Indian J. Genet., **67**(2): 173-176 (2007)

Short Communication

DNA marker-based genetic variation in *Cassia fistula* L.

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(Received: August 2006; Revised: April 2007; Accepted: July 2007)

The taxonomy and nomenclature of various species and intraspecific taxa have been quite complex and intriguing. They are not easily differentiated from closely related species due to great range of similarities. This usually leads to mis-identification and misinterpretation of the components. *Cassia* L. is a large and predominantly tropical genus of the subfamily *Caesalpinioideae*, family *Caesalpiniaceae*. Taxonomy of *Cassia* is based primarily on morphology [1], which often makes identification of its species difficult, because many species are separated on the basis of differences in morphological traits. Morphological characters and behaviournal pattern can sometimes evolve independently in unrelated species in response to common environmental changes [2] or by hybridization of related species in favorable conditions.

During evolution, characters evolve from primitive state to advanced state within a group of related organisms, in which a character shows more than one state, only one of these can be the ancestral state and others are derived [3]. Which have been found in *Cassia* group where seven stamen strands species are at higher evolutionary level than the species with ten stamens [4].

A survey of *Cassia fistula* growing in Lucknow and the neighbouring districts of Uttar Pradesh and Uttaranchal of India has been done which cover different geographical locations and found a very attractive form of the plant which produces totally white flowered racemes except a portion at the tip which bears golden yellow flowers with nodulated filament. *C. fistula* is a moderate sized, deciduous tree about 10 meters tall. Variation in shape and size of leaves, size of flowers and their colour shades are common in this species. It is however not clear whether these variations are hybrids or forms of the species. It is widely distributed and planted along roadside, garden and cultivated due to its ornamental as well as medicinal properties [5]. The flowers are golden yellow and in hanging branches of up to 40 cm long known as "Golden shower" and "AMALTAS" in India. There are two distinct group one has larger leaflets and bright flowers, the other smaller leaflets and paler flowers. There are also variations in the shades of yellow and in the size of the flowers [6].

The cultivar identification and estimation of diversity using phenotypic markers have several limitations especially in perennial crops as because morphological characters, specially quantitative traits are subject to environmental influences. Now it is possible to single out differences present at the molecular level which is more authentic and less affected by environmental factors. Hence characterization of genotypes at the genetic level supplemented with phenotypic characters could be the first step towards efficient conservation, maintenance and utilization of the existing genetic diversity. When dealing with morphologically similar taxa, study of molecular markers exemplified a definitive approach than morphological observation. Among the methods used in studies, DNA markers have proved to be excellent parameter to resolve the problems of identification of critical taxa and to understand their relationships and taxonomic status [7].

Random amplified polymorphic DNA (RAPD) is a powerful technique, which can be used to identify and determine specific genomes or to estimate the genetic relationship among the individual genomes of *Cassia fustula* L. RAPD is based on the Polymerase chain reaction (PCR) [8]. In addition to the technical simplicity and speed of RAPD methodology, the polymorphic DNA amplified by using random 10-mer oligonucleotides primers can generate many useful genetic markers for the analysis of genetic diversity and to study the phylogenetic relationship [9].

Nineteen selections located in different parts of Uttar Pradesh and Uttaranchal in India in a range of 2000 km, in which some are wild and some maintained by local peoples were selected from the 30 accessions collected in the study. After the morphological studies

it is found that plants were variable in their morphological characters like shape and size of leaves, size of flowers and their colour shades. One plant of *C. fistula* in NBRI Lucknow (India) was a white flowered raceme except a portion at the tip, which bears golden yellow flowers with nodulated filament. Young and healthy leaves from single tree of each accession were used for DNA Isolation by CTAB method with minor modifications [10]. The extraction buffer composition was 2% w/v CTAB, 1.4 M NaCl, 100 mM Tris-HCl (pH-8.0), 20 mM EDTA, and 0.2% 2β-mercapto-ethanol v/v. DNA was treated with bovine pancreatic RNase and extracted once with phenol, followed by phenol: chloroform (1:1) and twice with chloroform: iso-amyl alcohol (24:1). After precipitation with iso-propanol, a 70% ethanol wash was given. DNA was dissolved in TE buffer and quantified by fluorometer (DyNA Quant 200).

DNA prepared from all the selections was used for PCR reactions. RAPD variations were assessed using individual DNA. Conditions have been optimized to allow reproducible RAPD amplification. PCR reactions were individually primed with different primers. To survey a large number of loci, 45 decamer primers supplied commercially by Operon technologies Inc. USA, were first tested on two DNA samples. Thirty seven primers produced reproducible bands. These primers were then used to analyse the full set of DNA samples representing nineteen individuals.

