Journal of Plant Genomics
ISSN: 0976-8823 & E-ISSN: 0976-8831, Volume 3, Issue 1, 2013, pp.-39-41.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000281

RAPD PCR FINGERPRINTING OF *Artocarpus lakoocha* Roxb AND *Pterospermum acerifolium* Willd TO UNDERSTAND PHYLOGENETIC LINKAGE

TUSAMDA N.I., GORANIYA S.P., SHIROLKAR A.R., MURTHY S.N. AND PAWAR S.D.*

National Research Ayurvedic Institute of Basic Ayurvedic Sciences, Department of Biochemistry, Pune- 411038, MS, India.
*Corresponding Author: Email- sd_pawar@yahoo.com

Received: July 26, 2013; Accepted: August 12, 2013

Abstract- *Artocarpus lakoocha* Roxb and *Pterospermum acerifolium* Willd are the important anti- glycation, antioxidant and antiulcer agents in Ayurveda which is a comprehensive healthcare system known for its immense potential. There is a need to preserve and explore their quantum of genetic variation by analysing the polymorphism between these plants. Literature suggests that there have not been detail classification of these plants, hence we aim to analyse the interrelationship and genetic polymorphism by using RAPD markers. In this study a set of 25 plants RAPD universal primers (RPI 1- RPI-25) were used. Total Genomic DNA extracted from the leaves was used for PCR reactions. The study suggests that the genetic pattern of the two species showed the distinct variation in polymorphism at amplicons level. Each of the two plants reproduced 6 highly polymorphic bands. This approach will help in identifying genetic variation among different species and developing ways to conserve the medicinal aspects of *Artocarpus lakoocha* Roxb and *Pterospermum acerifolium* Willd. Moreover, Random amplified polymorphic DNA (RAPD) markers were used to assess genetic diversity in *Artocarpus lakoocha* Roxb and *Pterospermum acerifolium* Willd.

Keywords- RAPD, PCR, Phylogenetic, DNA, UPGMA, *Artocarpus lakoocha* Roxb., *Pterospermum acerifolium* Willd

Citation: Tusamda N.I., et al (2013) RAPD PCR Fingerprinting of *Artocarpus lakoocha* Roxb and *Pterospermum acerifolium* Willd to Understand Phylogenetic Linkage. Journal of Plant Genomics, ISSN: 0976-8823 & E-ISSN: 0976-8831, Volume 3, Issue 1, pp.-39-41.

Introduction

Ayurveda is also known as the science of longetivity and an ancient available system that consists of treatments for various diseases [1]. Ayurveda comprises plants that are main source for medicines, with proper integration of modern scientific techniques and traditional knowledge that helps to explore various facets of cure in the field of medicine [2]. *Artocarpus lakoocha* Roxb is distributed throughout the Indian Subcontinent and South-East Asian countries. The tree is well known for its wood; its fruits are edible and are believed to have medicinal value. The *Artocarpus lakoocha* Roxb species are found to be rich in phenolic compounds including flavonoids, stilbenoids and Arylbenzofurons [3]. Antiglycation and antioxidant activities of oxyresveratrol extracted from the heartwood of *Artocarpus lakoocha* Roxb have also been reported [4]. Determination of β-sitosterol and Lupeol both simultaneously in *Artocarpus lakoocha* Roxb leaf powder has shown earlier [5]. *Pterospermum acerifolium* Willd (karnikara tree) is an angiosperm, indigenous to Southeast Asia that is traditionally included in the Sterculiaceae family. But it is also grouped in the expanded Malvaceae family. The flowers of the tree provide a number of medicinal uses such as tonic, a cure for inflammation (Topical application), ulcers, blood related problems, and tumors. Phytochemical investigation of ethanol extracts of the *Pterospermum acerifolium* Willd flowers led to the isolation and identification of two newly reported flavones, 4'-(2-methoxy-4-(1,2,3-trihydroxypropyl) phenoxy luteolin and 5,7,3'-trihydroxy-6-O-β-D-glucopyranosyl flavone, and one new lactone, 3,5-dihydroxyfuran-2 (5H)-one along with 14 known compounds [6]. RAPD stands for Random Amplification of Polymorphic DNA. It is a variant of PCR reaction which is used to amplify DNA sequences randomly. The standard RAPD technology utilises short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR [7]. The Present study is to develop fingerprints and assess the inter-relation between the genes present in *Artocarpus lakoocha* Roxb and *Pterospermum acerifolium* Willd.

