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Molecular Authentication of the Traditional Medicinal Plant *Nyctanthesarbor-tristis* (Linn.) by RAPD –DNA Fingerprinting Technique

Kore H.R.* [1], Kondawar S.M. [2],Ghurghure M.S. [3]

[1]D.S.T.S. Mandal's College of Pharmacy, Vijapur Road Jule Solapur-1, Maharashtra
[2]AppasahebBirnale College of Pharmacy, Sangali, Maharashtra
*Email : kore.rundramani@rediffmail.com

ABSTRACT

The RAPD (Random Amplified Polymorphic DNA) technique was employed in the present study for determination of the components in an Ayurvedic herbal medicine. The RAPD approach is appealing to a large number of scientists since, it may be implemented in a variety of settings with little effort. Most importantly, Random Amplified Polymorphic DNA analysis does not require any cloning, sequencing, or other molecular characterization of the genome in order to extract a large number of genetic markers from a small amount of DNA. Fresh leaves of the*Nyctanthesarbor-tristis* (Linn.) plant were gathered in the month of June in the surroundings of Solapur. Researchers used OPAB11, OPT12, OPQ15, and OPC10 4 decamer primers to conduct a RAPD study, which measures genetic variation at random. Pure and RNA-free high-molecular-weight DNA was extracted from the leaves. The DNA was amplified with repeat primers and 4PCR primers, and then analysed on agarose gels.

Keywords : *Nyctanthesarbor-tristis* (Linn.); RAPD analysis; DNA: Primer; RAPD markers

INTRODUCTION

The RAPD (Random Amplified Polymorphic DNA) technique was employed in this study for determination of the components in an Ayurvedic herbal medicine. India is home to an abundance of flora and fauna that should be protected and studied. Having access to a large genome will help us overcome the obstacles to food production caused by various biotic and abiotic stressors. Only if there is a large diversity of genes within these kinds they can be crossed successfully. Unfortunately, bio-wealth is under constant attack despite our reliance on it. Recent developments in molecular biology have laid the groundwork for the discovery of an almost infinite variety of DNA markers. DNA-based markers' value is often established by the methods employed to uncover polymorphism in DNA. The Restriction Fragment Length Polymorphism (RFLP) assay, which measures genetic diversity and creates a genetic linkage map, is currently the method of choice for a wide range of species. To identify DNA polymorphism, the Restriction Fragment Length Polymorphism (RFLP) technique uses restriction enzyme digestion and DNA hybridization, both of which take a considerable amount of time and effort. Polymerase chain reaction (PCR) technology has quickly proliferated over the past decade, allowing for the creation of a number of novel genetic assays based on the selective amplification of deoxyribonucleic acid (DNA). The high success rate and seeming ease of polymerase chain reaction (PCR) are two of the main reasons for its widespread use.

Unfortunately, Polymerase chain reaction (PCR) tests are restricted in their utility due to the requirement of DNA sequence information. The establishment of genetic markers for various purposes was aided by the realization that Polymerase chain reaction (PCR) with random primers can be employed to amplify a set of randomly dispersed loci in any genome.

Principal of Random Amplified Polymorphic DNA Methods:

Polymerase chain reaction (PCR) amplification of random portions of genomic DNA with a single primer of arbitrary nucleotide sequence yields decamer (10-nucleotide length) DNA fragments that can differentiate between genetically separate individuals, albeit not always in a reproducible manner. It employs a random order of primers to examine the ancestry of a given individual. Several of the most prevalent uses of Random Amplified Polymorphic DNA markers are reviewed, as well as their concepts, operating mechanism, characteristics, laboratory techniques, data processing and interpretation, advantages, and limitations. Many researchers are curious about the Random Amplified Polymorphic DNA method. The ability to obtain a large number of genetic markers from very little amounts of DNA without the need for cloning, sequencing, or any other type of molecular characterization of the genome of the species in question is perhaps the most important factor in the success of Random Amplified Polymorphic DNA analysis.

