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Research Note

Diversity analysis among opium poppy (*Papaver somniferum* L.*)* crosses and parents using RAPD

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Abstract
The RAPD analysis was carried out with 28 crosses, 8 parents and 2 checks of opium poppy. Purified and isolated DNA was subjected to PCR based marker (RAPD) for assessment of genetic diversity. The quality of DNA was determined by calculating ratio between A_{260} and A_{280} observed between 1.857 to 2.167 which indicated a good quality of plant DNA. The concentration of DNA ranged between 123 µg/µl (UOP-60 x UOP- 99) to 750 µg/µl (UOP-79 x UOP- 80). In the RAPD analysis 12 primers gave good amplified products with template DNA. Polymorphism shown by 12 primers ranged between 50 per cent (OPP-02) to 100 per cent (OPA-01, OPA-08, OPB-06 and OPD-05). Average polymorphism was found to be 84.80 per cent. From RAPD profiling similarly matrix was obtained and Jaccard's similarity coefficient lies between 0.57 (UOP-69 x UOP-80) to 0.95 (UOP-53 x UOP-79) with an average of 0.79. On this basis a dendrogram was constructed with UPGMA method. Dendrogram differentiate 28 crosses, eight parents and to checks of opium poppy into one major and four minor groups. Further genetically diverse parents and crosses can be alternatively used for accumulating favourable genes so as to finally improve the productivity.

Key words
Opium poppy, RAPD, genetic diversity

The opium poppy (2n=22, *Papaver somniferum* L.) belonging to the family *Papaveraceae*, is an annual medicinal herb. The medicinal value of opium poppy is due to presence of numerous alkaloids. It contains more than two dozen alkaloids out of which morphine, codeine, narcotine, thebaine and papaverine, are frequently used as pain killer, sedative, an analgesic, anti-tussive and anti spasmodic in modern medicine.

Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Lal *et al.*, 2014; Yadav *et al.*, 2007; Acharya and Sharma, 2009). It is correlated with the genome fraction surveyed by each kind of marker, their distribution throughout the genome and the extent of the DNA target which is analyzed by each specific assay (Davila *et al.*, 1999).

RAPD markers are commonly used because they are quick and simple to obtain enabling genetic diversity analysis in several types of plant material such as natural populations, population in breeding programmes and germplasm collections (Lal *et al.*, 2013; Patel *et al.*, 2011; Ferreira and Grattupaglia, 1996). RAPD markers are superior when simplicity and costs were considered (Udaya kumar *et al*., 2013; Mir *et al.*, 2011; Sangwan and Sangwan, 2001). RAPD (William *et al*., 1990) has been used in analysis of genetic distance in different plant species (Sharma *et al*., 1996; Lashermes *et al*., 1996; Samec and Nasinec, 1996; Tripati *et al*., 2011 and Shukla and Singh, 2006). The present investigation is thus made on 28 crosses, eight parents and two checks of poppy from to assess their genetic diversity using RAPD analysis.

The present investigation was conducted on leaves collected from the crosses of opium poppy grown under AICRP project on Medicinal and Aromatic Plants, Department of Plant Breeding and Genetics, RCA, Udaipur. Twenty eight crosses, eight parents and two checks of opium poppy were characterized during present study.

DNA isolation, purification and quantification: A total of five gram of leaf tissues were collected from a single plot of each cross, parent and check. The leaf tissues were frozen in liquid nitrogen and ground to a fine powder in a pestle and mortar. The genomic DNA was isolated from powdered leaf tissue using the CTAB method described by Doyle and Doyle (1990) and treated with RNase to eliminate RNA. DNA concentration was measured by UV-absorbance method. The concentration of DNA preparation varied from 123 µg/µl (UOP-60 x UOP- 99) to 750 µg/µl (UOP-79 x UOP- 80) respectively. The integrity of the isolated DNA was verified by visualization of DNA on 0.8% Agarose gel with DNA. The quality of DNA was determined ranged from 1.857 to 2.167, which indicating the good quality of DNA (Table 1).

RAPD analysis: Amplification of polymorphic DNA was done by using 30 primers obtained from Department of Molecular Biology and Biotechnology, Rajasthan college of Agriculture,

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Udaipur. The details of PCR reaction mixture is given below:

For RAPD marker analysis, PCR amplification was performed in a volume of 20 µl reaction mixture containing 50 ng of template DNA, 0.5 µM of primer, 2.0 µL 10X assay buffer, 1.6 µL dNTPs, 0.33 µL Taq (1 U) DNA polymerase and 13.07 µL of ddH_2O. PCR reactions were initiated with an initial denaturation step of 94°C for 5 min, followed by 2-45 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min and extension at 72°C for 2 min. After 2-45 cycles of PCR reaction, a final extension step at 72°C for 5 min was performed.

