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**RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MOLECULAR MARKER
BASED GENETIC ANALYSIS OF *ALOCASIA INDICA* AND *TEPHROSIA PERPUREA***

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ABSTRACT

The present investigation is mainly focused on developing the genetic relationship between *Alocasia indica* and *Tephrosia purpurea* plant samples (05 each) collected from various zone of Chhattisgarh using fifteen 10 decamer random amplified polymorphic DNA (RAPD) molecular marker. The result revealed that *A. indica* had a similarity coefficient ranged from 0.23 to 0.43 whereas it ranged from 0.51 to 0.69 in case of *T. purpurea* plant. The dendrogram showed that all selected plants of *A. indica* grouped in one; except samples collected from Lundra Surguja which separated alone at a coefficient value of 0.23, it was seen that, sample collected from Achanakmar forest Bilaspur and Pakhanjur Kanker were firmly related together. Similarly 05 plants of *T. purpurea* grouped in a single cluster; except sample collected from Lundra Surguja which separated alone at a coefficient value of 0.51, sample collected from the University campus, Pt. RSU, Raipur and Pakhanjur Kanker was firmly related together. A set of differentiating markers viz. OPB-06, OPC-01, OPD-01 and OPD-03 may be used as diagnostic markers to identify the particular plant. The finding, suggested that genetic characterization be useful for distinguishing variation at molecular level and rapid identification of any plant species.

Keywords: *Alocasia indica*, *Tephrosia Purpurea*, Molecular marker, RAPD, Genetic relationship

INTRODUCTION

Alocasia indica (Family: Araceae) is extensively cultivated in tropical and sub-tropical regions, widely grown in a few areas of India including West Bengal, Maharashtra, Assam, and Southern India. It is a perennial herb and its tuber is generally consumed as a common vegetable. It has an extended history of getting used as medicine in treatment of spleen, abdomen, related disorders, inflammatory diseases including rheumatism and bruises [1]. Alcoholic extract of this plant leaves has hepatoprotective, antioxidant, antimicrobial [2], antiprotozoal and antidiarrheal activities [3]. *A. indica* exhibit alkaloids, flavonoids, phenolics and tannins, and which play pivotal role in deactivation of free radicals [4, 5]. Similarly *Tehrosia purpurea* (Family: Fabaceae) commonly popular as wild indigo is globally distributed in arid, tropical and sub-tropical areas. It is used as a crucial component of various pharmaceutical preparations viz. Yakrifit and Tephroli; traditionally this plant is used for treatment of diarrhea, dyspepsia, jaundice, rheumatism, urinary disorders and asthma, [6]. Phytochemical investigation revealed the presence of chalcones, flavonoids, flavanols, flavanones, glycosides, isoflavones, and sterols in *T. purpurea* [6].

Assessment of medicinal properties of such potent medicinal plant based on biochemical markers has limitations; because of biochemical properties and their effect are greatly influenced by development stage of the plant and environmental factors and the. In contrast introduction of polymerase chain reaction (PCR) has invented many DNA based markers viz. random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), inter-simple sequence repeats (ISSRs), sequence-characterized amplified region (SCAR), and amplified fragment length polymorphism (AFLP).

Among these, RAPD marker which detects genetic variation at DNA level by using a single primer of arbitrary nucleotide sequence, analysis is an extensively used technique and such advantages over biochemical makers have made a rapid impact in genetic relationship studies and molecular delineation of various medicinal plants e.g., *Scutellaria* [7], *Cuscuta reflexa* [8], and *Dendrobium officinale* [9, 10]. Besides the DNA markers techniques have very unique features like ubiquitous nature, detection at any developmental stage and independent of environmental effects and management practices, and hence have direct applicability in authentication of traded

potential ethanomedicinal plants. Recently, this technology has been used for genetic diversity studies and molecular characterization of many medicinal plant species [11, 12].

For scientific exploration and sustainable utilization of most potential ethnomedicinal plants like *A. indica* and *T. purpurea* it is utmost important to characterize these plants genetically with the help of most effective molecular marker. Nevertheless, till date a very less study explored genetic variation at DNA level and reported polymorphism for these plant species, which made it very difficult for identification and quality control in pharmaceutical industries. Thus, it is expected that the outcome of this study would allow a researchers for more efficient utilization of plant characters in developing suitable varieties and exploration of better pharmaceutical quality.