A complex DNA template is utilised to amplify random sequences using a single, short oligonucleotide primer that binds to many distinct loci. This indicates that the size of the amplified fragment produced by Polymerase chain reaction (PCR) is proportional to both the length and size of the primer and the genome being amplified. An underlying premise of Polymerase chain reaction (PCR) amplification is that a given DNA sequence (complementary to that of the primer) would exist in the genome on opposite DNA strands in opposite orientation at a distance that is amenable to amplification by Polymerase chain reaction (PCR). These enlarged products (up to 3.0 kb) are typically separated on agarose gels (1.5-2.0%) and ethidium bromide stained for visualization. The use of a single decamer oligonucleotide stimulates the production of many discrete DNA products and these are believed to come from separate genetic loci. The presence or absence of a specific Random Amplified Polymorphic DNA band indicates the presence or absence of polymorphisms caused by mutations or rearrangements at or between the primer binding sites[7].

Many researchers are interested in the Random Amplified Polymorphic DNA method because of how easy it is to use and how broadly it may be applied[8]. The ability to obtain a significant number of genetic markers from very little amounts of DNA without the need for cloning, sequencing, or any other type of molecular characterization of the genome of the species in question is perhaps the most important factor in the success of Random Amplified Polymorphic DNA analysis[9].

In this study, we tested how well Random Amplified Polymorphic DNA analysis worked at identifying polymorphism in*Nyctanthesarbor-tristis* (Linn.).Our primary goal was to ascertain whether or whether this strategy could be used to generate polymorphic markers in

Nyctanthesarbor-tristis (Linn.)for the purposes of genotype identification, phylogeny analysis, and the creation of a genetic map of *Nyctanthesarbor-tristis* (Linn.)

MATERIALS AND METHODS

Collection and authentication of plants

The fresh leaves parts of *Nyctanthesarbor-tristis* (Linn.) were collected from local areas of Solapur District in month of June. The collected plant materials were authenticated at P.G. Department of Botany and Research Centre D. B. F. Dayanand College of Arts and Science, Solapur, India.DNA fingerprinting of plant is performed at the Centre for DNA fingerprinting and diagnostic, Hyderabad.

Procedure

Random amplified polymorphic DNA (RAPD) analysis was performed with 4 decamer primers like OPAB11, OPT 12, OPQ 15, and OPC10.

DNA Extraction

DNA was extracted from the leaf of *Nyctanthesarbor-tristis* (Linn.) using Gsure plant mini kit and quantitated using Nanodrop. PCR was performed in 25µl reaction volumes containing 1X PCR buffer [10mM Tris HCl (pH 8.3), 50mM KCl, 1.5 mM $MgCl_2$], 200µM dNTPs, 50ng of template DNA, 5 pmol of each primer, and 5 unit of Taq polymerase (Thermo scientific). PCR conditions included an initial Denaturation step at 94°C for 5 min, followed by 40 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 (Lonza Inc., USA) along with 1kb molecular marker (Thermo scientific). The reproducibility of the bands generated by these 04 primers was confirmed by replicating the amplification twice and if needed thrice. Only the bands showing reproducible amplification were considered for scoring and for further analysis.

The plant and DNA fingerprinting profile of*Nyctanthesarbor-tristis* (Linn.) is shown in Figure 1. Comparison of primers and DNA bands from the*Nyctanthesarbor-tristis* (Linn.) accession shown in table 1.

RESULTS AND DISCUSSION

High molecular weight DNA extracted from the leaf material was found to be pure and free of RNA. The DNA was diluted and was amplied using 4 Polymerase chain reaction (PCR) primers, repeat primers and were analysed on 1% agarose gels.



Fig.1: Image of *Nyctanthesarbor-tristis* (Linn.)

