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Genetic variations assessment in opium poppy using RAPD marker

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Abstract
Present investigation was carried out at department of molecular biology and biotechnology, Rajasthan College of Agriculture, Udaipur to determine the genetic variations in the most valuable medicinal crop Opium poppy (*P. somniferum* L.). Total 24 diverse accessions of Opium poppy were evaluated for genetic diversity assessment using RAPD (Random Amplified Polymorphic DNA) marker. 15 primers yielded 130 amplified loci, of which 108 were polymorphic. The polymorphism amongst 24 genotypes of *P. somniferum* was 83.07% and the overall size of PCR amplified products ranged between 100 bp to 2700 bp. The Jaccard's similarity coefficient value 0.43 to 0.86 certified the variation distribution is diverse. UPGMA based phylogenetic tree depicting the genetic relationship among 24 opium poppy accessions could be grouped into two major clusters. These cluster further again classified into two sub-clusters. This RAPD assay provides significant heterogeneity information of opium poppy accessions for further exploitation in crop improvement.

Keywords: Molecular marker, RAPD, *Papaver somniferum* L. etc.

1. Introduction
Opium poppy (*P. somniferum* L.:2n=22), is an annual medicinal rabi sown crop grown in India, belong to the family Papaveraceae, genus 'papaver'. It is an important valuable crop due to the presence of three major alkaloid groups, Phenanthredene (morphine, codeine, thebaine), true Benzylisoquinilone (papaverine) and Phthalideisoquinilone (narcotine), (Hagel and Facchini, 2013) [6]. Differentiation based on the morphological characters among plant species is too difficult hence genetic variation play an important role in the study of inter and intra-specific genetic variation among plant species (Bharathi *et al.*, 2017) [2]. DNA-based markers provide a more specific analysis of estimating the genetic relationship between genotypes and taxonomic groups as compared to morphological and biochemical markers. Among the various DNA marker–assisted techniques RAPD, (Randomly Amplified Polymorphic DNA) (Williams *et al.*, 1990) [15] has been most popular because of speed, low cost and the use of only minute amounts of plant matter for study. Molecular markers applied in plant breeding to establish the need for information on variation in DNA sequences even in those crops, which contain little classical genetic and cytogenetic information. Due to the high level of polymorphism, now days several markers are used for diversity analysis in crop improvement programme (Weising *et al.* 2005; Nybom *et al.* 2014) [14, 9]. Polymerase chain reaction based technology has led to the development of simple and quick RAPD techniques, which identify PCR fragment polymorphisms, using a single primer of arbitrary nucleotide sequence. The evaluation based on RAPD profiles would be suitable for providing such information by virtue of high level of DNA polymorphism by this technique (Srivastava *et al.*, 2011) [13]. RAPD based technique has been reported in various crops such as opium poppy, ociumum etc. (Acharya and Sharma 2009, Darokar *et al.*, 2014, Chikkaswamy 2015, Patel *et al.*, 2015, Khatik *et al.*, 2017) [1, 4, 3, 10, 8]. With this background, we have attempted genetic characterization in opium poppy genotypes using RAPD marker.

2. Material and Methods
2.1 Plant materials and isolation of genomic DNA
In the present investigation, fresh young leaves samples of 24 accessions of Opium (*P. somniferum* L.) were collected from the field of AICRP on Medicinal, Aromatic Plants and Betel vine, Rajasthan College of Agriculture, Udaipur. These 21-28 DAS of Opium poppy accessions leaves were used for the genomic DNA isolation following the CTAB (Doyle and Doyle, 1987) [5] method further Leaf tissues were transferred into pre-chilled mortar and grind

it further this fine powder was allowed to thaw in the presence of 10 ml of pre-heated extraction buffer and incubated. An equal volume of chloroform: isoamyl-alcohol mixture (24:1 v/v) was added. The precipitated DNA was pipette into an eppendorf tube 500 µl of TE buffer was added to dissolve the DNA followed by addition of 10 µl of RNase and incubated at 37ºC for 30 minutes. This mixture was centrifuged and the supernatant was discarded. The DNA pellet in 150-250 µl of TE (depending on the pellet size) and stored at 4ºC. The quality of DNA was judged from the ratio of the two OD values recorded at 260 and 280 nm.

