British Biotechnology Journal
9(2): 1-6, 2015, Article no.BBJ.18727
ISSN: 2231–2927

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Evaluation of Genetic Variability in *Adansonia digitata* L. by RAPD Analysis

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Authors' contributions

This work was carried out in collaboration between all authors. Author LG carried out the analysis *and* managed the literature searches*. Author SAG designed the study, drafted the manuscript and interpreted the data. Authors RKA and RS managed and supervised the study. All authors read and approved the final manuscript.*

Article Information

DOI: 10.9734/BBJ/2015/18727
Editor(s):
(1) Chung-Jen Chiang, Department of medical laboratory Science and Biotechnology, China Medical University, Taiwan.
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(4) Anonymous, Mexico.
(5) Anonymous, Vietnam.
Complete Peer review History: http://sciencedomain.org/review-history/10616

Original Research Article

Received 7th May 2015
Accepted 6th August 2015
Published 19th August 2015

ABSTRACT

Baobab (*Adansonia digitata* L.) belonging to Bombacaceae family, is one of the most widely used indigenous tree species in sub-Saharan Africa. The objective of the present study was to assess molecular variation among *A. digitata* and to determine the level of genetic similarity among them. The yield of DNA ranged from 15-40 µg/mg of leaf tissue and the purity was between 1.1- 2.9, indicating minimal levels of contaminating metabolites. The technique was ideal for isolation of DNA from *A. digitata* and the DNA isolated was used for randomly amplified polymorphic DNA (RAPD) analysis using the primer OPB07. The bands obtained ranged in size from 54-795 bp. Two clusters were observed, one group with 8 bands and the other with 11 bands. Present study could be important in domestication, conservation, management & improvement strategies of *A. digitata*.

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Keywords: Amplification; Baobab; conservation; genetic diversity; RAPD.

ABBREVIATIONS

RAPD: Random Amplified Polymorphic DNA.
PCR : Polymerase Chain Reaction.

1. INTRODUCTION

Medicinal plants play a vital role in the maintenance of human health. *Adansonia digitata* is one of the important medicinal plants, extensively used by traditional practitioners in India for its medicinal value.

It is important to understand the pattern of variation existing in populations of economically important trees, for use in domestication, conservation, management and plant breeding. Such a distribution should result in formation of distinct geographical races that are adapted to various ecological conditions [1]. Baobab has a long history of traditional uses as treatment against fever, dysentery and bleeding wound. Every part of the baobab tree is reported to be useful [2]. Since variability is a prerequisite for selection program, it is necessary to detect & document the amount of variation existing within and between populations.

One of the molecular marker techniques for studying genetic variability is Random Amplified Polymorphic DNA (RAPD). The technique requires no prior knowledge of the genome and needs only a small amount of DNA. Using the technique, polymorphism can be detected in closely related organisms. RAPD is preferred by many researchers as an effective method to use for identification of genetic variation within and among populations in forest trees. The RAPD technique, a quick and relatively inexpensive method, is extensively used to analyze the genetic variability among bacteria, fungi and plants. The study demonstrated that RAPD markers are useful tools to compare the genetic relationship and pattern of variation among such prioritized and endangered medicinal plants [3]. In addition, RAPD analysis is efficient, economical and produces genetic markers suited to the assessment of population, race and species-specific genetic variation. The present study aims to assess the genetic relationship between different *A. digitata* plants collected from various parts of Agra district.

2. MATERIALS AND METHODS

2.1 Plant Materials and DNA Extraction

A. digitata leaves were used for RAPD analysis which was collected from different regions of Agra district of Uttar Pradesh (Table 1). Deoxyribonucleic acid (DNA) extraction was improved by modifying some of the steps in the original CTAB DNA isolation protocol [4]. 1 g fresh young leaves were homogenized in a pestle mortar with liquid nitrogen. Then 5 ml extraction buffer (Tris-HCl 100 mM, pH 8.0; EDTA 20 mM, pH 8.0; NaCl 1.4 M) was added. Each sample was shaken and equal volume of chloroform-isoamyl alcohol (24:1) was added. The phases were separated by centrifugation at 15,000 rpm for 15 min. The nucleic acid present in the upper aqueous phase was precipitated by using isopropanol. RNA contamination was removed by RNase enzyme and DNA was recovered using absolute alcohol and stored and resuspended in 120 mL Tris EDTA.

Table1. Site of collection of *Adansonia digitata* (Baobab) from Agra district

Sample	Site of collection
1	Khandari
2	Bhagwan cinema
3	Sikandra
4	Paschimpuri
5	Fatehpursikri
6	Tundla road
7	Keetham
8	Gwalior road
9	Tajganj
10	Fatehabad

2.2 Quantitative and Qualitative Analysis of Genomic DNA

DNA quantity and purity was estimated by measuring the OD (optical density) at 260 and 280 nm [5].