Following the amplification, the PCR products were loaded on 0.8 % Agarose gel which was prepared in 1 x TAE buffer containing 0.5 µg/ml of the ethidium bromide. The Amplified products were electrophoresed for 2.5-3 hrs at 50 V with cooling. After separation the gel was viewed under UV transilluminator and photographed with the help of gel documentation system (Alpha DG DOC).

Scoring the RAPD products: In order to score and preserve banding pattern photograph of the gel was taken by a Gel Documentation System, under UV transilluminator. RAPD bands were designated on the basis of their molecular sizes (length of polynucleotide amplified). 100bp DNA ladder (hiper himedia 50 lane) loaded simultaneously with primer products in the gel was used to estimate the molecular sizes. The distance run by amplified fragments from the well was translated to molecular sizes with reference to molecular weight marker. The presence of each band was scored as '1' and its absence as '0'.

Statistical Analysis for Similarity Coefficient: The scores (0 or 1) for each band obtained from photograph were entered in the form of a rectangular data matrix (qualitative data matrix). The pair-wise association coefficients were calculated from qualitative data matrix using Jaccard's similarity coefficient. The equation for calculating Jaccard's similarity coefficients 'F' between two samples A and B is:

$$f = n_{x\,y} / (n_1 - n_z)$$

n_{xy} = Number of bands common to sample A and sample B; n_1 = Total number of bands present in all samples; n_z = Number of bands not present in sample A or B but found in other samples.

Cluster analysis for the genetic distance was then carried out using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the clones using computer program NTSYS pc version 2.02 (Rohlf, 2004).

The Genetic diversity assessment can increase the effectiveness of breeding programmes. The 28 opium poppy crosses, 8 parents and 2 checks were successfully discriminated on the basis of their RAPD patterns. Out of 30 primers used in this study, 12 primers gave amplified products. Each RAPD products was assumed to represent a single locus and data were scored as (1) and (0) for presence and absence, respectively.

Out of 30 primers used in this study which 12 primers gave amplified products. Polymorphism shown by 12 primers ranged between 50 per cent (OPP-02) to 100 per cent (OPA-01, OPA-08, OPB-06 and OPD-05). Average polymorphism was found to be 84.80 per cent. Each RAPD products was assumed to represent a single locus and data were scored as (1) and (0) for presence and absence respectively. Electrophoresis pattern of RAPD profile on 1.6 percent Agarose gel is illustrated in plate 9 with OPP-01 primers gave 10 bands in the range of 300 bp to 1300 bp with 100% polymorphism. The primer OPD-05 gave 9 bands in the range of 100 bp to 1500 bp with 100% polymorphism. The representative photographs of electrophoresis gels showing RAPD profiles after amplification with RAPD primers were depicted in Plate -9.

The 12 RAPD primers on 28 opium poppy crosses, 8 parents and 2 checks generated total 85 bands, out of which 72 were polymorphic. The number of DNA amplified fragments per primer ranged from 0 to 8. The most informative primers were found to be OPA-01, OPA-08,OPB-06 and OPD-05 with 10 polymorphic bands respectively while least informative was OPP-02 with only 2 polymorphic bands out of 4 bands. The average size of fragments obtained was between 100-3200 bp. The maximum numbers of amplified bands were seen in crosses UOP-53 x UOP- 80, UOP-69 x UOP- 20 and UOP-20 x UOP- 99 with 57 bands while parents UOP-60 had 56 bands.

Genetic relationship among the Germplasm and cluster analysis: The banding pattern generated and polymorphism reflected in these patterns was used to calculate the diversity among accessions taken for present study. Genetic similarity estimates based on RAPD banding patterns were calculated using method of Jaccard's coefficient analysis (1980). The similarity coefficient matrix generated for the primers was subjected to algorithm UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and clusters were generated using NTSYS 2.02 pc program (Rohlf, 2004). The dendrogram showing relationships among various varieties was constructed using these clusters (Fig. 1.). Jaccard's similarity

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coefficient values ranged from 0.57 (UOP-69 x UOP-80) to 0.95 (UOP-53 x UOP-79) with an average of 0.79.

