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## Analysis of Genetic Differentiation of Few Edible *Cassia* Species Using RAPD Molecular Markers



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### ABSTRACT

As several plants of Genus *Cassia* of Family Caesalpinaceae are medicinally important and even edible, it is necessary to identify exact species of the plant. In the present investigation, Randomly Amplified Polymorphic DNA (RAPD) markers are used to study genetic variation of some edible *Cassia* species. The four informative primers were used to evaluate degree of polymorphism. These primers produced multiple band profiles and bands were found in the size range of 900kb to 1250kb. Of the four plants used, though three are of *Cassia tora* type and one is *Cassia sophera*, they exhibited phylogenetic differences as revealed by the dendrogram. Intraspecific genetic variability and physical distances were exhibited by three plants of *Cassia tora* collected from different localities which may be useful in proper identification of the plants.

## INTRODUCTION

Many plants of Genus *Cassia* are widely used in traditional medicine and highly valued in industries as they possess various pharmacological activities (Mohanty S, 2010). Genus *Cassia* of family Caesalpinaceae is one of twenty five largest genera of plants in the world (Acharya Laxmikanta, 2010). This genus exists in all terrestrial habitats and species under this Taxon have wide variability in habit ranging from diverse groups of flowering plants which include delicate herbs, shrubs, treelets and even trees. There has been great divergence of opinion concerning the delimitation and taxonomic status of this group of plants, as the *Cassia* plants exhibit a great deal of diversity and is taxonomically complex (Mohanty S, 2006).

*Cassia tora* and *Cassia sophera* are two wild edible plants and are used in medicine. These plants are rich sources of pharmacologically important compounds like anthraquinones, some can be used as laxatives. Leaves and roots are employed to treat skin diseases, dysentery and ophthalmia as they have antimicrobial activities and also possess purgative properties. Due to this plants are greatly in demand by national and international industries (Vivek Tripathi, 2009).

Morphological as well as biochemical markers used in the authentication of wild medicinal plants have many limitations due to the impact of environmental conditions. The molecular approach for the identification of plant species seems to be very effective than morphological markers as it allows direct access to the genome and makes it possible to understand the relationships between individuals (Niraj Tripathi, 2012). Of the several advantages of DNA profiling techniques, independence of DNA sequence to the environmental conditions, delimitations of identification at any stage of plant growth, discrimination of very similar genotypes, including clonal variants are few which can be listed out. RAPD marker technique has gained importance due to quick screening ability, simplicity, efficiency and ease of performance. RAPD markers provide an efficient assay for polymorphism which allows rapid identification and isolation of chromosome specific DNA fragments, thus used for the analysis of genetic variation and identification in *Cassia* species (Nada BH, 2009).

Molecular markers have increased our understanding of spatial and temporal patterns of genetic variations and of the evolutionary mechanisms that generate and maintain variation (Kumar A, 2007). In investigation of genetic diversity within and between populations of wild, known and

unidentified plants, RAPD plays a key role. RAPD is the most useful tool in detection of polymorphism and has the advantage that the material is processed by an efficient and inexpensive technique without requiring prior knowledge of the genome (John De Britto, 2010). So, the present study has been planned to analyze the genetic variation between *Cassia tora* from different regions and *Cassia sophera* using RAPD markers.

## **MATERIALS AND METHODS**

### **Plant material**

Healthy, young and disease free *Cassia tora* and *Cassia sophera* plants were collected from various locations of Karnataka and Telangana states. Leaves were separated, washed and used immediately for DNA isolation.

### **Genomic DNA isolation**

Total genomic DNA was isolated from fresh leaf tissue of four different *Cassia* plants using modified CTAB method (Nada BH, 2009). After isolation DNA pellet was dissolved in TE buffer. The quantity and quality were measured using UV-VIS spectrophotometer. The DNA stored at 4<sup>0</sup>C for further use and diluted to 25ng/μl with TE buffer before used for PCR.

### **RAPD analysis and Primer selection**

Purified genomic DNA was subjected to PCR amplification using random primers (RAPD). PCR amplification of 25ng of genomic DNA carried out using four primers, OPA01, OPA07, OPA14, and P1. RAPD-PCR analysis was performed as per the standard method of William et al, 1990 (Nada BH, 2009, Kumar A, 2007).

