated a total of 201 bands comprising both polymorhphic as well common loci (Table 3). Of the 105 loci revealed by all the five RAPD primers, fifteen were found to occur as a common locus in all the three genotypes and thirty six loci revealed were unique for either of the mutants and the parental genotype. Of the 96 bands generated by ISSR primers, 43% of them were found to occur as common locus in all the three genotypes. Though the extent of genetic distinctness or unique locus revealed by ISSR in specific genotypes was similar to that reveled by RAPD; extent of common locus detected among

Table 1. Primer sequences, amplified bands, and polymorphic information content by RAPD analysis

Primer	Amplified bands	Polymorphic information content (%)
OPE-1	27	81.48
OPE-2	26	80.77
OPE-3	27	85.19
OPE-4	24	95.83
OPE-5	1	100.00
Total	105	85.71

Table 2. Primer sequences, amplified bands, and polymorphic information content by ISSR analysis

Primer	Amplified bands	Polymorphic information content (%)
ISSR-1	2	50.00
ISSR-2	10	60.00
ISSR-3	5	20.00
ISSR-4	7	42.85
ISSR-5	7	14.28
ISSR-6	3	66.66
ISSR-7	2	50.00
ISSR-8	4	75.00
ISSR-9	10	80.00
ISSR-10	2	50.00
ISSR-11	7	85.71
ISSR-12	7	0.00
ISSR-13	6	83.33
ISSR-14	1	0.00
ISSR-15	2	100.00
ISSR-16	2	50.00
ISSR-17	3	100.00
ISSR-18	0	0.00
ISSR-19	7	14.28
ISSR-20	9	66.66
Total	96	53.12

genotypes by ISSR was higher than that revealed by RAPD. The results suggest the superiority of ISSR in detecting the genetic similarity.

The entire 105 bands, generated from five RAPD primers, and 96 bands generated from ISSR primers were subjected to calculate the genetic similarity index (GS) among the 3 genotypes (Table 4). Coefficient of genetic similarity estimated as per Nei and Lei for RAPD ranged from 0.64 to 0.72 and for ISSR ranged between 0.65 and 0.84. Based on Jaccard's coefficient, genetic similarity coefficient ranged from 0.53 to 0.77 using RAPD and from 0.51 to 0.56 with ISSR. Though the values of the coefficient changed depending on type of marker and type of the coefficient utilized for estimation, the trend remained uniform indicating the highest similarity coefficients between CG-109 and IIHRP-1.

Nei and Li index are based on the infinite allele model. In the evolutionary processes, mutations occur stepwise and the most probable cases are change in one or two tandem repeats [4]. Considering the vegetative propagating nature of carnation and with that the potential of the crop to contain the higher amount of heterozygosity; one can expect a huge amount of molecular variation being present in the nuclear material of each genotype. RAPD has the potential to catch genetic variation present between the genotypes that might not be expressed morphologically. RAPD primers were able to detect the differences that genotypes under study must have accumulated due to chromosomal aberrations and mutations over the time because of their vegetative propagation. ISSR markers are more specific compared to RAPD and detects sensible mutations [5]. ISSR markers were observed to be superior to RAPD markers in estimating the genetic similarity.

In conclusion, the study indicated the utility of RAPD markers for discrimination, and particularly the utility value of these markers in charcterising and conserving the maximum diversity of germplasm accessions originating through mutations. The study also reveals the utility of ISSR markers and estimation of genetic similarity based on Nei and Li index for identification of varieties while according the Plant Breeder's rights to mutants and selections.

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Table 3. Unique and common bands revealed by RAPD and ISSR primers in IIHRP-1, IIHRS-1 and CG-109

		Unique and common bands between and individual genotypes							
		12	13	23	123	1	2	3	Total
ISSR	No.	48	51	44	43	1	9	29	96
	%	50	53.1	45.8	44.7	1.0	9.38	30.2	100
RAPD	No.	24	18	18	15	2	7	27	105
	%	22.8	17.1	17.1	14.3	1.9	6.67	25.7	100

Note: 1 = CG109, 2 = IIHRP1, 3 = IIHRS1

Table 4. Genetic similarity according to Jaccard(1908) and Nei and Li (1979) coefficients between different genotypes

	CG-109	IIHRP-1	IIHRS-1
I. Nei and Lei similarity matrix			
Based on RAPD similarity			
CG-109	1	-	-
IIHRP-1	0.72	1	-
IIHRS-1	0.65	0.64	1
Based on ISSR similarity			
CG-109	1		
IIHRP-1	0.84	1	
IIHRS-1	0.76	0.65	1
II. Jaccard similarity matrix			
Based on RAPD similarity			
CG-109	1	-	-
IIHRP-1	0.77	1	-
IIHRS-1	0.68	0.53	1
Based on ISSR similarity			
CG-109	1		
IIHRP-1	0.56	1	
IIHRS-1	0.53	0.51	1

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