The PCR reaction were performed using Gene Amp PCR System 9700 with 44 cycle of 94°C for 60 sec., 36°C for 90 sec. and 72°C for 90 sec. followed by a five min final polymerization step and reactions were held at 4°C until further processing. PCR reaction was preceded by a hot start at 94°C for 5 min. Each reaction contained 10X thermostable PCR buffer 25 mM $MgCl_2$, 0.2 mM dNTPs in equimolar ratio, 1 Unit *Taq* Polymerase (Genei, Banglore), 0.5 mM primer (Operon USA) and 50ng template DNA to a final volume 20µl.

The reactions were then electrophoresed through 1.4% agarose gels under constant volts for over night. A EcoRI and Hind III digested λ DNA standard were used to assign molecular weight to individual RAPD bands. After completing electrophoresis the gels were stained in a dilute solution of ethidium bromide. For RAPD analysis, the reactions were performed at least twice and only those bands that were consistently produced were scored for presence/absence (1/0). Percentage polymorphism was calculated as the proportion of amplification products that were polymorphic across all the lanes to the total number of amplification products. Dendrogram based upon unweighted pair group with arithmetic mean method (UPGMA) was constructed using NTSYSpcv-2.0 [11].

PCR analysis using random 10-mer primers revealed significant differences in RAPD profiles in *C. fistula* accessions derived from different geographic locations. It has also detected small differences between accessions.

A total of 45 decamer primers were used to amplify DNA extracted from the nineteen accessions used in this study. Some of the primers, however produced either no amplification or unreadable gel smears. Thirty seven primers yielded scorable amplification pattern (Fig. 1) primer OPC-8 amplified similar profile in all 18 accessions of one group and one band at M.W 800bp is missing in white flower with nodulated filament. (Fig. 1A, shown by arrow) and primer OPAP-3 generated different profile for all 19 accessions (Fig. 1B).

A total of 5092 amplified products were scored in all 19 accessions using 37 random primers. In total 412 polymorphic and monomorphic bands were generated of which 306 bands were polymorphic (74.27%). The average number of bands per primer was 7.2 and the number of amplified products/primer varied from 3 (OPC-7) to 20 (OPC-11). Analysis of band data indicated that there was no accession specific band within *Cassia fistula*. This indicates a high level

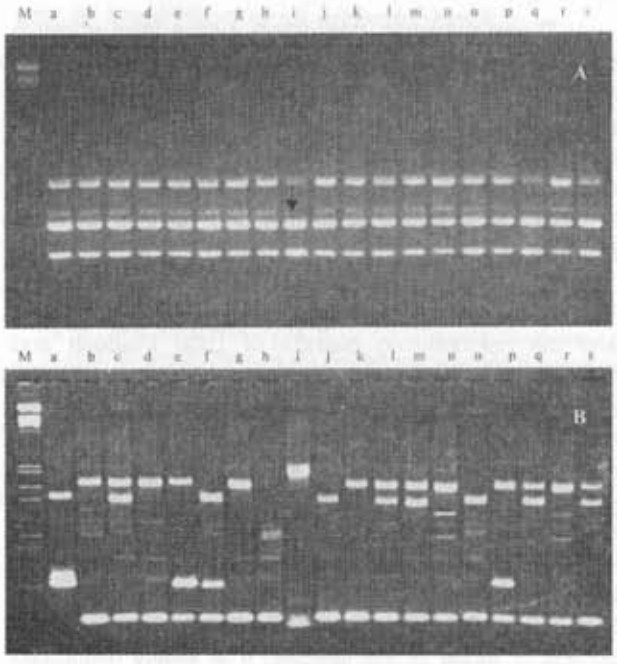

Fig. 1. The representative polymorphic profiles of all the accessions with primer OPC-8 (A) and OPAP-3 (B). Lane M: λ DNA marker digested with *Hind* III and *Eco*RI; Lane a-s: Accessions of *Cassia fistula* of different places. Arrow indicate accession with nodulated filament

of natural hybridization between accessions of two main groups. The population diversity were detected based on the presence/absence of RAPDs bands. Highest similarity (0.86%) was observed between N and R accessions and least similarity between H and I (Table 1). The dendrogram showed 2 major clusters that included plants from all 19 different populations and formed one groups of 18 and other of one which is white flowered and nodded filament (Fig. 2). The dendrogram of first group of 18 showed very little differentiation and formed subgroups.