MATERIALS AND METHODS

Plant material

The plant material for this investigation comprised of 05 samples of each plant *Alocasia indica* and *Tephrosia perpurea* which were collected from different agro-climatic zone of Chhattisgarh (Table 1).

Extraction of plant genomic DNA

DNA from selected plant samples were extracted following CTAB (Cetyltrimethyl

ammonium bromide) method [13] with required modification. 2 gm fresh young leaves of *T. purpurea* and 2 gm tuber of *A. indica* were grounded with preworned CTAB buffer (2% CTAB, 1.5 M NaCl; 20 mM EDTA, 100 mM Tris-HCl, pH 8.0, 0.6% β -mercaptoethanol) using mortar and pestle and transferred to a 2 ml tube containing 1 ml CTAB buffer. After mixing for 2-5 min. RNAase (10 mg/ml) was added in tube then the mixture was incubated at 65 °C for 1 hour. Further sample was centrifuged at 12,500 rpm for 12 min, then supernatant was collected and equal to this volume phenol:chloroform:isoamyl alcohol mixture (25:24:1) were added in tube, and after mixing thoroughly again sample was centrifuged at 12,500 rpm for 12 min at 10°C. Upper aqueous layer was transferred in new tubes further it was repeated again and added same amount of chloroform: isoamyl alcohol (24:1). Upper aqueous layer containing DNA was retransferred in fresh new tubes, 1 ml of 3 M sodium acetate and 2/3 vol. of chilled isopropanol was added in tube. The DNA was washed with 70% ethanol, dried and dissolved in 1x buffer (TAE pH 8.0). Extracted DNA was kept at -20°C till further use. DNA quality was measured by using electrophoresis and concentration of DNA was measured by

using spectrophotometer at wavelength 260 and 280 nm.

PCR amplification

DNA amplification of *A. indica* and *T. purpurea* was performed using 15 random 10 decamer RAPD primer following the method as reported by [14], with required minor modifications. It was amplified in 25 µl PCR reaction mixture comprising 50 ng plant genomic DNA, 1x reaction buffer (50 mM KCl, 10 mM Tris HCl, pH 8.3), 1.5 U *Taq* DNA polymerase, 100 µM dNTPs and 200 µM primer. Amplification was performed in dome shaped PCR tubes in Bio-Rad (Gradient T-100) thermal cycler, programmed as one cycle of initial denaturation at 94°C for 2 min. 30 seconds, followed by 38 cycles of denaturation at 94 °C for 1 min. 30 seconds, annealing 36 °C for 1 min. 30 seconds, elongation 72 °C for 1 min. and a last elongation at 72°C for 10 min. After the completion of PCR amplification, sample was kept at -20 °C until gel electrophoresis. The amplified DNA bands were resolved with help of electrophoresis in 1.4 % (w/v) agarose gel (0.5X TAE), stained by ethidium bromide (10 µg/ml). After electrophoresis the PCR amplified DNA bands were visualized and photographed under a gel documentation system. During study the 1kb standard DNA ladder was used

as molecular size marker. The PCR amplification study was repeated 2-3 times to confirm that the amplified DNA fragments obtained with the particular primers is reproducible and predictable.

RESULTS

RAPD marker analysis in *A. indica* and *T. purpurea*

Both *A. indica* and *T. purpurea* plant samples cumulatively produced the different RAPD profile. Out of 15 random decamer primers, 08 primers (**Table 2**) amplified a total of 68 fragments, of which 56 (82.3%) were found polymorphic. An average 17.7% of total fragments were constantly present among all selected plant samples. Among the 15 RAPD primer used, the banding pattern of OPB-06 and OPD-01 were unique to *A. indica* plant collected from Lundra block Ambikapur, Chhattisgarh and the banding pattern of OPC-01 and OPD-03 were unique to plant sample collected from Achanakmar forest Bilaspur Chhattisgarh. In *A. indica* plant samples the size of amplified DNA fragments from all the primers varied between 200-2700 bp. The minimum size of 200 bp DNA fragment was generated with primer OPD-03, while the maximum size of 2700 bp was generated with primer OPD-01. Primers OPA-03, OPB-05, OPB-06 and OPB-04 generated only seven (minimum)

DNA fragments, whereas a maximum of 13 DNA fragments were generated with primer OPD-01. On an average each primer generated 8.5% polymorphic DNA fragments. Some primers viz., OPA- 01 and OPC-04 generated 100% polymorphic DNA fragments. Primers, OPB-06 and OPD-03 are reported as most informative marker based on the DNA fragments generated by that primer. Representatives RAPD banding profile using various RAPD primers is shown in **Figure 1**.