PCR reaction mixtures of 20μl were prepared containing 25ng template DNA(1μl), 0.5U Taq polymerase(0.5μl), 10 picomoles of random primers (2μl ), 100mm each dNTPs (2.5μl ) 50mm MgCl<sub>2</sub> (1.5μl ml), 1X PCR reaction buffer (2.5μl ). Volume was made up to 20μl with double distilled water. These PCR mixtures were optimized in thermo cycler. Three steps were set in PCR, which are as follows.

Step 1: Initial denaturation at 94<sup>0</sup>C for 5mins.

Step 2: Run for 40 cycles, each starting with denaturation at 94<sup>0</sup>C for 1min, followed by annealing at 36<sup>0</sup>C for 1min and ended by extension at 72<sup>0</sup>C for 1min.

Step 3: Final extension cycle performed at 72<sup>0</sup>C for 7 minutes.

The PCR thermocycler is optimized to hold the product at 4<sup>0</sup>C. The amplified PCR product was mixed with 3 µl of tracking dye (0.25% Bromophenol Blue), 0.25% glycerol and 40% sucrose and then spun in microcentrifuge. The PCR products and 1kb DNA ladder were electrophoresed using 2% agarose gel at 100 volts, visualized by Ethidium Bromide and UV trans-illuminator. Gel is photographed using Gel Documentation system. Amplifications were repeated three times to ensure reproducibility.

## RESULTS AND DISCUSSION

DNA obtained was of good quality as determined from Table 1 data.

Quantity and quality were estimated by spectrophotometer by taking OD at 260nm and 280nm.

**Table 1: Quantitative and qualitative estimation of DNA**

Samples	OD at 260nm	OD at 280nm
Ct1	0.227	0.128
Ct2	0.265	0.144
Ct3	0.131	0.072
Cs	0.165	0.093

PCR – based molecular marker namely RAPD employed to study polymorphism pattern between different *Cassia* species indicate that though *Cassia* species used show genetic variability, Ct1 is more related to Ct3 than Ct2. Ct4 i.e. Cs is also related to Ct3 genetically. This suggests that all species from *Cassia tora* and *Cassia sophera* are related but show phylogenetic distances and physical distances.

Arbitrary 10mers primers were selected. The four informative primers were selected and used to evaluate the degree of polymorphism and genetic relationship within and between *Cassia*

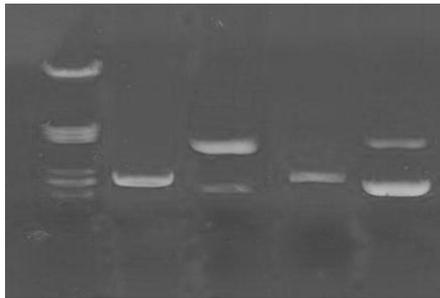
species. These primers produced multiple band profiles with number of amplified DNA fragments. Gel pictures are shown in Figure 1.

