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DNA Finger Printing of Species and Accessions of *Plumbago* L. Using Issr Markers

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ABSTRACT

Genetic diversity among species and accessions of Plumbago L. were estimated using ISSR marker. In this study the three species and two accessions were collected from different sites, DNA was isolated from each plant. Out of the 10 primers used 5 primers produced highly polymorphic DNA fragments. The total number of amplified DNA fragments was 126. Polymorphic Information Content (PIC), Resolving power (Rp) were also calculated. The PIC values for ISSR primers ranged from 0.768166 to 0.877915. Rp values calculated ranged from 6.8 to 11.6. The highest Rp value was observed in UBC-820 i.e. 11.6, whereas lowest in UBC-841 i.e. 6.8. Nei's overall genetic diversity or heterozygosity was calculated and found 0.2194. The genetic identity between the population ranged from 0.51 to 0.86 and the genetic distance ranged from 0.14 to 0.49. The overall percentage of polymorphism was 55.00. Primer UBC 810 exhibit highest polymorphism i.e. 100%.

KEYWORDS : DNA Fingerprinting, ISSR, Primers.

INTRODUCTION:

Plumbagin is an important napthoquinone showed various activities including anticancer (Parimal and Sachdanadam 1993), antimutagenic and insecticidal activity (Kubo *et a.,*1983), antibacterial, antifungal (Didry *et al.,* 1994). The most exploited source of plumbagin is *Plumbago* species (Komaraiah *et al.,* 2003). Three species were found in India i.e. *P. auriculata; P. rosea* and *P. zeylanica.* Study of genetic diversity and the genetic relationship among species and accessions of *Plumbago* L. are important for the sustainable conservation and increased use of plant genetic resources. Hence, in the present Study, ISSR markers were employed to study the extent of genetic diversity among three species and two accessions of *Plumbago* L, collected from different locations.

MATERIAL AND METHODS

Plant materials

The plant samples were collected from different locations of Maharashtra i.e. Chikhaldara, Amravati Dist., Dapoli Dist. and Parbhani Dist. The samples were randomly collected from each population. The voucher specimens are deposited in the department and identified by BSI Pune, Maharashtra. Identified species and accessions were coded for convenience (Table No. 1)

Genomic DNA isolation and purification

Tender unfolded leaf samples were collected from three species and two accessions locations and stored at -70°C for DNA extraction. The total genomic DNA was extracted from the samples using the CTAB method (Gadge, and Nathar 2015). Concentration of the purified genomic DNA in each case was adjusted to 10 ng/ µl and stored at -70° C for PCR amplification.

PCR amplification

Five out of ten ISSR primers were used for PCR amplification of the genomic DNA of *Plumbago zeylanica* GC content and Tm were calculated for each primer (Table - 2). PCR reactions were carried out in a final volume of 25 µl, which contained 2.5 µl 10 X Taq polymerase buffer, 4.0 µl of deoxyribonucletides (dNTPs), 0.5 µl $MgCl_2$, 0.1 µl of Taq DNA polymerase, 2.5 µl of deca oligonucleotide primer, 3.0 µl of template DNA and 12.4 µl of sterile distilled water. The reaction mixture was subjected to programmed PCR-amplification in a gradient PCR thermocycler machine (Biometra UNO Thermo block). The PCR program includes initial denaturation of DNA at 94°C for 7 minutes, denaturation at 94°C for 1 minute, annealing temperature varied from 47-52°C for each primer for 45 seconds and extension at 72°C for 2 minutes. The program was repeated for 35 cycles with final extension at 72°C for 10 minutes. The amplified DNA was stored at 4°C till electrophoresed. The products were resolved by electrophoresis on 1.5 % agarose gel containing ethidium bromide along with 1 Kb ladder DNA as a standard molecular weight size marker. The gels were visualized under UV transilluminator and image was captured using gel documentation system (Genei).

Data analysis

The photographic plate was transformed into binary matrix using '1' and '0' characters. The band was scored '1' for its presence and '0' for its absence. Genetic similarity index among populations was calculated using the standard coefficient method (Nei and Li, 1979). The dendrogram was constructed using the UPGMA (Unweighed Pair Group Method with Arithmetic Average) (Sneath and Sokal, 1973) algorithm in SHAN clustering module of NTSYS-pc software version 2.02 (Rohlf, 1997). The polymorphism information content (PIC) value was calculated using the formula,

$$PIC = 1 - \Sigma pi\ 2,$$

where *pi* is the frequency of the *i*th allele (Smith *et al.,* 1997).

Resolving power RP value was calculated as

$$Rp = \Sigma\ I\ b$$

$$I\ b = 1 - [2 \times (0.5 - pi)]$$

where I b is band informativeness, and *pi* is the proportion of accessions containing band *i* (Prevost and Wilkinson, 1999).

RESULTS AND DISCUSSION:

The genetic diversity and the relationship among three species and two accessions of *Plumbago* L. were analyzed with five ISSR random primers (10-mer) which produced resolved amplified fragments with average 25.2 per primers, total of 126 ISSR fragments were observed. Overall percentage of polymorphism was 55%, with 100% maximum observed in UBC-810 (Fig. 1) and the lowest in UBC-827 i.e. 33.33 (Table 3).