The cross UOP-69 x UOP-80 was most dissimilar and scored values of lower order with other crosses (average similarity co-efficiency being 0.57 over rest of the crosses and hence, maximum diverse from the rest) followed by UOP-53 x UOP-99, UOP-20 x UOP-99 and UOP-53 x UOP-80 with average similarity co-efficiency of 0.58,0.58 and 0.59. The cross UOP-53 x UOP-79 was most similar and score higher values with other crosses (average similarity co-efficiency being 0.95 over rest of the crosses and hence, minimum diverse from the rest) followed by UOP-80 x UOP-20, UOP-53 x UOP-80, UOP-69 x UOP-60 and UOP-80 x UOP-1185 with average similarity co-efficiency of 0.93, 0.92,0.92 and 0.92.

The dendrogram based on RAPD analysis has generated one major group A and four minor groups B, C, D and E, respectively. The major group A consists of two sub groups (A1 and A2). The sub group A1 consists of two sub group (A-I and A-II). The sub group A-I consisted of 11 crosses, 3 parents and 2 checks whereas sub group A-II consisted of 5 crosses and 2 parents. However the class within the sub group A2 consisted of two crosses had higher within group similarity of 89.4% while it is 89.2% for sub group A1. The sub group A1 and A2 joined together at the similarity level of 76.1% whereas four minor groups B, C, D and E. The B minor group consisted of 4 crosses and 1 parent had higher within group similarity of 89.1% for group B. However the group C consisted of 2 crosses and one parent had higher within group similarity of 82.2% for group C. The minor group D consisted of 2 crosses and 1 parent the similarity level of 82.0% respectively. The minor group E consisted of only 2 crosses, joined major group (A) at the similarity level of 76.1% and 69.2% respectively (Fig. 2).

On the basis of present study it may be concluded that RAPD profile of opium poppy the crosses *viz.* UOP-20 x UOP-60, UOP-69 x UOP-80, UOP-79 x UOP-60, UOP-79 x UOP-99, UOP-53 x UOP-69, UOP-80 and UOP-60 can be used for the diversity studies. Further the groups/clusters obtained by dandrogram could also be distinguished by similarity for the morphological characteristics within each group/cluster. Therefore molecular based selection provided more information about genetic diversity and could be used as a selection tool for advance generations in opium poppy. Such an association may help in framing more effective breeding program as well as genetically diverse parents and crosses can be alternatively used for accumulating favourable genes so as to finally improve the productivity.

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Table 1. Concentration of DNA in Opium poppy crosses, parents and checks

S. No.	Crosses)/ Parents/Checks	Ratio of A_{260}/A_{280}	Conc. of DNA (µg/µl)
1	UOP-53 x UOP-69	1.909	360.0
2	UOP-53 x UOP-79	1.966	284.0
3	UOP-53 x UOP-80	1.948	277.0
4	UOP-53 x UOP-20	1.926	321.0
5	UOP-53 x UOP-1185	1.891	297.0
6	UOP-53 x UOP-60	2.052	387.0
7	UOP-53 x UOP-99	1.947	635.0
8	UOP-69 x UOP-79	1.894	218.0
9	UOP-69 x UOP-80	1.958	566.0
10	UOP-69 x UOP-20	1.900	140.0
11	UOP-69 x UOP-1185	1.962	250.0
12	UOP-69 x UOP-60	1.902	238.0
13	UOP-69 x UOP-99	2.133	314.0
14	UOP-79 x UOP-80	2.110	750.0
15	UOP-79 x UOP-20	2.082	373.0
16	UOP-79 x UOP-1185	2.080	382.0
17	UOP-79 x UOP-60	1.989	458.0
18	UOP-79 x UOP-99	2.096	375.0
19	UOP-80 x UOP-20	2.044	225.0
20	UOP-80 x UOP-1185	2.000	162.0
21	UOP-80 x UOP-60	2.019	257.0
22	UOP-80 x UOP-99	2.000	186.0
23	UOP-20 x UOP-1185	2.118	188.2
24	UOP-20 x UOP-60	2.154	168.6
25	UOP-20 x UOP-99	2.062	162.0
26	UOP-1185 x UOP-60	2.081	189.0
27	UOP-1185 x UOP-99	2.167	131.9
28	UOP-60 x UOP-99	1.923	123.0
29	UOP-53 (P1)	1.957	225.0
30	UOP-69 (P2)	1.862	132.0
31	UOP-79 (P3)	1.872	358.0
32	UOP-80 (P4)	2.000	230.0
33	UOP-20 (P5)	1.872	179.0
34	UOP-1185 (P6)	1.888	495.0
35	UOP-60 (P7)	1.857	159.0
36	UOP-99(P8)	1.929	331.0
37	CHETAK APHIM (C_1)	1.902	148.5
38	MOP-540 (C_2)	1.940	243.0