The amount of DNA can be calculated by using the formula:

*Yield of DNA = Absorbance 260*50* dilution factor 1000 g/ml.*

2.3 RAPD Analysis

RAPD analysis was carried out by following the protocol of Williams et al. [6].

A PCR tube contained 2 µl DNA, Taq DNA polymerase 0.5 unit, 100 mM dNTPs, 5 µl 10x polymerase buffer and 2 µl of primers. Forty cycles were programmed in a thermal cycler (Bio-Rad} with initial denaturation at 94ºC for 4 min. In each cycle, denaturation for 1 min. at 94ºC, annealing for 2 min. at 35-36ºC and extension for 2 min. at 72ºC was performed with the final extension after 40 cycles for 7 min. Following amplification, the samples were stored at 4ºC prior to electrophoresis.

2.4. Electrophoresis

The RAPD-PCR reactions were electrophoresed in 1% agarose gel. Agarose was dissolved in 1x TAE buffer and boiled in water bath. Ethidium bromide (0.5 ug/ml) was added in the solution and disposed it in electrophoretic unit. Samples were loaded in the wells to run at 50 V for 2 hours. The bands were visualized in gel documentation system.

3. RESULTS

3.1 Quantitative and Qualitative Analysis of DNA

The purity of DNA was estimated by comparing the absorbance at 260 and 280 nm. If the ratio of absorbance at 260 and 280 is between 1.7 and 1.9 the DNA sample is relative free from impurities. If the ratio of absorbance at 260 and 280 is less than 1.7 and 1.9 the DNA sample is contaminated with protein. If the ratio is greater than 1.9 the DNA sample may have Phenol, EDTA and other impurities like cell debris or RNA. The quantity and purity of the isolated DNA was determined by spectrophotometry. An average DNA yield of 15-40 µg/mg was observed. The maximum yield (40 µg/mg) & minimum yield (15 µg/mg) was observed in sample 5 and sample 8, respectively (Table 2).

Table 2. Quantitative analysis of *Adansonia digitata* DNA

Sample no.	Amount of DNA (µg/mg)
1	20
2	22
3	18
4	35
5	40
6	25
7	17
8	15
9	18
10	16

The purity of isolated DNA was observed from extinction ratio (260/280 nm). The purity ranged from 1.1 to 2.9 that indicate variable purity range (Table 3).

3.2 RAPD Analysis

The RAPD technique was used to find out the extent of genetic diversity in *Adansonia digitata* and primer OPB07 was used for amplification.

Primer sequence:

OPB 07 5' GGT GAC GCA G 3'
M = Marker (100 – 2000 bp)
2000, 1000, 800, 700, 600, 500, 300, 200, 100 bp

Table 3. Qualitative analysis of *Adansonia digitata* DNA

Sample	O.D at 260 (nm)	O.D at 280 (nm)	Ratio	Quality
1	0.22	0.16	1.3	Contaminated with protein
2	0.62	0.54	1.1	Contaminated with protein
3	0.25	0.15	1.6	Contaminated with protein
4	0.29	0.18	1.6	Contaminated with protein
5	0.23	0.10	2.3	Other impurities
6	0.19	0.12	1.5	Contaminated with protein
7	0.19	0.10	1.9	Free from impurities
8	0.12	0.12	1.0	Contaminated with protein
9	0.10	0.10	1.0	Contaminated with protein
10	0.25	0.25	1.0	Contaminated with protein

The bands obtained ranged in size from 54-795 bp. Two clusters were observed in the RAPD analysis (Fig. 1). Sample 1-5 with 8 bands were genetically different from sample 6-10 with 11 bands (Fig. 2 and Table 4).

