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Analyzing variability in *Coleus forskohlii* Briq. using RAPD markers

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ABSTRACT

***Coleus forskohlii* Briq. is an indigenous medicinal plant with high traditional use in India. Genetic analysis of 37 diverse *C. forskohlii* genotypes was performed using 25 RAPD primers, which yielded 117 bands, of which 60 (51.28%) were polymorphic providing an average of 3.75 bands per primer. There were no genotype-specific products. The number of bands per primer varied from 1 (OPZ 8 & 16) to 7 (OPZ 11). Similarity matrix was constructed using Jaccard's Coefficient and the data matrix of coefficient of similarity was subjected to cluster analysis using unweighted pair group methodology with arithmetic average (UPGMA). Cluster analysis resulted in grouping of 37 genotypes into two major clusters. The results indicated that RAPD could be used for genetic diversity analysis in *C. forskohlii* using higher number of primers as it is reliable, easy, rapid and cost-effective.**

Key words: *Coleus forskohlii*, genetic diversity, RAPD

INTRODUCTION

Coleus forskohlii Briq. family Lamiaceae, is the only naturally occurring species in *Coleus* to have fasciculated tuberous roots and it is indigenous to the Indian sub continent. It is a perennial, aromatic herb that grows wild on sun exposed arid and semiarid hill slopes of the Himalayas, Deccan Plateau, Eastern Ghats, Eastern Plateau and rain shadow regions of the Western Ghats in India. The tuberous root extracts of *C. forskohlii* are good source of a diterpene, forskolin which is exclusive to this species (Shah *et al,* 1980). Forskolin is used for the treatment of eczema, asthma, psoriasis, cardiovascular disorders and hypertension where decreased intracellular cAMP level causes disease development (Rupp *et al,* 1986). In Ayurveda, the tuberous roots of *Coleus* are used as drug for heart diseases, abdominal colic, respiratory disorder, insomnia and convulsions (Ammon and Muller, 1985). It also contains essential oil in tubers which has very attractive and delicate odour with spicy note (Misra *et al,* 1994). It has potential uses in food flavouring industry and can be used as an antimicrobial agent (Chowdhary and Sharma, 1998). Variation in *Coleus* genotypes based on forskolin and essential oil content has been reported earlier (Vishwakarma *et al,* 1988; Hegde, 1992; Nanaiah, 1993; Prakash and Krishnan, 1994). Since chemical characters are dependent on environment, it is essential to characterize this medicinally important plant at genetic level.

For any crop improvement programme, the assessment of genetic diversity and identification of superior genotypes are indispensable. Earlier, only morphological markers were used to assess the genetic diversity and for the identification of superior genotypes. Though they are important for initial genetic evaluation studies, morphological characters may change with environmental conditions. The diversity analysis of the 37 *C. forskohlii* genotypes by using morphological markers did not reveal any clear understanding (Kavitha *et al,* 2007). Hence, markers based on differences in DNA sequences between individuals, generally detecting more polymorphisms than morphological markers are preferred (Bostein *et al,* 1980 and Tanksley *et al,* 1989). Among the DNA-based markers, Randomly Amplified Polymorphic DNA (RAPD) provides excellent tool to study the genetic diversity and genetic relationship and to eliminate duplicates in germplasm (Virk *et al,* 1995).

RAPD technique has been successfully used to genetically profile many different medicinal plant species. The present work was taken up to systematically characterize the *C. forskohlii* germplasm collection by RAPD markers and this is probably the first attempt to estimate genetic diversity in *C. forskohlii* at molecular level.

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MATERIAL AND METHODS

Plant material

Thirty seven genotypes collected from different *Coleus* growing regions of Tamil Nadu and Karnataka and maintained in the Botanical Garden of Tamil Nadu Agricultural University, Coimbatore were taken for the diversity analysis (Table 1).

DNA isolation and PCR amplification

DNA was isolated from 40 mg of young leaf tissue following the protocol of Wilkie (1997). Mercaptoethanol (1%) and Polyvinyl Pyrrolidone (PVP) (2%) were added to the extraction buffer to remove the phenolics contaminants. To check the quality and quantity of the isolated genomic DNA, gel electrophoresis was carried out on 0.8% agarose gel. DNA concentration for PCR amplification was estimated by comparing the band intensity of a sample with the band intensities of known dilutions that gave good amplifications. The dilutions were carried out by dissolving the genomic DNA in appropriate quantity of TE buffer (pH 8.0).

DNA from the 37 genotypes of *C. forskohlii* was amplified in PCR using a set of 25 arbitrary oligonucleotide decamer primers (Operon Technologies, Alameda, California, USA). 15 ìl reactions containing 10-20 ng of

Table 1. Morphological description of *Coleus forskohlii* genotypes for genetic diversity analysis