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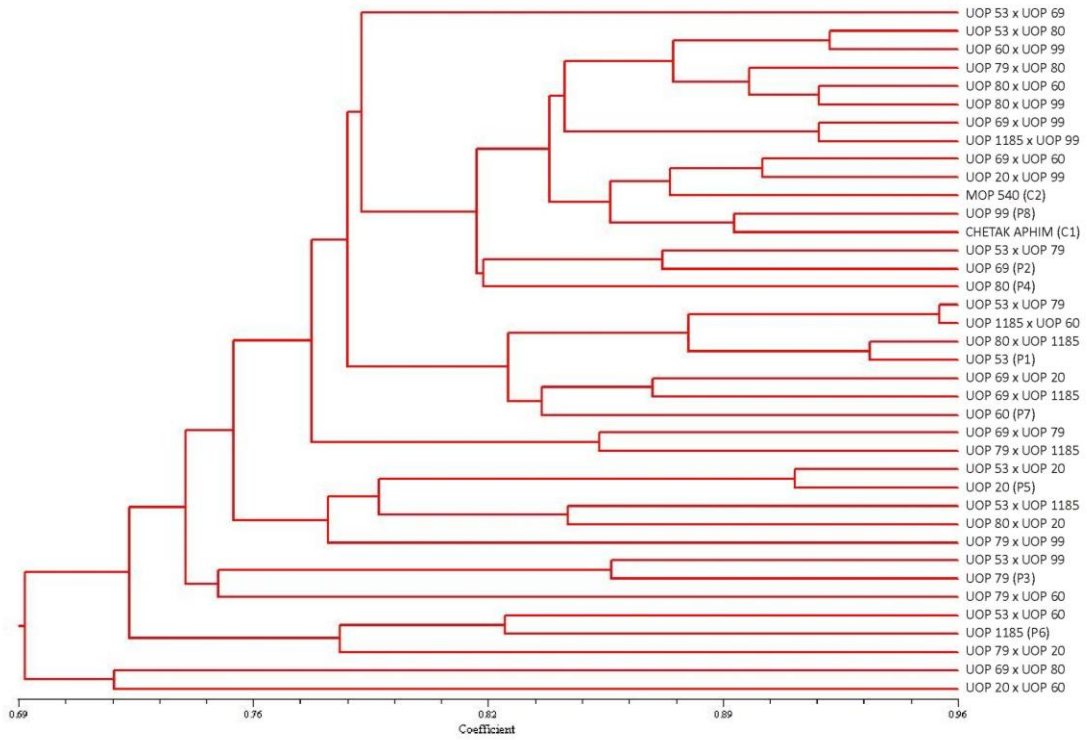

Fig. 1. Dendrogram generated for opium poppy crosses, parents and checks using UPGMA cluster based on Jaccard's similarity coefficient

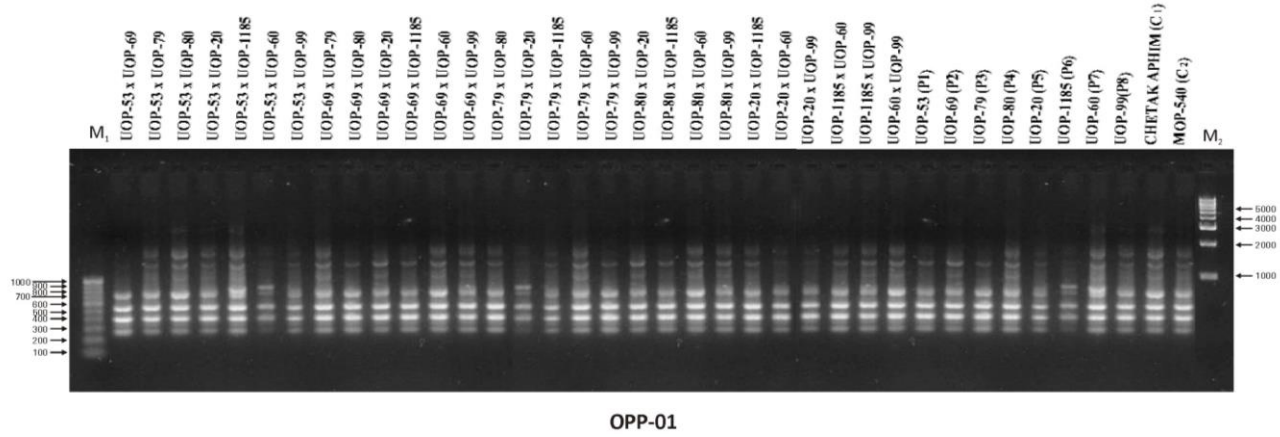

Fig. 2. RAPD profile of opium poppy crosses, parents and checks generated with primer OPP-01