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ISSR-Based Molecular Characterization of Opium poppy (*Papaver somniferum* L.) Genotypes

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A B S T R A C T

Opium poppy (*Papaver somniferum* L.) is an important medicinal crop in the world. In the present investigation 24 prominent opium accessions were assessed by ISSR molecular markers for the genetic diversity analysis. A total of 116 amplified bands were obtained from the 15 primers, out of which 98 were polymorphic. Overall size of PCR amplified products had ranged between 300 bp to 2800 bp. The polymorphism percentage ranged from 16.66% to 100% for nine primers. The ISSR similarity matrix revealed the Jaccard's similarity values lie between 0.35-0.90. The 24 genotypes could be divided into two major clusters at a similarity coefficient of 0.47, Chetak Aphim sole genotype made single cluster while Cluster II included 23 genotypes. UPGMA method used for dendrogram exhibited the proper genetic diversity in all the 24 accessions of opium poppy. The present investigation revealed that significant diversity existed in all the genotypes of Opium poppy.

Introduction

Opium poppy (*P. somniferum* L.:2n=22), locally known as 'Aphim' belongs to the family Papaveraceae, genus 'papaver'. The genus consists of naturally spreading more than 100 species of annuals, biennials and perennials all over the world. Asia Minor is believed to be the origin of opium poppy, (Terry and Pellens, 1928) and later on spread to Egypt, Greece, Arab, China, India and Europe, (Schiff Jr, 2002).. *P. somniferum* L. is known due to its specialized opiate having more than 80 alkaloids present in laticiferous vessels of its wall. Opium poppy is mainly cultivated for commercial purposes due to its

high opium yield potential, (Rastogi *et al.,* 2012). Alkaloids like morphine, codeine, narcotine, the baine and papaverine, are frequently used in medicinal preparations as painkiller, sedatives, analgesics, anti tussive and anti-spasmodic in modern medicinal formulations.

ISSR is a commonly used PCR-based DNA technique to compare the genetic relationships and the patterns of variation amongst accessions of crops. ISSR technique is highly effective in plant fingerprinting and phylogenetics studies, (Vaillancourt *et al.,* 2008). Diversity analysis in opium poppy using ISSR has also been studied by (Acharya and Sharma, 2009), (Gurkok *et al.,* 2013), (Guclu *et al.,* 2014), (Das *et al.,* 2015), (Kaur *et al.,* 2015), (Mohseni *et al.,* 2015). Therefore, in the present investigation 24 accessions of opium analyzed for the evaluation to measure the extent of genetic diversity and for further crop improvement of opium poppy.

Materials and Methods

Plant materials and isolation of genomic DNA

In the present study, leaf samples of 24 genotypes of Opium poppy (*P. somniferum* L.) were procured from the field of AICRP on Medicinal, Aromatic Plants and Betel vine, RCA Udaipur. Genomic DNA was extracted from young leaves of 21-28 DAS of Opium poppy genotypes following the CTAB method (Doyle and Doyle, 1987), with slightly manipulation. 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 pipetted 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 37^{o}C 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 4^{o}C. The quality of DNA was judged from the ratio of the two OD values recorded at 260 and 280 nm.

Screening of ISSR primer and genotyping

Initially a total 20 UBC series primers (Bangalore Genei Pvt. Ltd.) having 60% or more GC content were used for appropriateness of each primer for the further genotyping. Based on the distinctness and resolving property of amplified product finally, a total of 15 primers exhibited good 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 ISSR 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 42.9-55.9^{o}C 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.

Data scoring and statistical analysis

The scores (0 or 1) for each ISSR 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) Cluster analysis for the genetic distances was then carried out using UPGMA clustering method (Sneath and Sokal, 1973). 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).

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.

Results and Discussion

Extracted DNA ranged from 1715 to 3395 ng/µl. 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 ISSR primers only 15 exhibited good amplification in all genotypes. Frequently amplified loci were selected for data analysis. 15 primers yielded 116 scorable loci, of which 98 were polymorphic. The total number of amplified bands varied between four (UBC-836) and 14 (UBC-811) with an average of 7.73 per primer.

The polymorphism percentage ranged from 16.66% (UBC-854 and UBC-873) to 100% for nine primers. Average polymorphism across the 24 genotypes of *P. somniferum* was found to be 84.48%. Overall size of PCR amplified products had ranged between 300 bp to 2800 bp (Table 1). Six unique bands were detected in only nine genotypes *viz.*, UOP-02, UOP-53, UOP-79, UOP-80, UOP-88, UOP-117, UOP-128, UOP-150 and UOP-185 with the size range of 900-2500 bp. The average PIC value was 0.398 ranging from 0.084 to 0.665 with lowest and highest PIC values recorded for primers UBC-810 and UBC-822 respectively (Fig 1). Study by (Gurkok *et al.*, 2014), on Oxytona section of genus *Papaver* by ISSR molecular marker system also given the same results.