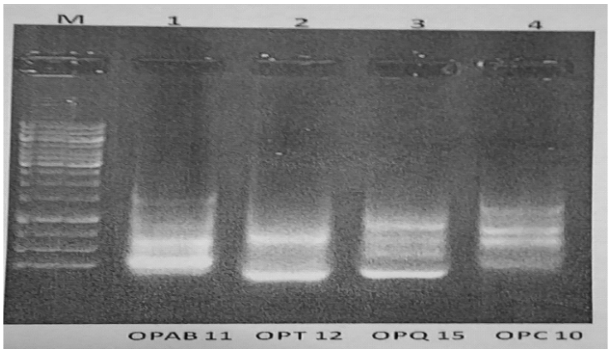

Fig.2: DNA fingerprinting profile of *Nyctanthesarbor-tristis* (Linn.) using RAPD markers.Lane 1: OPAB11, Lane 2: OPT 12, Lane 3: OPQ 15, Lane 4: OPC 10. M is the 1Kb molecular weight size standard.

Table 1: DNAfingerprinting profile of *Nyctanthesarbor-tristis* (Linn.)

Primers	Size (Base Pairs)	Primer sequence
OPAB 11	~230 ~250 ~650 ~1300	GTGCGCAATG
OPT 12	~200 ~670	GGGTGTGTAG
OPQ15	~210 ~400 ~670 ~910	GGGTAACGTA
OPC10	~250 ~670 ~850 ~1200	TGTCTGGGTG

This research shows that the Random Amplified Polymorphic DNA method may be used to successfully uncover useful DNA polymorphisms in species with extremely large genomes, such as *Nyctanthesarbor-tristis* (Linn.) Species. The high-molecular-weight DNA isolated from the leaves was found to be RNA-free and pure. Using 4 PCR primers and repeat primers, the DNA was amplified and then analysed 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 Random Amplified Polymorphic DNA 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 Random Amplified Polymorphic DNA reactions. Under the relaxed annealing conditions used in the 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. Our results show that phylogenetic analysis of species is possible through the application of Random Amplified Polymorphic DNA markers. A less biassed and more thorough dendrogram might be generated by the study of a larger number of cultivators within each species, as well as other wild relatives of cultivated *Nyctanthesarbor-tristis* (Linn.). Single sample for DNA barcoding analysis is reliable and convenient for authenticating medicinal species. Additionally; this would permit a more precise measurement of the differences in genetic makeup between different species. A more precise reconstruction of the *Nyctanthesarbor-tristis* (Linn.) Genus may be possible with the use of such an analysis and data from other cladistic methods. The use of such a method could also be useful in locating other wild*Nyctanthesarbor-tristis* (Linn.) Species that could be of use in genetic improvement efforts. DNA Barcoding method is effective utility in quality control of commercially important medicinal herbs which are adulterated. Application of these DNA barcoding techniques will help in identifying the authentic samples of herbal drugs used in herbal formulations. This also helps to identify the potent medicaments in the preparation.

CONCLUSION

This study shows that the Random Amplified Polymorphic DNA method can find useful DNA polymorphisms in large-genome species like *Nyctanthesarbor-tristis* (Linn.). High-molecular-weight leaf DNA was RNA-free and clean. DNA was amplified and analysed on 1% agarose gels

using 4 Polymerase chain reaction (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 Random Amplified Polymorphic DNA profile complexity does not correlate with genome size. According to scientists, Random Amplified Polymorphic DNA 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. Random Amplified Polymorphic DNA markers allow phylogenetic analysis of*Nyctanthesarbor-tristis* (Linn.) species. Studying multiple cultivators within each species and other wild relatives of farmed*Nyctanthesarbor-tristis* (Linn.) may produce a more accurate dendrogram. This would also enable more accurate species-specific genomic measurements. Such an analysis and other cladistic methods may help reconstruct *Nyctanthesarbor-tristis* (Linn.) more precisely. This strategy could potentially help to find wild*Nyctanthesarbor-tristis* (Linn.) species for genetic improvement.

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