Materials and Methods

The leaves of *Artocarpus lakoocha* Roxb and *Pterospermum acerifolium* Willd were collected from the herbarium at National Research Ayurvedic Institute of Basic Ayurvedic Sciences, Pune in January 2013. The plant materials were verified by Mrs. A. G. Mhase, the botanist and the specimens were preserved in the herbarium for reference.

DNA Extraction

The leaves samples were crushed in liquid nitrogen to a fine powder and kept at -20°C for further use. The total genomic extraction from the leaves of both the plants was done using Black Bio kit using approximately 100-120 mg of powdered sample. The DNA of both plants was then quantified by checking the absorbance at A_{260} nm with a UV Vis Spectrophotometer. The DNA was then subjected

to agarose gel electrophoresis and bands were observed. DNA was then kept at 4°C until further use.

RAPD- PCR

For RAPD PCR the reaction mixture was standardised to total volume of 20 µl containing MilliQ water (13µl), 10X PCR Buffer (2µl), $MgCl_2$ (1.5µl), dNTP (1µl), Primers (1µl), Template DNA (1µl), Taq polymerase (0.5µl). 25 different primers were used for each of the plants [Table-1]. The amplification conditions were 94°C for 3 min, 94°C for 45 sec, 44°C for 30 sec, 72°C for 1 min, 72°C for 5 min, and 4°C for hold. After the amplification step, the PCR product was analyzed by loading 20µl sample with 2 µl loading dye. 2µl Marker ladder (100-1000bp) was also loaded into 2% Agarose gel and was run at 75 Volts for approximately 60 mins. In reaction mixture of negative control (NC) DNA was omitted and in positive control (PC) primer was omitted to check if contamination was excluded.

Statistical Analysis

Gelquest® and Clustervis® software were used to construct dendrograms using the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) by comparing the bands that were observed common in both plants to specific primers.

Results

Genomic Extraction

The DNA obtained was 46.7ng/µl (*Pterospermum acrefolium* Willd) and 20.5ng/µl (*Artocarpus lakoocha* Roxb).

RAPD PCR Profiling

The RAPD PCR resulted in amplicons from the specific sequences of the primers giving rise to bands in both plants. [Fig-1] shows the RAPD Profile of *Artocarpus lakoocha* Roxb. The lane 3, 5, 7, 8, 10, 12, 13, 14, 15, 17, 18, 19, 20, 21, 23 show distinct band patterns for respective primers indicated in [Table-1] above. [Fig-2] shows RAPD Profile of *Pterospermum acerifolium* Willd. The lane 1, 5, 19, 21, 22, 23, 25 resulted in distinct band patterns for respective primers as indicated in [Table-1]. Only the primers which displayed prominent, reproducible and distinguishable bands were considered for analysis, as we can observe in [Fig-2]. Marker ladder was used as reference to outline the ancestral linkage between the two plants. The Phylogenetic tree analysis UPGMA method and similarity index in this study showed genetic variations in the bands reproduced by both the plants as shown in [Fig-3].

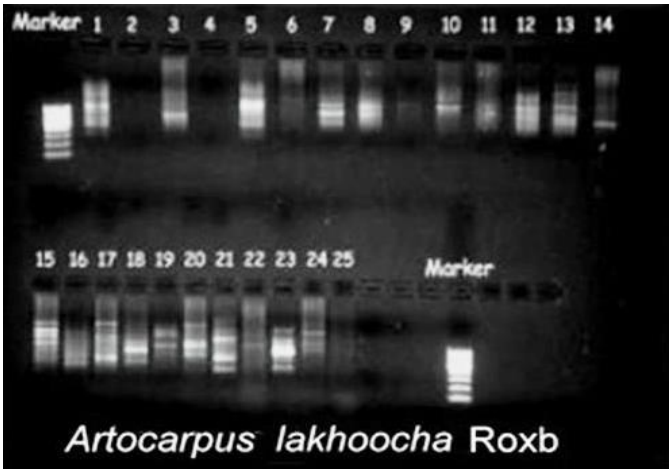

Fig. 1- RAPD-PCR of *Artocarpus lakoocha* Roxb and *Pterospermum acerifolium* Willd

