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Research Article

RAPD PATTERNS OF SOME IMPORTANT MEDICINAL PLANTS AND THEIR SUBSTITUTES USED IN AYURVEDA TO IDENTIFY THE GENETIC VARIATIONS

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ABSTRACT

Ayurveda and other traditional medicinal systems use whole plants or their parts for combating a great number of disorders. Hence maintaining the purity and quality of herbal drugs are necessary to identify the substitutes and adulterants. Using traditional identification methods to identify the correct plant part can be difficult especially in case of barks and roots. Bark of *Polyalthia longfolia* Thw. is mostly used as substitute of *Saraca asoca* (Roxb.) De Wilde, the best drug for the treatment of all type gynaecological disorders. *Wrightia tinctoria* R. Br. and *Wrightia rothii* (G. Don) Ngen. are also used as adulterants/ substitutes of important medicinal plant *Holarrhena pubescence* (Buch. Ham) Wall. ex. G. Don. RAPD–PCR analysis involving 8 decamer random primers was used to assess the quantum of genetic variation at genomic level. Two primers showed appreciable intra-species variation or molecular polymorphism at amplicon levels. Variation in *S. asoca* and *P. longfolia* was observed to be 30% by each primer. Despite close identity, a great deal of polymorphism was observed among the accessions. *W. tinctoria* and *W. rothii* also showed significant genetic variations with *H. pubescence*. UPGMA analysis showed ~30% variation in the collections, which is deemed to be useful in identification of plant material and formulating conservation strategies for these precious medicinal plant species

INTRODUCTION

In Ayurveda plants are the main source for medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The chromatographic techniques and marker compounds which are used to standardize Ayurvedic formulations has limitations because of variable sources, chemical complexity and the amounts of secondary metabolites which is influenced by the environmental factors. DNA fingerprinting distinguishes different varieties according to their DNA variations at a set of genetic loci. Powerful and convenient molecular marker system, randomly amplified polymorphism of DNA (RAPD) analysis is widely used for the Genetic mapping, taxonomic and polygenic studies of many plants. Their environmental stability and nearly unlimited availability have made RAPD markers an ideal tool for plant. RAPD maps have been or are being constructed for the genomes of various major crop plants.[1, 2] RAPD markers also provide useful tools for both evolutionary studies and the characterization of germ plasm stocks.[3, 4] Tightly linked RAPD markers may serve in turn as starting points for the characterization of genes without prior knowledge of their products or may render possible the physical characterization of large DNA fragments by pulsed field gel electrophoresis.[5] An indispensable prerequisite for the application of these techniques is the availability of an RAPD map of appropriate resolution.

The plant *Saraca asoca* (Roxb.) De Wilde, mentioned in Ayurveda as Ashoka is considered one of the best drug for the treatment of all type of Gynecological disorders. It's antimicrobial and effects on PGH2 synthetase enzyme complex has been reported earlier.[6, 7] Ashokarista is an important formulation for the same. Stem bark of the plant is used as medicine.[8] *Polyalthia longfolia* Thw. commonly known as nakli Asoka or Kasthadaru is mostly used as substitute or adulterate in place of Asoka. The *Wrightia tinctoria* R. Br. and *Wrightia rothii* (G. Don) Ngen. are also used as adultrant/substituents of important medicinal plant *Holarrhena pubescence* (Buch. Ham) Wall. ex. G. Don. The aim of the present study was to develop the genomic markers/ finger prints for authentication and identification of these plants.

MATERIALS AND METHODS

Collection of Plant Materials

The stem bark of *Saraca asoca* (Roxb) De wilde, *Polyalthia longfolia* Thw, *Wrightia tinctoria* R. Br. and *Wrightia rothii* (G.Don) Ngen were collected form Regional Research Institute (AY.), Pune in the month of April and material was identified by Dr. T. K. Mandal and voucher samples of plants were preserved in the Herbarium.

DNA Extraction

Stem bark tissues of plants were used for extraction of total genomic DNA. Five to thirty mg of bark tissue were frozen in liquid nitrogen and ground in a micro-centrifuge tube using a fitted pestle. The extraction of genomic DNA was perfume using the GeNei™ Ultra pure plant Genomic DNA prep kit (Bangalore Genei, India), after that isolated genomic DNA were estimated by measuring absorbance at A^{260} nm with a VU Vis Spectrophotometer SL 159 (ELICO, India). Typically, 0.2- 0.8 mg of DNA was obtained per mg of fresh stem barks. DNA samples were diluted to working solutions of 25 ng/mL, confirmed by agarose gel electrophoresis and stored at 4°C until use.