2.2 RAPD primer screening and genotyping

A total of 20 decanucleotide RAPD primers (Bangalore Genei Pvt. Ltd.) were used for PCR amplification. The PCR reaction mixture contain Quantity of DNA was diluted to a final concentration of 25 ng/µl using TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0). Master mixture contain dNTP mix 200 µM, *Taq* DNA polymerase 1U, Reaction buffer (10X), Primer 0.5 µM. PCR conditions for RAPD analysis included an initial pre-denaturation step of 5 minutes at 94°C and following 35 cycles of amplification. Denaturation 94°C for 1 minute, annealing temperature 32ºC for 1 minute, Extension temperature for 2 minute. The amplified products were loaded on 0.8% agarose gel the gel was visualized on a UV-transilluminator and photographed using gel documentation system.

2.3 Data scoring and statistical analysis

The scores (0 or 1) for each RAPD band obtained 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 (Jaccard, 1908) [7]. Cluster analysis for the genetic distances was then carried out using UPGMA clustering method (Sneath and Sokal, 1973) [12]. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the genotypes using computer program NTSYSpc version 2.02 (Rohlf, 2004) [11]. A two-dimensional and three dimensional principal component analysis (PCA) was constructed to provide another means of testing the relationship among the cultivars using the EIGEN programme (NYSTS-pc). Polymorphism Information Content (PIC) values was also calculated for ISSR marker system.

3. Result and Discussion

Twenty four accessions of opium analyzed for the evaluation to measure the extent of genetic diversity and for further crop improvement of opium poppy. Extracted DNA ranged from 1715 to 3395 ng/µl showed adequate quality. UOP-119 exhibited the maximum quantity of DNA (3395 ng/µl). Gel electrophoresis and Absorbance ratio (A260/A280) ranged from 1.62 to 1.89 also certified that obtained DNA had high quality for further PCR based amplification. Out of Twenty RAPD primers only 15 exhibited good amplification in all accessons. Frequently amplified loci were selected for data analysis. 15 primers yielded 130 scorable bands, of which 108 were polymorphic. The total number of amplified bands varied between five (primer OPA-11, Fig 1) and 12 (primer OPA-15 and OPB-03) with an average of 8.67 bands per primer. The polymorphism amongst 24 genotypes of *P. somniferum* was 83.07% and the overall size of PCR amplified products ranged between 100 bp to 2700 bp. The percent polymorphism ranged from as low as 12.50 (OPB-11) to as high as 100 (OPA-05, OPA-07, OPA-09, OPA-10, OPA-11, OPA-15, OPA-16, OPB-03, OPB-04). The average PIC was 0.257 ranging from 0.069 to 0.447. The lowest and the highest PIC values were recorded for primers OPB-11 and OPA-10, respectively (Table 1). Chikkaswamy (2015) [3] also reported in their observations wherein three out of ten decamer primers had generated 250 RAPD fragments with 232 being polymorphic (96.84%). RAPD markers exhibited different levels of genetic polymorphism of which some were useful for species identification and discrimination. Likewise, Patel *et al.* (2015) [10] in their studies showed using 20 RAPD primers that in 17 *Ocimum* genotypes, a total of 506 loci were generated, of which 490 (96.47%) loci were found polymorphic. Based on RAPD similarity matrix data, the value of similarity coefficient ranged from 0.43 to 0.86, *i.e.* 43-86% (Table 1). Maximum similarity value of 0.86 was observed between genotypes UOP-2 and UOP-20 followed by UOP-145 and UOP-150 (0.85) and UOP-53, UOP-54, UOP-88, and UOP-99 (0.84). Contrarily, minimum similarity value of 0.48 was observed between genotypes UOP-60 and UOP-80. Khatik *et al.* (2017) [8] have been reported similar findings in opium genotypes with value of Jacaard's similarity coefficient ranging between 0.57 to 0.95 reports.

Table 1: DNA amplification pattern and polymorphism generated in *P. somniferum spp.* Using 15 RAPD primers

S. No.	Primer code	Molecular weight range (bp)	Total number of scorable bands (a)	Total number of polymorphic band (b)	Polymorphism (%) b/a X 100	PIC valve
1.	OPA-01	350-2400	10	6	60	0.2623
2.	OPA-05	350-2400	10	10	100	0.3704
3.	OPA-07	300-2500	8	8	100	0.396
4.	OPA-09	250-1950	6	6	100	0.32
5.	OPA-10	350-1500	10	10	100	0.447
6.	OPA-11	600-2100	5	5	100	0.224
7.	OPA-14	225-1775	8	4	50	0.1381
8.	OPA-15	200-2000	12	12	100	0.4232
9.	OPA-16	400-2700	9	9	100	0.257
10.	OPB-02	350-1900	8	6	75	0.1242
11.	OPB-03	100-1700	12	12	100	0.3824
12.	OPB-04	200-1200	8	8	100	0.1869
13.	OPB-07	350-2100	7	5	71.42	0.1717
14.	OPB-11	350-1900	8	1	12.50	0.0694
15.	OPC-08	300-1900	9	6	66.66	0.0951
	Total		130	108	83.07	0.2578