Table 1- Plant RAPD primer sets

Sl. No.	Name of Primer	Accession Numbers
1	RPI 1	AM765819
2	RPI 2	AM750044
3	RPI 3	AM773310
4	RPI 4	AM773769
5	RPI 5	AM773770
6	RPI 6	AM773771
7	RPI 7	AM773312
8	RPI 8	AM773773
9	RPI 9	AM773315
10	RPI 10	AM750045
11	RPI 11	AM911709
12	RPI 12	AM773316
13	RPI 13	AM750046
14	RPI 14	AM773774
15	RPI 15	AM773775
16	RPI 16	AM773776
17	RPI 17	AM911710
18	RPI 18	AM765830
19	RPI 19	AM773777
20	RPI 20	AM773317
21	RPI 21	AM765820
22	RPI 22	AM911711
23	RPI 23	AM911712
24	RPI 24	AM765821
25	RPI 25	AM750054

**RPI 1 - RPI 25 indicates the Universal primers.*
Source : 3B Blackbio Biotech Biotools India [8].

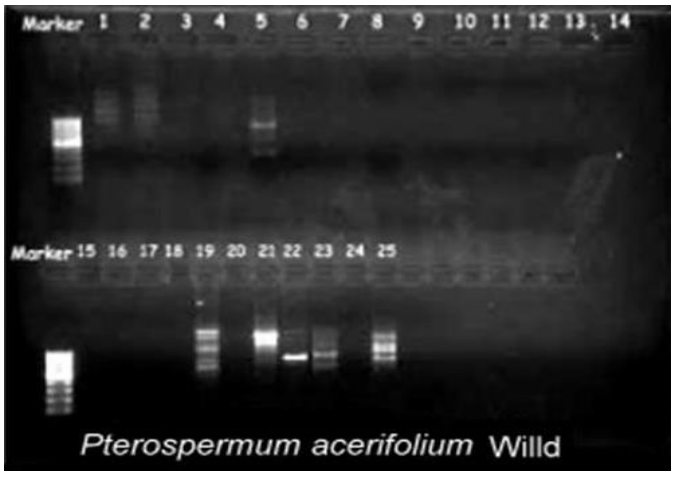

Fig. 2- Common bands found in *Artocarpus lakoocha* Roxb and *Pterospermum acerifolium* Willd by RAPD PCR analysis with the marker (100-1000 bp).

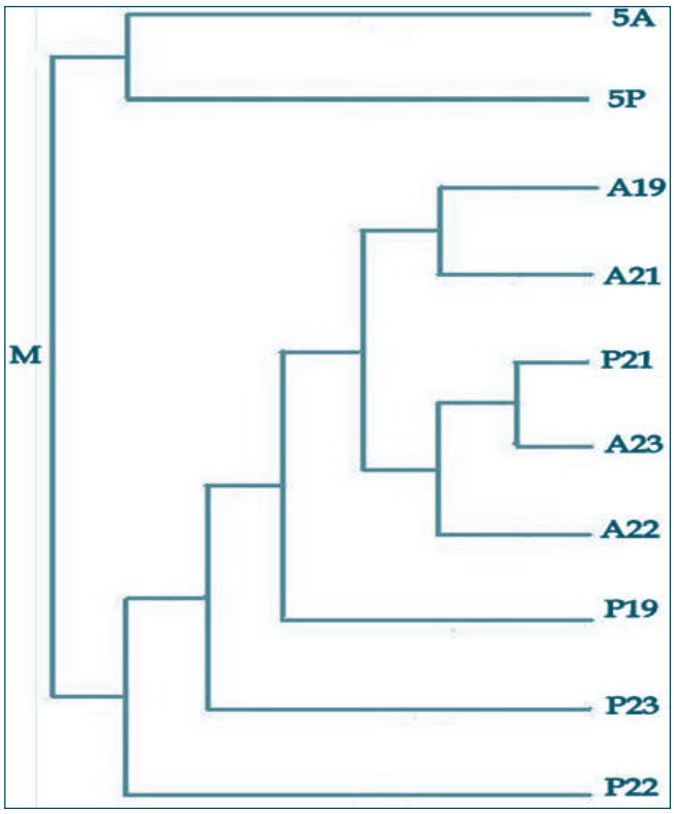

Fig. 3- Phylogenetic tree representing the linkage between *Artocarpus lakoocha* Roxb and *Pterospermum acerifolium* Willd, using UPGMA Software.