RAPD- PCR

For RAPD analyses reactions were carried out in a final volume of 25 mL containing 20 mM tris- HCL pH 8.4, 50 mM KCl, 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 mM primer, primer RPLA6A (Accession Number AM 773771) and PRL18A (Accession Number AM 765830), 50 ng of genomic DNA and 1 unit of Taq DNA polymerase (invitrogen). Amplifications were performed in a Peltier- Based Thermal Cycler Model P25+ (Cyberlab, USA). Programmed for RPLA6A having initial denaturation cycle (7 min at 94°C) followed by 10 cycles of denaturation (1 min at 94°C), annealing (2min at 35°C) and extension (1min at 72°C) with a final extension of 5 min at 72°C. RPL18A having initial denaturation cycle (7 min at 94°C) followed by 35 cycle of denaturation (1 min at 94°C), annealing (1min at 38°C) and extension (1 min 30 sec at 72°C) with a final extension of 7 min at 72°C. Each reaction was performed at least thrice to assess the consistency of the band profiles. Reaction products (10 µL) were resolved by electrophoresis in 1.2% agarose gels stained with ethidium bromide in TAE buffer at 90 V for 140 min. All the reactions included negative controls in which DNA or primer was omitted in the amplification reaction mixture.

Statistical Analysis

Similarity index (SI) was calculated based on *Rf* values for individual primer. Dendrograms were constructed using the Unweighted Pair Group Method with Arithmetical Averages (UPGMA).

RESULTS AND DISCUSSION

The PCR protocol as adopted in the study, resulted in reproducible pattern of amplicons using specific combination of accession and primer. Only the primers which displayed reproducible, scorable and clear bands were considered for analysis. The image profiles of banding patterns were recorded and molecular weight of each band was determined by Molecular Analyst software. The banding patterns

were scored based on the presence or absence of clear, visible and reproducible bands. The results were analyzed based on the principle that a band is considered to be 'polymorphic' if it is present in some individuals and absent in others, and 'monomorphic' if present in all the individuals or accessions. The most intense monomorphic band from each accession with each primer was used as reference to calibrate different lanes for the amount of DNA present. When there was no monomorphic band, the band with maximum frequency in each accession was used for calibration. In each lane, bands were scored present if their intensity was at least 10% of the monomorphic reference band within the same lane.

RAPD profiles from the leaves of *S. asoca* have been reported by Padmalatha and Prasad (2005).[9] However, RAPD profiles from the bark of these plants have never been reported which is main part of use in Ayurvedic medicines. In this study, two unique bands were observed in the bark DNA of *S asoca* using PCR primer RPL6A. One specific band was found in the RAPD profile developed using RPL18A primer (Fig 1). Therefore, three bands are specific bands in the *S. asoca* which may be utilized to distinguish this plant from others. On the other hand 5 unique bands in *W. tinctoria*, two bands in *H. pubscence* and two bands for *W. rothii* were observed using RPL6A primer (Fig 1).

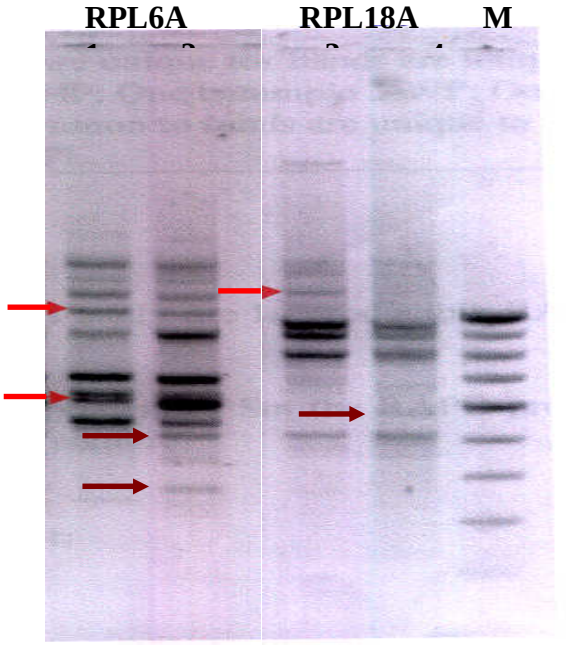

Fig. 1: RAPD profiles of two plant bark samples, using RPL6A and RPL18A primers. Lane 1 and 2: RAPD profiles of *S. asoca* and *P. longifolia* using RPL6A primer. Lane 3 and 4: RAPD profiles of *S. asoca* and *P. longifolia* using RPL18A primer. Lane M: Molecular weight marker of 100 base pair ladder.