In *Cassia*, where floral structure is adapted for outbreeding, where isolating mechanism appear to be weak and where hybridization is undoubtedly of frequent occurrence, sexual reproduction would be expected and allowing high levels of gene recombination and rapid production of new genotypes and wide segregation [12]. The study was undertaken to evaluate the extent and range of genetic diversity available in *Cassia fistula*.

Accurate estimates of diversity are a prerequisite for optimizing sampling strategies and for conserving tree genetic resources [13]. The high diversity revealed by RAPD is in agreement with the conclusion that outbreeding woody plants retain considerable variability [14]. Variation within the species suggests that this species has large effective population size or large mutation rate due to longer generation time since genetic diversity is proportional to the product of the two quantities [15]. Another possibility is interspecific gene flow with other species of *Cassia*. Such mutations or hybridizations are mainly reflected in change in the colour of flowers.

Table 1. Similarity matrix of 19 individual plants of *Cassia fistula* based on polymerase chain reaction (PCR) profiling of their genomic DNAs using 37 random primers

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1.000																		
0.757	1.000																	
0.763	0.766	1.000																
0.783	0.769	0.765	1.000															
0.750	0.737	0.754	0.778	1.000														
0.765	0.752	0.775	0.788	0.729	1.000													
0.798	0.773	0.785	0.809	0.798	0.765	1.000												
0.690	0.673	0.711	0.699	0.697	0.681	0.722	1.000											
0.489	0.495	0.484	0.482	0.455	0.475	0.477	0.446	1.000										
0.757	0.717	0.730	0.776	0.695	0.753	0.774	0.688	0.495	1.000									
0.746	0.717	0.714	0.749	0.764	0.726	0.774	0.747	0.476	0.745	1.000								
0.683	0.724	0.694	0.698	0.701	0.716	0.716	0.672	0.504	0.708	0.724	1.000							
0.756	0.753	0.733	0.747	0.725	0.740	0.761	0.691	0.521	0.766	0.749	0.717	1.000						
0.707	0.759	0.744	0.726	0.709	0.724	0.745	0.702	0.481	0.732	0.749	0.680	0.753	1.000					
0.773	0.710	0.712	0.731	0.725	0.708	0.761	0.685	0.483	0.737	0.743	0.700	0.759	0.719	1.000				
0.790	0.753	0.728	0.758	0.741	0.773	0.778	0.718	0.490	0.721	0.777	0.723	0.758	0.736	0.753	1.000			
0.700	0.688	0.659	0.678	0.677	0.686	0.702	0.663	0.483	0.671	0.688	0.784	0.691	0.655	0.696	0.719	1.000		
0.707	0.721	0.723	0.721	0.709	0.719	0.729	0.687	0.476	0.716	0.743	0.669	0.764	0.865	0.698	0.747	0.676	1.000	
0.638	0.631	0.624	0.612	0.613	0.665	0.633	0.636	0.509	0.607	0.649	0.654	0.643	0.627	0.653	0.678	0.686	0.638	1.000

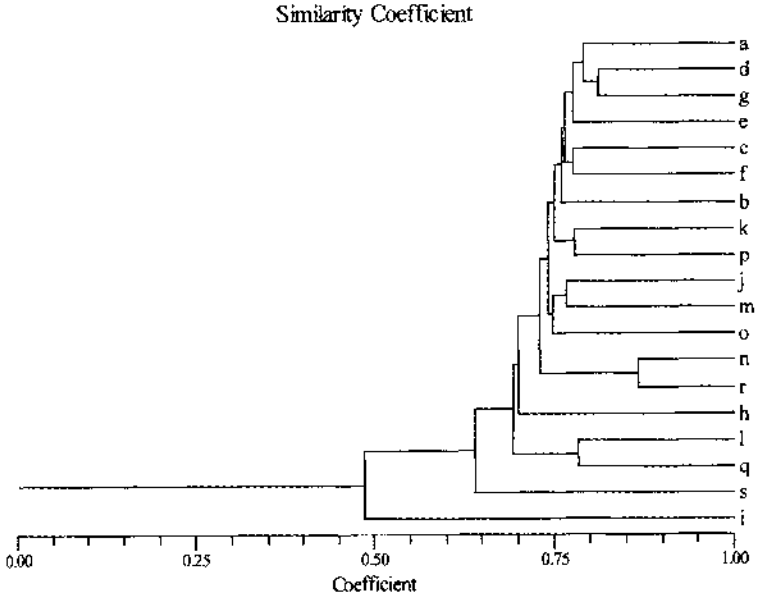

Fig. 2. UPGMA dengrogram showing the relationship among 19 accessions of *Cassia fistula* L.

Acknowledgement

This project was supported by a grant from the Department of Biotechnology, Govt. of India, New Delhi.

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