**LINE 1: MARKER- 1500kb, 1300kb, 1250kb, 1000kb, 900kb, 800kb.**

**LINE 2: CT1, LINE 3: CT2, LINE 4: CT3, LINE 5: CS**

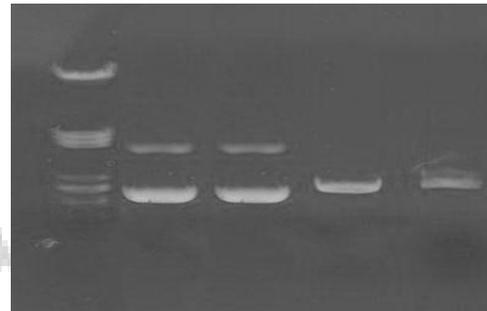
Primer 1: OPA 01

Sequence: 5' – 3' : CAGGCCCTTC



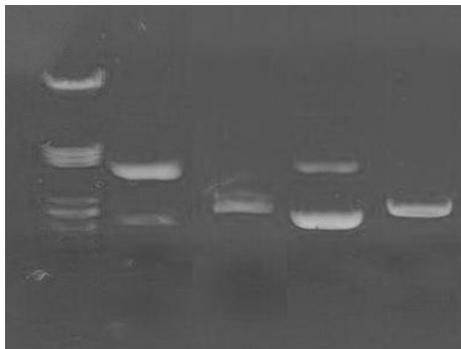
Primer 3: OPA 14

Sequence: 5' – 3' : CCAGCCGAAC



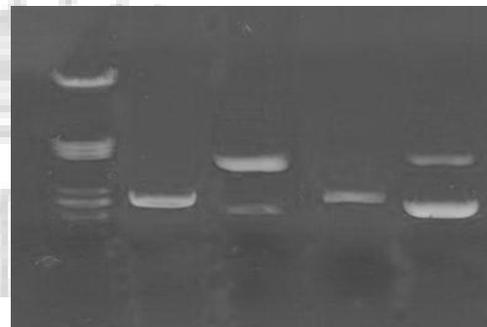
Primer 2: OPA 07

Sequence: 5' – 3' : GAAACGGGTG



Primer 4: P1

Sequence: 5' – 3' : CGCTGTCGCC



**Figure 1: Gel pictures for RAPD analysis of *Cassia* plants.**

The observed bands were found in the size range of 900kb to 1250kb and their number per primer varied from one to two. The genetic diversity scores range from 0.00 to 0.577 (Figure 2). The Dendrogram showed two main clusters (Figure 3). The first cluster contained 6 groups. The second cluster contained 7 groups.

Using RMSD values for tree construction

	CT1OPAF	CT2OPAF	CT3OPAF	CT4OPAF	CT1OPAS	CT2OPAS	CT3OPAS	CT4OPAS	CT1OPAT	CT2OPAT	CT3OPAT	CT4OPAT	CT1P1	CT2P1	CT3P1	CT4P1
CT1OPAF	0	0.408	0.000	0.408	0.408	0.000	0.408	0.000	0.408	0.408	0.000	0.000	0.000	0.408	0.000	0.408
CT2OPAF		0	0.408	0.577	0.577	0.408	0.577	0.408	0.577	0.577	0.408	0.408	0.408	0.577	0.408	0.577
CT3OPAF			0	0.408	0.408	0.000	0.408	0.000	0.408	0.408	0.000	0.000	0.000	0.408	0.000	0.408
CT4OPAF				0	0.000	0.408	0.000	0.408	0.000	0.000	0.408	0.408	0.408	0.000	0.408	0.000
CT1OPAS					0	0.408	0.000	0.408	0.000	0.000	0.408	0.408	0.408	0.000	0.408	0.000
CT2OPAS						0	0.408	0.000	0.408	0.408	0.000	0.000	0.000	0.408	0.000	0.408
CT3OPAS							0	0.408	0.000	0.000	0.408	0.408	0.408	0.000	0.408	0.000
CT4OPAS								0	0.408	0.408	0.000	0.000	0.000	0.408	0.000	0.408
CT1OPAT									0	0.000	0.408	0.408	0.408	0.000	0.408	0.000