Using RPL18A primer no unique band was observed in RAPD profile of *W. tinctoria* but at the same time 6 unique bands in *H. pubscence* and 1 band for *W. rothii* were observed (Fig 2).

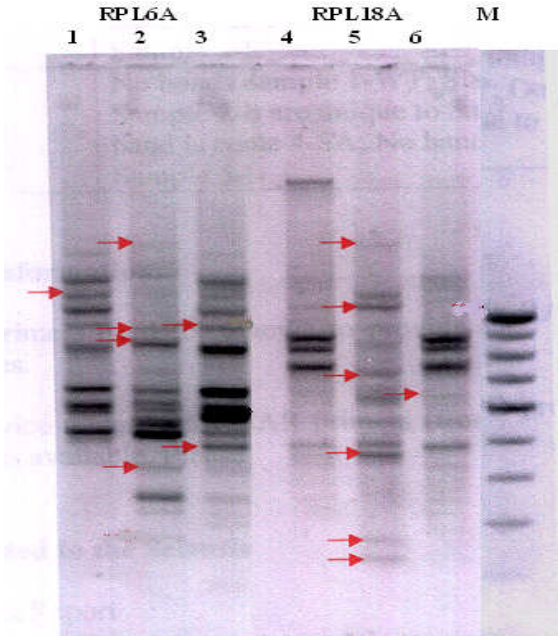

Fig. 2: RAPD profiles of three plant bark samples, using RPL6A and RPL18A primers. Lane 1, 2 and 3: RAPD profiles of *W. tinctoria*, *H. pubscence* and *W. rothii*. using RPL6A primer. Lane 4, 5 and 6: RAPD profiles of *W. tinctoria*, *H. pubscence* and *W. rothii*. using RPL18A primer. Lane M: Molecular weight marker of 100 base pair ladder.

Phylogenetic tree by both the primer used in this study showed no difference in same species. However, genetic difference is noticed in between *S. asoca* and *P. logifolia* (Fig 3). *H. pubscence* and *W. rothii* are observed to closely related plants than *W. tinctoria* as per UPGMA analysis and similarity index (Fig.4). In this study, the large of similarity values revealed by RAPD markers provides greater confidence for assessment of genetic relationship among the species and adultrants/substitutes. Principle component analysis was also done to visualize genetic relationship among the species. The results were similar to UPGMA analysis.

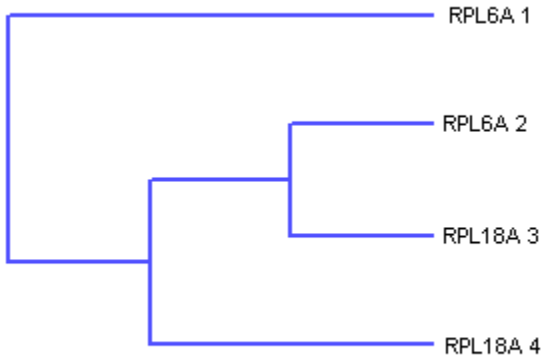

Fig. 3: The Phylogenetic tree representing the relationships between the two primers RPL6A and RPL18A constructed by UPGMA tree software.

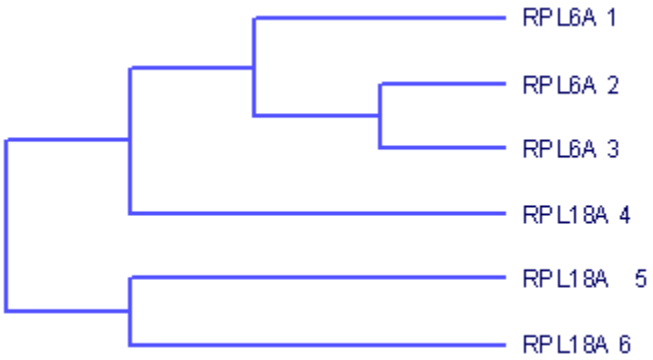

Fig. 4: The Phylogenetic tree representing the relationships between the two primers RPL6A and RPL18A of *W. tinctoria* (1, 4), *H. pubscence* (2, 5) and *W. rothii* (3, 6) constructed by UPGMA tree software.

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. The practical approach developed in the study will be useful in DNA fingerprinting and identification of plant species from the adulterants and substitutes. This will also makes identification and characterization of genotype very easy.

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