Genetic similarity and cluster analysis

The NTSYS SAHN Clustering program [15] used the UPGMA method for pair wise genetic similarity study and clustering 05 plants of *A. indica* and 05 plants of *T. perpurea* collected from different places of Chhattisgarh. Final output is represented in the form of dendrogram (**Figure 2 a, b**). This study revealed that all 05 plants of *A. indica* had a Jaccard's similarity coefficient [16] ranged from 0.23 to 0.43 whereas it was

ranged from 0.51 to 0.69 in case of plant samples of *T. perpurea*. Dendrogram depict that whole 05 plants of *A.indica* grouped in one cluster; except sample collected from Lundra Surguja which separated alone at a coefficient value of 0.23. In this study sample collected from Achanakmar forest, Bilaspur and Pakhanjur, Kanker had the highest similarity (43%) as compared to other sample; these two samples were highly related to sample collected from University campus, Pt. RSU, Raipur at coefficient value 0.40. Similarly 05 plants of *T. perpurea* grouped in one cluster; except sample collected from Lundra Surguja which separated alone at a coefficient value of 0.51. Sample collected from Achanakmar forest, Bilaspur and University campus, Pt. RSU, Raipur had the highest similarity (69 %) as compared to other sample; these two samples were highly related to sample collected from Pakhanjur, Kanker at coefficient value 0.62.

Table 1: List of plant samples collected from different Agro-climatic zone of Chhattisgarh

S. No.	Collection Site	District Name	Agro-Climatic Zone
1	Lundra	Surguja	Northern Hills zone
2	Achanakmar forest	Bilaspur	Chhattisgarh plains
3	University campus, Pt. RSU, Raipur	Raipur	Chhattisgarh plains
4	Pakhanjur	Kanker	Bastar plateau
5	Bailadila	Dantewada	Bastar plateau

Table 2 List of RAPD primers names, their sequence, number of DNA fragment amplified, no. of polymorphic DNA fragment and percentage of polymorphic DNA fragment generated by 8 selected primers

S. No	Primer name	Primer sequences	GC %	Tm (°C)	Total number of DNA fragment	Number of polymorphic DNA fragment	Percent of polymorphic DNA fragment
1	OPA-01	CAGGCCCTTC	70	38	10	10	100
2	OPA-03	AGTCAGCCAC	60	35	07	06	85.7
3	OPB-05	TGCGCCCTTC	70	42	07	06	85.7
4	OPB-06	TGCTCTGCC	70	40	07	06	85.7
5	OPC-01	TTCGAGCCAG	60	38	09	08	88.8
6	OPC-04	CCGCATCTAC	60	38	07	07	100
7	OPD-01	ACCGCGAAGG	70	42	13	06	75
8	OPD-03	GTCGCCGTCA	70	39	08	06	67

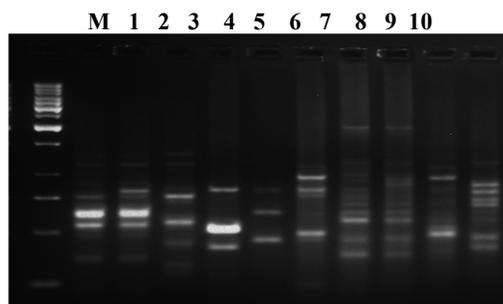


Figure 1: RAPD banding pattern 1-5 *A. indica* and 6-10 *T. perpurea* generated by the RAPD primer OPB-06. The lane M represents 1Kb molecular size marker