The constructed dendrogram showed that three species and two accessions were placed in two major clusters. Cluster I and Cluster II. Cluster I is further divided into two sub-clusters, Ia and Ib. Sub-cluster Ia comprised of species *P. auriculata* (*Pa1*) and its accessions (*Pa2*). Sub-cluster Ib consisted species *P. zeylanica* (*Pz1*) and its accessions (*Pz2*). Whereas *P. rosea* was grouped as separate cluster i.e. cluster II (Fig. 2).

Similarity matrix among two accessions and three species was calculated using the Jaccard's coefficient to the data obtained from ISSR analysis. It is clear from the obtained data and calculations that *P. auriculata* with its accession and *P. zeylanica* with its accession contribute the highest similarity index 0.86. The lowest similarity index i.e. 0.51 was observed among *P. rosea* (Pr) – and accession of *P. zeylanica* (*Pz2*) (Table - 3).The genetic distance was observed negligible i.e. 0.14

shared by species *P. auriculata* with its accession (*Pa1* and *Pa2*) and among *P. zeylanica* and its accession (*Pz1* and *Pz2*).

Calculated PIC values ranged from 0.768166 to 0.877915 in primer UBC-841and UBC-10 respectively. The average of PIC calculated showed 0.835893. Highest RP value 11.6 was observed in primer UBC-820 and lowest i.e. 6.8 in primer UBC-841. Average RP value was observed 9.36.

CONCLUSION:

Analysis of ISSR data can be used to detect genetic diversity among species and accessions of *Plumbago*. Thus in the present study the population which exhibited high percentage of polymorphism was considered to be the superior genotypes. Development of molecular technique makes it easy to analyze genetic characteristics of a specific species in varying environmental conditions. The accessions and species collected from various sites had the highest percentage of polymorphism. Such superior genotypes of these medicinal plants could be collected and conserved through *ex-situ* and *in-situ* conservation.

Table - 1: PLANT CODE AND COLLECTION SITES

Type	Species/accession	Code	Collection site
Species	*P. auriculata*	*Pa1*	Dist. Parbhani
Accession	*P. auriculata*	*Pa2*	Dist. Amravati
Species	*P. rosea*	*Pr*	Dist. Dapoli
Species	*P. zeylanica*	*Pz1*	Dist. Parbhani
Accession	*P. zeylanica*	*Pz2*	Dist. Amravati

Table - 2: PRIMER DETAILS

Primers	Sequence	GC content	Tm	Total amplicon	Polymorphic amplicon	% Polymorphism	PIC	Rp
UBC-10	GAGAGAGA-GAGAGAGAT	47 %	50°C	27	27	100	0.877915	9.2
UBC-811	GAGAGAGA-GAGAGAGAC	52%	52°C	23	8	34.78	0.816635	9.2
UBC-820	GTGTGTGTGT-GTGTGTG	52%	50°C	29	14	49.00	0.865636	11.6
UBC-827	ACACACACA-CACACACG	52%	52°C	30	10	33.33	0.851111	10
UBC-841	GAGAGAGA-GAGAGAGAC	52%	52°C	17	12	71.00	0.768166	6.8
	Total			126	71	55.00	0.835893	9.36

Table - 3: SIMILARITY MATRIX AND GENETIC DISTANCE.

Species/ Accession	Pa 1	Pa 2	Pr	Pz 1	Pz 2
Pa 1	***	0.14	0.41	0.33	0.46
Pa 2	0.86	***	0.44	0.30	0.38
Pr	0.59	0.56	***	0.46	0.49
Pz 1	0.67	0.70	0.54	***	0.14
Pz 2	0.54	0.62	0.51	0.86	***

Similarity matrix (below) and genetic distance (above).

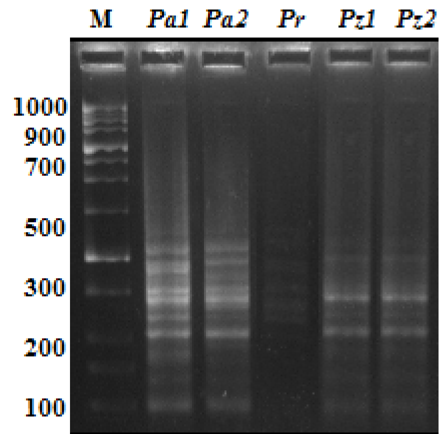

Fig. 1 ISSR pattern generated by UBC-810

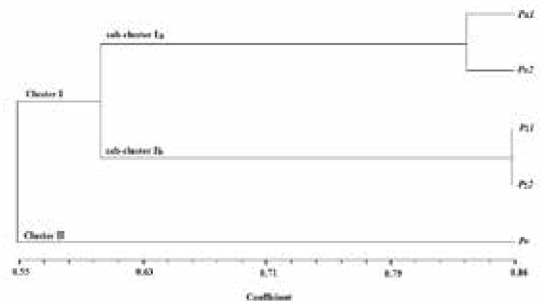

Fig. 2 Dendrogram of three species and two accessions of *Plumbago***.**

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