Figure 2: Genetic diversity score of *Cassia* species

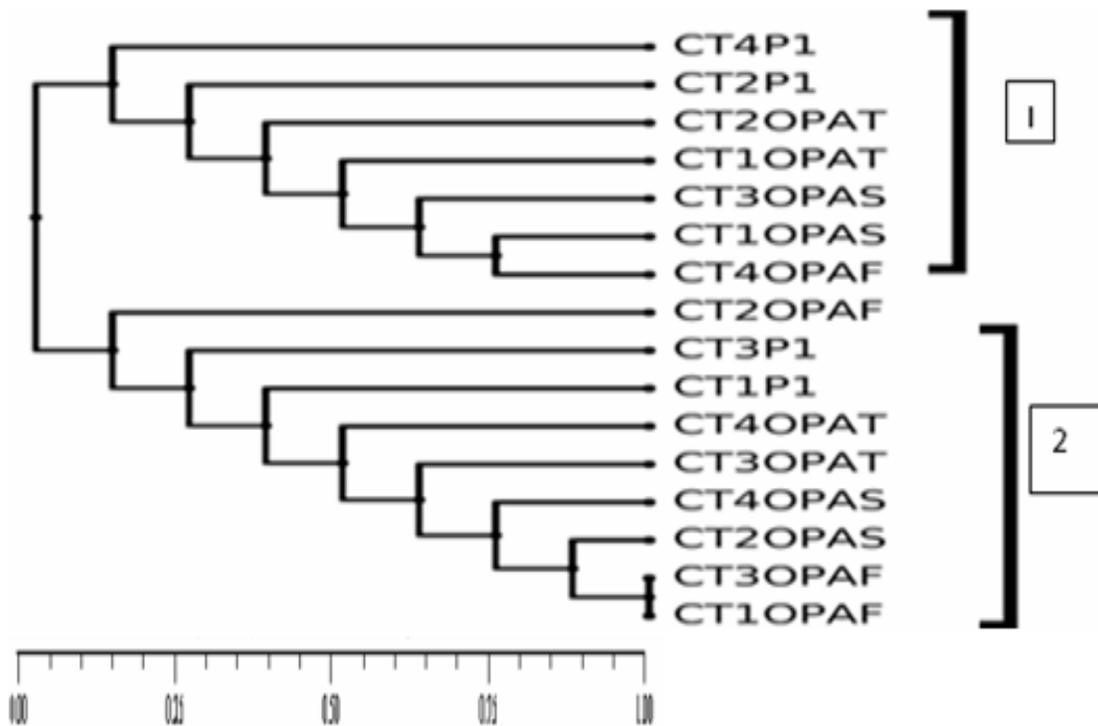


Figure 3: Dendrogram showing genetic relationships among *Cassia* species

Numerous novel DNA markers like RAPD, RFLP, SSR, ISSR etc. have been rapidly integrated as tools for genome analysis during the last decade. Molecular markers are superior to morphological markers to study intra and inter-specific genetic variations (Kalita MC, 2007). Genus *Cassia* is now under Family Leguminosae, Subfamily Caesalpinioideae. Due overlapping morphological characters of various species of the Genus *Cassia*, the Taxonomy and

Nomenclature are quite complex. Besides medicinal importance the Genus has also taxonomic importance. Irwin and Barneby, 1981, 1982 proposed an improved classification and raised the Genus *Cassia*. L to the level of Subtribe Cassiinae. Data obtained from RAPD and ISSR analysis revealed high degree of genetic diversity among the different taxa of Cassiinae (Laxmikanta A, 2001).

Dendrograms constructed from RAPD data revealed DNA marker based genetic identification in *Cassia*. The broad adaptation of some species of *Cassia* implies a large probability that nuclear mutation occurred in past and that resulted in diversification of morphological characters and genetic polymorphism. The genetic diversity of *Cassia* plants is closely related to their geographical distribution. Species with a wide geographic area generally exhibit greater genetic diversity (Arya V, 2011). Though RAPD generated phylogeny include homology of bands showing the same rate of migration, cause of variation in fragment mobility and origin of sequence in the genome, it has the greatest asset in its capacity to scan across all regions of the genome, well suited for phylogeny studies at species level (reff 10).

The RAPD analysis revealed higher genetic variation in *Cassia tora* and *Cassia sophera* growing at different environments. Ct1 is from Mangalore, Karnataka. Ct2 from Julywada, Hanamkonda, Ct3 from Matwada, Warangal, both from Telangana. Cs from matwada Warangal. Within *Cassia tora* species, Ct1, Ct2 and Ct3 exhibited significant genetic divergence. These plants were selected as they exhibited good antimicrobial and antioxidant properties (Sumangala Rao, 2012). Their leaf extracts possess anticancer properties as they showed antiangiogenesis (Sumangala Rao, 2013). Thus they are good candidates for study and need to be exactly identified.

Through its technical simplicity and convenience RAPD has been successfully used in genetic diversity among species of Genus *Cassia* and is beneficial in crop improvement and detection of gene flow between the species. RAPD is sufficiently informative, more powerful and less restricting than other techniques like RFLPs in research (Elavazhagan T, 2010).

## CONCLUSION

In the current study, different edible plants of *Cassia* Genus, though morphologically similar exhibited genetic variation. RAPD revealed their intra specific genetic variability, which is

expressed clearly in dendrogram. This study may explore more details in taxonomy of closely related plants of Genus *Cassia*.

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