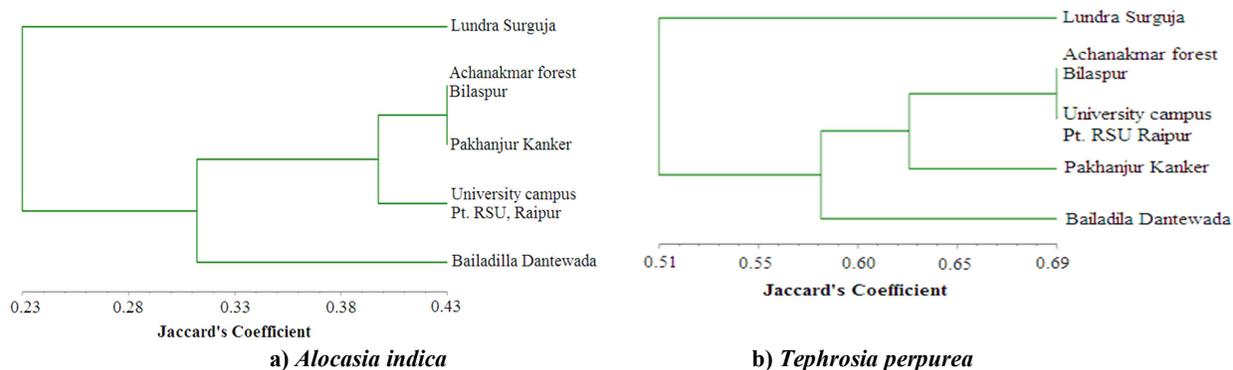


Figure 2: UPGMA cluster depicting genetic relationship among a) *A. indica* and b) *T. perpurea* plants based on RAPD fragments produced by 08 RAPD primers

DISCUSSIONS

Genetic characterization and identification of ethnomedicinal plant is very significant for their effective and efficient utilization. Earlier genetic relationship studies of any plant species was primarily based on morphological data, which was not sufficient and does probably reflected grouping of a

phyletic nature. But relationship studies in many species are very difficult only on the basis morphological data, even after comprehensive biochemical studies. Therefore, the data generated by DNA marker technology might be significant in taxonomic viewpoint. But now a days it is considered that other than single

morphological data; biochemical data pooled together with molecular data is more significant in sorting out the inter relationships between various plant species.

In this study, DNA based RAPD markers technique was used to examine the genetic variation between total 05 plants of each *A. indica* and *T. perpurea* collected from different places of Chhattisgarh. Presently for any genetic studies mostly DNA based markers are preferred because they relate variability directly at the DNA level and provide reliable and enormous data that permit a reproducible estimate of genetic variability among different plant species [17]. Many previous researchers have suggested RAPD as reliable methods and have been used for genetic relationships studies and identification of various medicinal plant species [12, 18, 19]. The diversity analysis, among different plant sample of *A. indica* collected from different location of Chhattisgarh generated 17.7% monomorphic bands (Table 2), indicates that they were genetically diverse. Using RAPD marker technique similar range of similarity coefficient values has been reported earlier by [20] in plant species *Saccharum* and other related genera. In this experiment, we reported some plant specific DNA fragment (OPC-01) in sample collected from

University campus, Pt. RSU, Raipur, Pakhanjur Kanker and Bailadila, Dantewada which may be used for better scientific exploration of a specific plant sample. Likewise various authors have reported RAPD markers as a powerful tool for finger printing a plant genetic DNA besides this marker has been successfully applied to assess genetic diversity studies in many plant species like *Leucaena* species [21], *Afgekia* species [22], *Oxytropis* species [23], *Fragaria* species [24], *Orobanche* species [25] etc. More number of polymorphic DNA fragments and wide range of similarity values (0.23-0.43 and 0.51-0.69) indicate that the species used in the present study possess less genetic similarity. Besides the plant sample collected from Achanakmar forest, Bilaspur (Figure 2a) and Bailadila, Dantewada (Figure 2b) separated from other remaining 04 samples and fall on the side of the dendrogram, indicating less genetic similarity among the various selected four plant samples. Despite the years of extensive investigation on the plant species *A. indica* and *T. perpurea*, except some morphological and pharmacological studies, very little research is known about its phylogeny. Until to-date, no work using molecular data (DNA fingerprinting technique) has been reported in evaluating genetic relationships among the

various *A. indica* and *T. perpurea* plant species, the only exception is the studies of species variation cytogenetically (based on G+C content) in eight species of *Tephrosia* [26-29].

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