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MOLECULAR AUTHENTICATION OF *CRINUM SOLAPURENSE* PLANT BY RANDOM AMPLIFIED POLYMORPHIC DEOXYRIBONUCLEIC ACID (RAPD) -BARCODING TECHNIQUE

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Abstract:

Background:

DNA fingerprinting is a simple technique is used to identification of nucleotide sequences of the medicinal plants and DNA sequences.

Aim and Objectives:

Molecular Authentication of *Crinum Solapurense* Plant by Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD) -Barcoding Technique. The main objective of this research work was to identification of Particular DNA sequencing from group of unknown and protection of biodiversity.

Results and Discussion:

We used AP l, AP4. AP11 and OPAB 11 decemer primers to identify the sequencing the genetic variation of ayurvedic medicinal plants.

Conclusion: The DNA was amplified with four decemer primers and polymerase chain reaction primers, and then analyzed on 1 % agarose gels.

Keywords: Deoxyribonucleic Acid, Primers, Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD),Barcoding Technique.

Introduction:

DNA fingerprinting is a way to identify a certain individual, rather than simply identifying a particular species or a particular trait. A technique used scientists to distinguish between individuals of the same species using only samples of their DNA. In the classical DNA fingerprinting method radiolabeled DNA probes containing mini satellite oligonucleotide

sequences are hybridized to DNA that has been digested with a restriction enzyme, separated by agarose electrophoresis and immobilized on a membrane by Southern blotting or - in the case of the oligonucleotide probes - immobilized directly in the dried gel[1]. The radio-labelled probe hybridizes to asset of minisatellite or oligonucleotide stretches ingenomic DNA contained in restriction fragments whose size differ because of variation in the numbers of repeat units[2].Molecular fingerprinting which have been frequently used for studying genetic diversity, population genetics and genetic characterization in various plant species and crop cultivars. The molecular markers are not influenced by the external environmental factors unlike that the morphological markers hence accurately detect the genetic relationship between among the plant species[3]

Crinum solapurense resembles *C. viviparum var. viviparum* and *C. lorifolium* but differs in having 1 – 10 bulblets on the mother bulb, sturdy and canaliculate 12 – 27 leaves, 10 – 30-flowered umbels, undivided stigma and 3 – 12-seeded fruits. *Crinum solapurense* belongs to family Amaryllidaceae.

Material and Methods:

Collection and authentication of plants: The whole plant *Crinum solapurense* was collected from the Bhima River between Machnur village in Solapur district of Maharashtra, India.

RAPD markers used: AP l, AP4. AP11 and OPAB 11

DNA fingerprinting of the plant is performed at centre for DNA fingerprinting and diagnostic centre, Hyderabad on 02.11.2023.

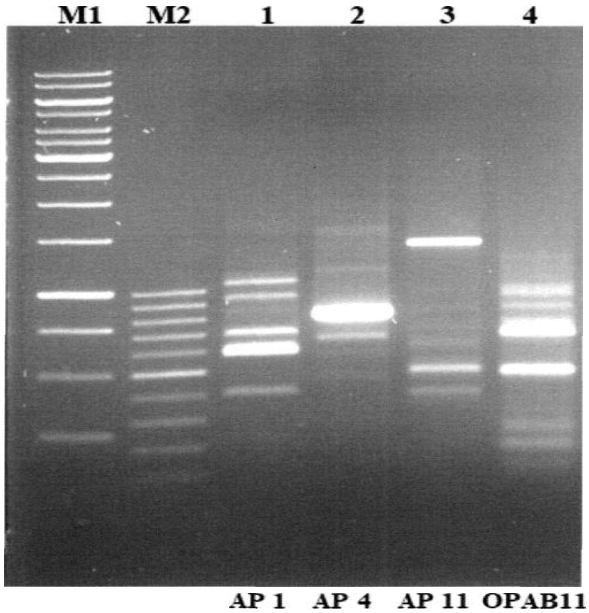
Method:

Procedure:

DNA was extracted from the leaf of *Crinum solapurense* using GSure plant mini kit and quantitated using Nanodrop. PCR was performed in 25 Ji1 reaction volumes containing 1 X PCR buffer [10 mMTris HCI (pH 8.3), 50 mM KCI,1.5 mMMgC L2], 200pM dNTPs, 50 ng of template DNA, 5 pmol of each primer, and 5 units of Taq polymerase (Thermo Scientific). PCR conditions included an initial denaturation step at 94°C for 5 min, followed by 45 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final extension at 72°C for 7 min. All amplified products were resolved in 1 % agarose gels (Agarose SERVA Wide Range) along with 1 kb molecular marker (Thermo Scientific).

Results: High-molecular weight DNA extracted from the plant materials was pure and free from RNA. The results are shown in figure 1 and table 1.

Figure 1: Profile of DNA fingerprinting of *Crinum solapurense* variety. In the figure Lane 1:



AP 1, Lane 2: AP 4, Lane 3: AP 11, Lane 4: OPAB 11. M 1 is the 1 Kb Molecular weight size standard. M2 is the l00bp Molecular weight size standard.

Primer Name	**Size (Base Pairs)**	**Primer sequences**
AP l	410	AGCCAGCGAA
	600	
	700	
	960	
	1200	
AP 4	680	CAAACGACGG
	800	
	1300	
AP 11	410	ATGCTCCGAG
	520	
	620	
	1400	
OPAB 11	250	GTGCGCAATG
	300	
	500	
	680	
	850	
	980	

Table 1: DNA Fingerprinting profile of *Crinum solapurense*

Discussion:

This research shows that the RAPD method may be used to successfully uncover useful DNA polymorphisms in species with extremely large genomes, such as *Crinum solapurense* Species. The high-molecular-weight DNA isolated from the leaves was RNA-free and pure. Using 4 PCR primers and repeat primers, the DNA was amplified and then analyzed on 1% agarose gels. The trials were repeated, and only the bands that could be replicated each time were used in the final statistical analysis. In a set of experiments using different dinucleotide

repeat primers, all primers successfully produced clear bands. It's not easy to explain why RAPD profile complexity is not correlated with genome size. Scientists believe that competition among priming sites in the template, rather than the total number of priming sites accessible, governs the composition of amplification products in RAPD reactions. Under the relaxed annealing conditions used in thermal cycling, primer-template mismatches are more likely to arise. Therefore, in the presence of several priming sites, competition leads to a greater proportion of initiations from perfectly matched sites and a lower total number of products.

Conclusion:

This study shows that the RAPD method can find useful DNA polymorphisms in large-genome species like *Crinum solapurense*. High-molecular-weight leaf DNA was RNA-free and clean. DNA was amplified and analyzed on 1% agarose gels using 4 PCR and repeat primers. Only repeatable bands were employed in the statistical analysis. All dinucleotide repeat primers produced clear bands in experiments. It is hard to explain why RAPD profile complexity does not correlate with genome size. According to scientists, RAPD reactions, amplification products are determined by template priming site competition rather than total priming site accessibility. Thermal cycling relaxes annealing, increasing primer-template mismatches. Thus, competition between priming sites increases the proportion of perfectly matched initiations and decreases the total number of products. RAPD markers allow the phylogenetic analysis of *Crinum solapurense* species.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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