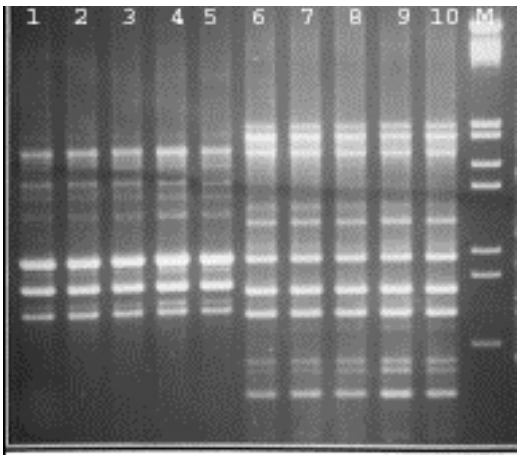

Fig. 1. A typical RAPD banding pattern amplified with primer OPB07

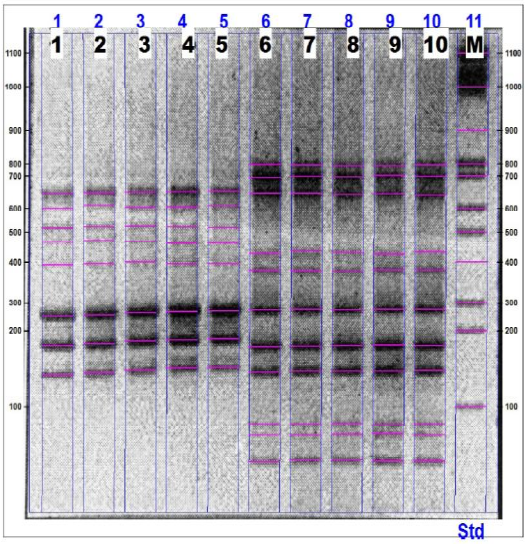

Fig. 2. Gel image of genomic DNA of *A. digitata*

4. DISCUSSION

In the present study 10 samples of *A. digitata* collected from different sites of Agra were analyzed for qualitative and quantitative DNA analysis and genetic diversity using RAPD analysis.

An average DNA yield of 15-40 µg/mg observed in the study is in concern with Stewart and Zhang [7], who reported an average DNA yield of 60 µg per 50 to100 mg of fresh leaf tissue of cotton, sufficient for 3000 to 6000 polymerase chain reactions.The DNA yield from the mini-prep method is comparable to that (100-500 µg/g) obtained from 0.5 g of fresh leaf tissue of *Vitis* species [8]. The proper yield of DNA can be attributed in part of the proper choice of leaf tissue for extraction. Young and fresh leaves are better for DNA extraction, as they are easier to crush to release DNA.

RAPD can be considered to be essential tool in cultivar identification (DNA typing), assessment of genetic variability and relationship management of genetic resources and bio-diversity, studies of phylogenetic relationship and in genome mapping [9]. In the present study 10 samples of *A. digitata* collected from different regions of Agra district showed two clusters of five each. The two groups had different genome size. Similar findings on genetic diversity in baobab were previously also reported by some workers. [10-12]. Assogbadjo et al. [10] observed that there was genetic structuring and low to high genetic diversity between baobab populations in different climatic regions of Benin (West Africa). High level of genetic structuring present in baobab at regional scale (Benin, Ghana) and within population was observed [11-12]. Several reports are available to demonstrate the use of RAPD markers for determination of genetic variation in plants. Jain et al. [13] studied molecular diversity in *Phyllanthus amarus* by RAPD profiling of 33 collections from different location using MAP primers. Batistini et al. [14] evaluated the genetic diversity of seven populations of *Anemopaegma arvense*, using random amplified polymorphic DNA markers. Ponnuswami et al. [15] used RAPD markers to identify the desirable traits in Palmyra palm. Maia et al. [16] used random amplified polymorphic DNA (RAPD) markers to detect polymorphism and to examine relationship among four table grape clones from North Western Parana, in Southern Brazil. Khurana et al. [17] evaluated the genetic variation in the *Jatropa curcas* using RAPD and ISSR markers. Suwanchaikasem et al. [18] demonstrated that *Thunbergia laurifolia* was successfully distinguished from its related species based on their molecular signatures. They showed that RAPD analysis is a technique that is able to examine the phylogenetic relationship of different plant species. Osman et al. [19] used RAPD marker for the identification of *Eucalyptus* species genome.

Table 4. RAPD profile of *A. digitata*

S. No.	Name of sample	No. of bands	Band size (bp)
1	Khandari	8	114-628
2	Bhagwan cinema	8	116-630
3	Sikandra	8	118-632
4	Paschimpuri	8	121-633
5	Fatehpursikri	8	122-634
6	Keetham	11	53-795
7	Tundla road	11	56-791
8	Gwalior road	11	56-787
9	Tajganj	11	54-787
10	Fatehabad	11	54-791

5. CONCLUSION

The present study is the first report that provides genetic information of *A. digitata* among individual plants. Two clusters were identified in 10 test plant samples. It is suggested that RAPD analysis could be successfully applied for detecting genetic variability in natural population of *A. digitata*. Moreover RAPD markers will have a major impact on the conservation & improvement of *A. digitata*. A good strategy to protect this critically endangered plant species is to protect more of their habitat.

ACKNOWLEDGEMENTS

Financial assistance provided by Govt. of U.P (Higher Education) in the form of Centre for Excellence to the Department of Botany is gratefully acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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