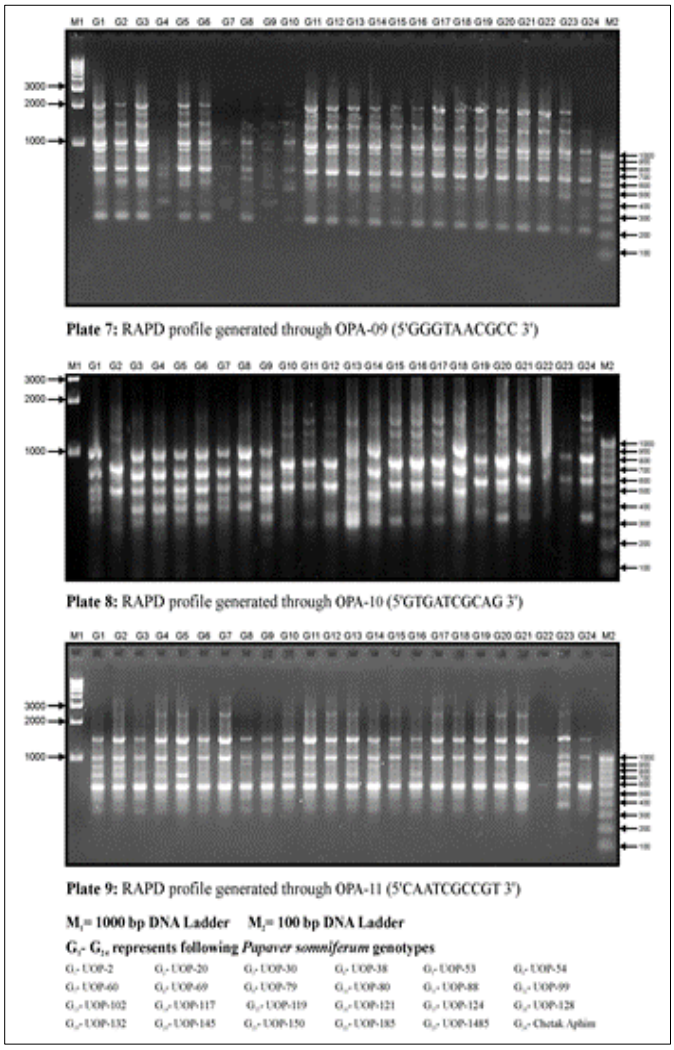

Fig 1: RAPD profile generated through OPA-09, OPA-10 and OPA-11.

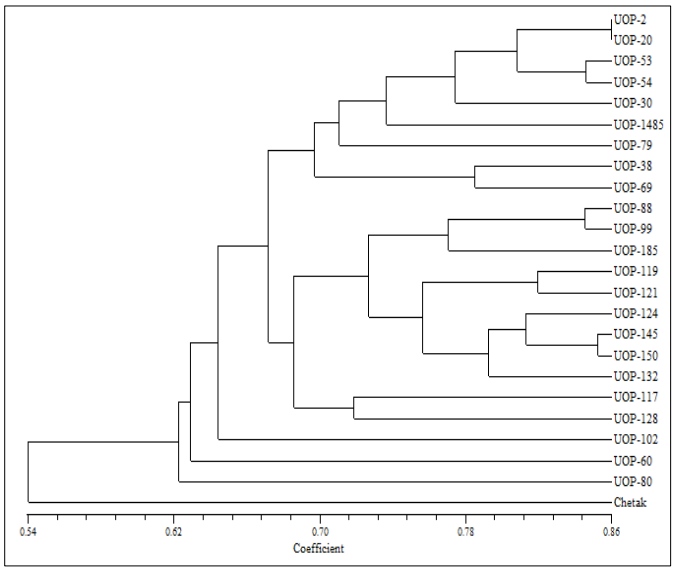

Fig 2: Dendogram generated for *Opium spp.* genotypes for RAPD using UPGMA cluster analysis based on Jaccard Similarity Coefficient

4. Conclusion

Twenty RAPD primers having 60% or more GC content were used for the present study. Out of 20 primers, only 15 showed a good amplification. RAPD analysis revealed out of 130 amplified bands 108 was polymorphic with 83.07% polymorphism. Study of genetic diversity paves a path for the selection of important germplams which can be exploited for further opium breeding improvement programe.

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