In their investigation, 20 ISSR primers yielded 82 bands, of which 80 were found polymorphic. (Guclu *et al.*, 2013) also exhibited relevant results like 15 primers yielded 55 bands, of which 44 were polymorphic. The value of similarity coefficient ranged from 0.35 to 0.90, *i.e.* 35-90%.

All the 24 genotypes could be divided into two major clusters at a similarity coefficient of 0.47 (Fig 2). Cluster I included one genotype Chetak Aphim at a similarity coefficient of 0.47.

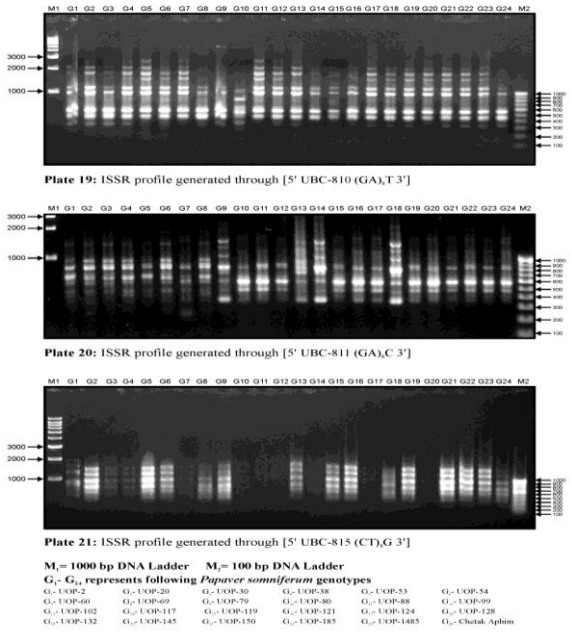
Cluster II included 23 genotypes. Cluster II could be further divided into 2 sub clusters at a similarity coefficient of 0.52. Sub cluster I included 15 genotypes while Sub cluster II included eight genotypes.

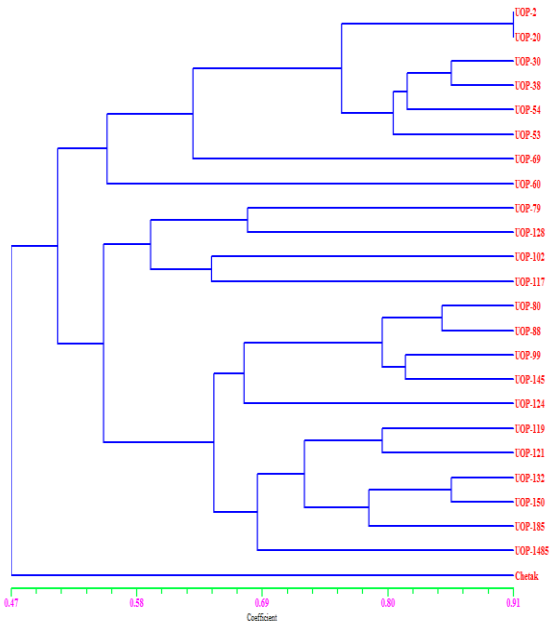
Hence, this investigation provides novel insights into the genetic diversity and population structure of *P. somniferum* L., which will helpful to the plant breeder for further resource utilization and the development of genetic improvement approach for *P. somniferum* L. the present study also proved that ISSR markers like UBC-815, UBC-822, UBC-840 with higher polymorphism and PIC value could be further used for the molecular characterization of opium poppy accessions.

Table.1 DNA amplification pattern and polymorphism generated in *P. somniferum spp.* Using 15 ISSR 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.	UBC-810	450-2500	9	4	44.44	**0.0843**
2.	UBC-811	350-2200	**14**	13	92.85	0.34
3.	UBC-815	600-1600	8	8	100	0.6607
4.	UBC-817	500-1900	8	8	100	0.3824
5.	UBC-820	500-900	5	5	100	0.5105
6.	UBC-822	900-2500	6	6	100	**0.6652**
7.	UBC-826	350-1250	9	9	100	0.4265
8.	UBC-834	525-2800	6	6	100	0.3571
9.	UBC-836	800-2000	**4**	4	33.33	0.28
10.	UBC-840	400-2000	9	9	100	0.6549
11.	UBC-848	400-1500	7	7	100	0.495
12.	UBC-854	300-1350	6	1	**16.66**	0.1116
13.	UBC-872	500-1800	11	11	100	0.4411
14.	UBC-873	300-1500	6	1	**16.66**	0.3564
15.	UBC-878	400-2200	8	6	75	0.3564
	Total		**116**	**98**	**84.48**	**0.398**

Fig.1ISSR profile generated through UBC-810, UBC-811 and UBC-815
Fig.2 Dendogram generated for *Opium spp.* genotypes for ISSR using UPGMA cluster analysis based on Jaccard Similarity Coefficient





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