**5A, A19, A21, A23, A22 denotes the primer number with 'A' as Artocarpus lakoocha Roxb respectively.*

**5P, P21, P19, P23, P22 denotes the primer number with 'P' as Pterospermum acerifolium Willd respectively.*

**M denotes the marker*

Discussion

The extracted genomic DNA of both the plants was quite efficiently extracted and so could be used as template for the RAPD profiling of both the plants. In this study, the large similarity values revealed by RAPD markers while making the phylogenetic tree, provide greater confidence for assessment of genetic relationship among the species.

RAPD profiling of these two plants have not yet reported. The UPGMA tree showed that the two plants were related and may have the common ancestral background. Even though the two plants have different uses in Ayurvedic sciences they still can be tracked back and may help in the evolutionary relationships of these two plants which can help to evaluate the further studies like proteomics, quantification of phytochemical analysis, etc to a greater extent.

Conclusion

Based on the study the large range of similarity and dissimilarity values for the plants using RAPD provides the greater confidence for assessment of genetic diversity and relationships. Thus, this approach will be helpful in ranking the species according to their genetic inter-relatedness. This can provide a better platform for identification and authentication of *Artocarpus lakoocha* Roxb. and *Pterospermum acerifolium* Willd.

List of Abbreviations

RAPD: Random Amplification of Polymorphic DNA

PCR: Polymerase Chain Reaction

dNTP: Deoxyribonucleotide triphosphate

Taq: *Thermus aquaticus*

UPGMA: Unweighted Pair Group Method with Arithmetical Averages

Acknowledgement

The authors would like to express special thanks to The Director General, Central Council for Research in Ayurvedic Sciences, New Delhi for his support and encouragement to carry out the above mentioned study.

References

[1] Gupta M., Shaw B.P. (2009) *Indian Journal of Traditional Knowledge*, 8(3), 372-378.

[2] Gahlaut A., Gothwal A., Hooda V., Dabur R. (2013) *Int. J. Pharm. Pharm. Sci.*, 5(1), 239-241.

[3] Hakim E.H., Achmad S.A., Juliawaty L.D. (2006) *J. Nat. Med.*, 60, 161-184.

[4] Povichit N., Phrutivorapongkul A., Suttajit M., Leelapornpisid P. (2010) *Int. J. Sci. Technol*., 4(3), 454-461.

[5] Raote A., Jangid A., Tale R., Vaidya V. (2011) *International Journal of Pharma and Bio Sciences*, 2(1), 860-66.

[6] Dixit P., Khan M.P., Swarnkar G., Chattopadhyay N., Maurya R. (2011) *Bioorg. Med. Chem. Lett*., 21(15), 4617-21.

[7] Bardakci F. (2001) *Turk. J. Biol.*, 25, 185-196.

[8] 3B Blackbio Biotech Biotools, *Plant RAPD Primer Set*, 25, Catalogue No. 3B760P.

[9] Padmalatha K., Prasad M.N.V. (2006) *African Journal of Biotechnology*, 5(3), 230-234.

[10] Vivekanand K., Rana D., Bodhisattwa C., Subhangkar N., (2012) *Int. J. Drug Dev. and Res*., 4(3), 220-227.

[11] Prashith Kekuda T.R., Raghavendra H.L., Mallikarjun N., Venugopal T.M., Anil Kumar H.S. (2012) *Int. J. Drug Dev. Res*., 4(1), 330-336.

[12] Bhalke R.D., Pal S.C. (2012) *Asian Journal of Pharmaceutical and Clinical Research*, 5(2), 23-26.

[13] Malik S.K., Khan Z.U., Ajaib M. (2012) *Pak. J. Bot*., 44, 105-109.

[14] Sairkar P., Vijay N., Silawat N., Garg R.K., Chouhan S., Batav N., Sharma R., Mehrotra N.N. (2012) *Science Secure Journal of Biotechnology*, 1(1), 1-8.