Research Paper

PCR Analysis of a Chinese Herbal in Some Indian States

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Abstract

Couroupita guianensis Aubl. commonly is the scientific name of cannon ball tree, which is belongs to Lecythidaceae family and is widely used in Chinese traditional medicine. This tree is in danger dau to vast destruction in their habitat almost in its native range because of human settlement and other developmental-related activities. Native populations of *C. guianensis* were collected from different parts of the Indian states. To study the genetic diversity of *C. guianensis* population we performed RAPD – PCR procedure with several primers.

Keywords: *Couroupita Guianensis*; Genetic Diversity; Primers; RAPD – PCR

1. Introduction

Couroupita guianensis Aubl. Which is commonly known as cannon ball tree, is a member of Lecythidaceae family and is widely used in Chinese medicine from the past [1]. this tree is originated from South Ameria, especially in Amazonian basin. It is grown as an ornamental plant in various countries. But usually grown in Lord Shiva temple of India is generally grown in Lord Shiva temple because of its special flowers which are looks like the hood of Naga (snake) so called as Nagalinga Pushpa [2].

Couroupita guianensis widely used for its medical properties and many researchers studied on its pharmacological properties originally its, fruit pulp is used to remedy canine and other animal diseases. Fruit pulp and oil extracts has Antibacterial activity and was demonstrated to be effective on gram-positive and gram-negative bacteria. Ethyl acetate in flowers aqueous extraction were effective in DPPH and superoxide radical scavenging activity [3]. Antianxiety and other neuropharmacological activities of flower extracts have been demonstrated in mice models previously. as mentioned before, traditional uses pharmacological application of *C. guianensis* is being threatened due to destruction its habitat and native territory because of human settlement and other developmental activities [4]. in spite of extensive planted area in Thailand, India and the United States it is almost grown as an ornamental tree and needs urgent conservation strategies to rescue the species. In addition, tomedicinal, ecological and economical importance of this tree, the genetic diversity should be studied to improve genetically and also germplam conservation in the wake of deforestation and adaptability of its exotic habitats. In different geographic and environmental consition, DNA based molecular markers have been successfully implemented because ofits numerous merits over morphological markers [5]. Between various molecular markers which are available to evaluate the genetic diversity of medical plants, Random Amplified Polymorphic DNA (RAPD) markers proved the simplest, rapid, handy and inexpensive method. It is also involved small and reliable samples, but still, genetic information of the population is lacking.

2. Materials and Methods

2.1 Sample collection

Plant materials (young leaves, clean and disease free) from different populations of Gujarat,

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Maharashtra, Karnataka, Goa, Kerala and Tamilnadu were collected in sterile polythene bags and transported to the laboratory. On immediately in the laboratory, leaf samples were sterilized with 70% alcohol and air dried. Then were processed for DNA isolation. The location details of Sampling and accession codes are given in table 1.

2.2 DNA isolation and quantification

DNA isolation from a leaf sample performed by DNeasy plant mini kit followed by manufacturers' instructions. Isolated DNA is separated on 0.8% agarose gel in 1X TAE running at 50V for 1hr. high molecular weight DNA separation is recorded using a BioRad gel documentation unit. DNA quality and quantity were assessed by reading in Biophotometer (Eppendorf). The genomic DNA extraction is stored at -20ºC for further use.

Table 1. Sample collection and accession code details

Sl No	Name of the place	Name of the state	Accession code
1	Vadodara	Gujarat	Vdr
2	Lonavala	Maharashtra	Lnl
3	Belgaum	Karnataka	Bgm
4	Mugalkhod	Karnataka	Mkd
5	Dharwad	Karnataka	Dwd
6	Davangere	Karnataka	Dvg
7	Mysore	Karnataka	Mys
8	Vasco	Goa	Vas
9	Thrissur	Kerala	Thr
10	Chennai	Tamilnadu	Chn

2.3 Primers

The OPA set of random decamer primers were purchased from Eurofins Genomics in India. The primers were then reconstituted in molecular biology grade water to 100pm/µl as a stock solution. For working solution 5 pm/ µl is diluted from a stock solution in molecular biology grade water and stored at -20ºC.

