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Short Communication

Genetic Diversity Analysis of *Couroupita guianensis* Aubl. based on RAPD Marker

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***Couroupita guianensis* Aubl. Commonly known as cannon ball tree, belongs to family Lecythidaceae and widely used in Chinese medicine. *C. guianensis* is being threatened due to habitat destruction in its native range for human settlement and other development related activities. Native populations of *C. guianensis* were collected from different locations of the India states and characterized through. To study the genetic diversity of *C. guianensis* populations we performed RAPD – PCR with several primers. The results were revealed in this study.**

Keywords : *Couroupita guianensis,* Genetic diversity, Primers, RAPD – PCR.

Couroupita guianensis Aubl. commonly known as cannon ball tree, belongs to family Lecythidaceae and widely used in Chinese medicine (Han, 1994). *C. guianensis* is native of South Ameria, specially to Amazonian basin. It is grown as ornamental plant in various countries and in India is generally grown in Lord Shiva temple because of its special featured flowers which look like hood of Naga (snake) protecting the shivalinga, hence is called as Nagalinga Pushpa (Kumar, 2011).

Couroupita guianensis widely used for its medicinal properties and is extensively studied for its pharmacological properties by various researchers from different countries. In its native range, fruit pulp is used to treat the canine and other animal diseases. Leaves used for the cure of odontalgia and various skin infections including protozon diseases in humans. Flowers are used for treatment of mood disorders, tumors and inflammation. Flowers are used for used for treatment of mood disorders, tumors and inflammation. Antibacterial activity of fruit pulp extracts Oil extracts was demonstrated against gram positive and gram negative bacteria. Ethyl acetate fractions of aqueous extracts of flowers proved effective in DPPH and superoxide radical scavenging (Azimi, 2012). Antianxity and other neuro-pharmacological activities of flower extracts have been demonstrated in mice models. With immense traditional uses and demonstrated pharmacological activities, *C. guianensis* is being threatened due to habitat destruction in its native range for human settlement and other development related activities (Bafna, 2011). Although its extensively planted in Thailand, India and United States as ornamental tree, there is an urgent need of conservation strategies for the species. Considering the medicinal, ecological and economical importance of this tree species, the genetic diversity needs to be studied for genetic improvement and germplasm conservation in the wake of

deforestation and adaptatability in its exotic habitats. The DNA based molecular markers have been successfully implemented due to its numerous merits over morphological markers against geographic and environmental variations (Williams, 1990). Among variety of molecular markers available to evaluate the genetic diversity of medicinal plants, Random Amplified Polymorphic DNA (RAPD) markers proved most simple, rapid, handy, inexpensive where small sample is involved and reliable when prior genetic information of population under study is lacking.

Materials and Methods

Sample collection

Plant materials (young leaves, clean and disese free) from different populations of Gujarat, Maharashtra, Karnataka, Goa, Kerala and Tamilnadu were collected in sterile polythene bags and transported to the laboratory. On arrival to the laboratory, leaf samples were sterilized with 70% alcohol and airdried. Then the samples were processed for DNA isolation. Sample collection location details and accession codes are given in table 1.

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

DNA isolation and quantification

DNA is isolated from leaf sample using DNeasy plant mini kit following manufacturers' instructions. Isolated DNA is separated on 0.8% agarose gel in 1X TAE running at 50V for 1hr. high molecular weight DNA separated is documented using BioRad gel documentation unit. DNA quality and quantity was assessed by reading in Biophotometer (Eppendorf). The genomic DNA isolated is stored at -20^{o}C for further use.

Primers

The OPA set of random decamer primers were purchased from Eurofins Genomics India. The primers were reconstituted in molecular biology grade water to 100pm/µl as stock solution. For working solution 5pm/ µl is diluted from stock solution in molecular biology grade water and stored at -20^{o}C for further use.

PCR amplification

The PCR amplification was done using gradient thermal cycler (Master cycler, Eppendorf). The reaction mixture was containing og 50ng genomic DNA, 5pm 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 is made up to 20µl using molecular biology grade water. The reaction conditions were, initial for 5 min at 95^{o}C followed by 35 cycles of cycle denaturation for 1min at 95^{o}C, primer annealing for 1 min at 38^{o}C and primer extension for 2 min at 68^{o}C and final extension for 30 min at 68^{o}C. The primers which are giving scorable polymorphic bands were selected for final amplification. The bands amplified were documentaed in gel documentation unit.

Scoring and Statistical Analysis

The each band amplified was considered as unit character and were scored as absent (0) or present(1). Based on the binary scoring data similarity matrix was constructed in accordance to Jaccard Similarty Coefficient. Dendrogram based on similar was

constructed by UPGMA method using NTSys PC Version 2.0.

Results and Discussion

A dendrogram was constructed for 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, the 11 population formed four clusters at similarity index of 0.69 for Operon H9 primer. It was observed that for H9 primer four clusters were formed in which 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 association 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, TRY 1 TP Pm 1 resppectively. In addition to the above observation for the primer H16 primer two clusters were formed. The cluster I comprises of 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.

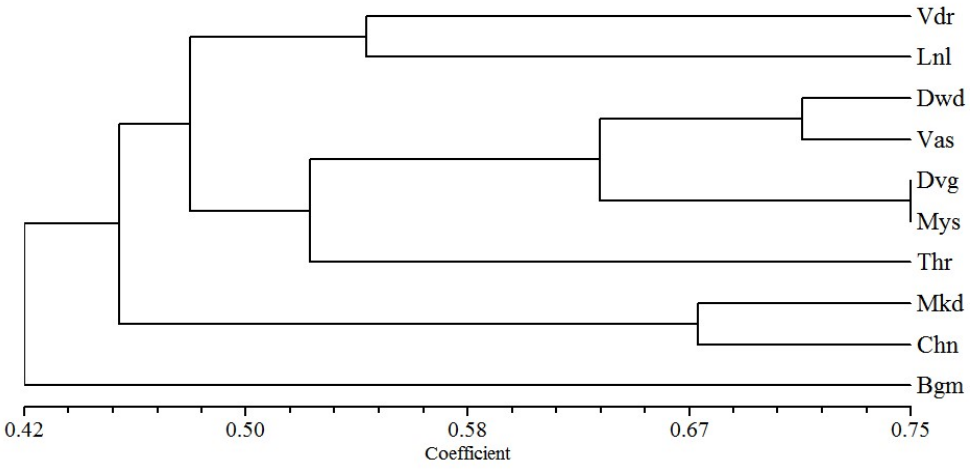

Figure 1: Phylogenetic relationship of *Couroupita guianensis*

Conclusion

RAPD is probably the easiest and cheapest methods for laboratory just beginning to use molecular markers. RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding. This is mainly due to the speed, cost and efficiency of the RAPD technique to generate large numbers of markers in a short period compared with previous methods for the analysis and establishing the phlogenetic relationships for *Couroupita guianensis*.

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