2.4 PCR amplification

The PCR amplification was performed using gradient thermal cycler (Master cycler, Eppendorf). The reaction mixture was contained og 50ng genomic DNA, 5 pm primer, 200µm dNTP mix (Bangalore Genie), 1.5 M MgCl2, 1X taf buffer and 1U Taq DNA polymerase (New England Biolabs). The final volume of the reaction mixture made up to 20µl using molecular biology grade water. The reaction conditions were, initiated for 5 min at 95ºC followed by 35 cycles of cycle denaturation for 1min at 95ºC, primer annealing for 1 min at 38ºC and primer extension for 2 min at 68ºC and final extension for 30 min at 68ºC. The primers which are giving scorable polymorphic bands were selected for final amplification. The amplified bands were documented in gel documentation unit.

2.5 Scoring and Statistical Analysis

Each amplified band was considered as a unit character and were scored as absent (0) or present (1) based on the binary scoring data. Matrix similarity was constructed in according to the Jaccard Similarity Coefficient.
Dendrogram diagram was constructed by UPGMA method using NTSys PC Version 2.0.

3. Results and Discussion

A dendrogram analysis was depicted for the population using Jaccard's similarity index values using the NTSYS- pc ver 2.02. In Sequential Agglomerative Hierarchical Non overlapping (SAHN) UPGMA were used to generate dendrogram. Based on the Dendrogram resuts the 11 populations formed four clusters at similarity index of 0.69 for Operon H9 primer. It was also, observed that for H9 primer four clusters were formed. in Cluster l comprises of CBE 1 TP Pm 1 and KKM 1 CT Pm 2 were grouped closely (Figure 1). Along with these the remaining clusters such as II, III and IV had close relation with KGR 1 PA Pm 2; TVM 1 TP Pm 1, KKM 1 TP Pm 1 , KGR 1 TP Pm 3, KKM 1 JA Pm 3, KGR 1 HS Pm 1 ; MDU 1 PA Pm 2 , MDU 1 HB Pm 1 and TRY 1 TP Pm 1, respectively. In addition to the above-mentioned results for the primer H16 , two clusters were formed. The cluster I comprise CBE 1 TP Pm 1, TVM 1 TP Pm 1, KKM 1 JA Pm 3, TRY 1 TP Pm 1, KKM 1 TP Pm 1, KGR 1 PA Pm 2, KGR 1 TP Pm 3, MDU 1 PA Pm 2, MDU 1 HS Pm 1 and Cluster ll comprises of KKM 1 CT Pm 2, KGR 1 HS Pm 1.

4. Conclusion

It could be concluded that the RAPD is the easiest and cheapest methods for laboratory just being used in of molecular markers. this marker has found wide applications in gene mapping, population genetics andmolecular evolutionary genetics in the plant, and animal breeding science. This is mainly due to the higher speed, low cost and efficiency of the RAPD technique to generate large numbers of markers in a short period of time rather than previous methods for the analysis and establishing the phylogenetic relationships for *Couroupita guianensis*.
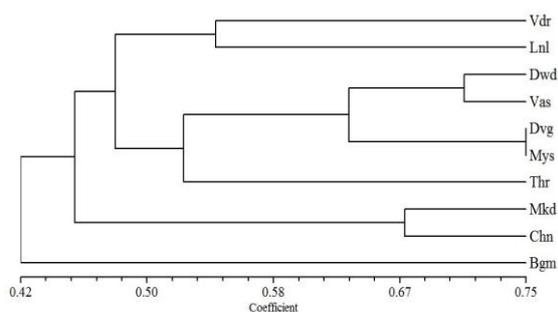
Figure 1. Phylogenetic relationship of *Couroupita guianensis*

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