Genotype	Salient features
CF 1	Sub-erect growth, glabrous stem, pale green round small leaves, pale purple flowers, sparse roots
CF 2	Sub-erect growth, glabrous stem, dark green round large leaves, pale purple flowers, dense roots
CF 3	Sub-erect growth, medium pubescence, dark green leaves, violet flowers, sparse roots
CF 4	Sub-erect growth, sparse pubescence, pale green leaves, violet flowers, sparse roots
CF 5	Sub-erect growth, sparse pubescence, pale green large leaves, violet flowers, sparse roots
CF 6	Erect growth, dense pubescence, pale green small leaves, violet flowers, dense fibrous roots
CF 7	Sub-erect growth, sparse pubescence, dark green large leaves, violet flowers, dense fibrous roots
CF 8	Sub-erect growth, sparse pubescence, pale green leaves, violet flowers, sparse roots
CF 9	Sub-erect growth, medium pubescence, pale green small leaves, violet flowers, dense fibrous roots
CF 10	Erect growth, sparse pubescence, pale green large leaves, violet flowers, sparse fibrous roots
CF 11	Erect growth, medium pubescence, pale green large leaves, violet flowers, sparse fibrous roots
CF 12	Sub-erect growth, sparse pubescence, pale green large leaves, violet flowers, sparse fibrous roots
CF 13	Erect growth, dense pubescence, pale green small leaves, violet flowers, dense fibrous roots
CF 14	Sub-erect growth, sparse pubescence, pale green large leaves, violet flowers, sparse fibrous roots
CF 15	Sub-erect, medium pubescence, dark green small leaves, lilac flowers, small tubers
CF 16	Sub-erect growth, sparse pubescence, pale green leaves, violet flowers, sparse fibrous roots
CF 17	Erect growth, medium pubescence, pale green small leaves, violet flowers, dense fibrous roots
CF 18	Erect growth, sparse pubescence, pale green leaves, violet flowers, sparse fibrous roots
CF 19	Sub-erect growth, medium pubescence, variegated leaves, non flowering, small tubers
CF 20	Sub-erect growth, dense pubescence, pale green large leaves, non flowering, tuberous roots
CF 21	Erect growth, dense pubescence, dark green small densely packed leaves, non flowering, tuberous roots
CF 22	Erect, sparse pubescence, dark green small leaves, non flowering, tuberous roots
CF 23	Very erect growth, medium pubescence, pale green small leaves, violet flower, dense fibrous roots
CF 24	Sub-erect growth, dense pubescence, dark green small densely packed leaves, non flowering, tuberous roots
CF 25	Erect growth, medium pubescence, pale green small densely packed leaves, non flowering, tuberous
CF 26	Sub-erect growth, dense pubescence, dark green small leaves, non flowering, tuberous roots
CF 27	Sub-erect, sparse pubescence, pale green large leaves, non flowering, tuberous roots
CF 28	Erect growth, dense pubescence, pale green leaves, non flowering, tuberous roots
CF 29	Sub-erect growth, dense pubescence, pale green leaves, non flowering, tuberous roots
CF 30	Erect growth, sparse pubescence, pale green leaves, non flowering, tuberous roots
CF 31	Erect growth, dense pubescence, dark green leaves, non flowering, tuberous roots
CF 32	Sub-erect growth, dense pubescence, dark green leaves, non flowering, tuberous roots
CF 33	Erect, sparse pubescence, pale green large leaves, non flowering, tuberous roots
CF 34	Sub-erect, medium pubescence, pale green leaves, non flowering, tuberous roots
CF 35	Sub-erect, dense pubescence, pale green leaves, non flowering, tuberous roots
CF 36	Sub-erect growth, medium pubescence, pale green leaves, non flowering, tuberous roots
CF 37	Sub-erect growth, dense pubescence, pale green leaves, non flowering, tuberous roots

genomic DNA, 1.5 mM of assay buffer. 0.5 mM each of dATP, dTTP, dGTP and dCTP, 1.5 ìM of primer, 0.25 mM $MgCl_2$ and 0.03 units of Taq DNA polymerase (Bangalore Genei Pvt Ltd, Bangalore). Amplifications were performed in PTC Thermal Cycler (MJ Research Inc.,) programmed for an initial denaturation at 94º C for 5 min., 44 cycles of 1 min. denaturation at 94ºC, 1 min. annealing at 37º C and 2 min. extension at 72ºC and a final extension of 10 min. at 72ºC and then at 4ºC till storage. PCR amplified products (15ìl) were subjected to electrophoresis in a 1.2 % agarose gel in 1X TBE buffer at 100 volts for 3.5h using Aplex submarine electrophoresis unit. The ethidium bromide stained gels were documented using Alpha Imager TM 1200 – Documentation and Analysis system (Alpha Innotech Corportion, USA).

Data analysis

RAPD bands were scored by considering only the clear and unambiguous bands. Markers were scored for the presence and absence of the corresponding band among the different genotypes. The scores '1' and '0' were given for the presence and absence of bands, respectively. Polymorphism information content (PIC) or expected heterozygosity scores for each RAPD marker were calculated based on the formula $Hn = 1- \text{“}pi^2$, where *pi* is the allele frequency for the i^{th} allele (Nei, 1973).

The data obtained for RAPD profiling was subjected to cluster analysis. Similarity matrix was constructed using Jaccard's coefficient for RAPD profiling and the similarity values were used for cluster analysis. Sequential agglomerative hierarchical non – overlapping (SAHN) clustering was done using unweighted pair group methodology with arithmetic averages (UPGMA) and data analysis was done using NTSYSpc version 2.02 (Rohlf, 1994).

RESULTS AND DISCUSSION

In the present study, *C. forskohlii* germplasm collection was assessed for diversity at DNA level, to define its core diversity and identify individual genotypes. As the first step, the RAPD analysis was carried out using a set of 25 decamer random primers for DNA amplifications through PCR. This profiling showed only 16 out of the 25 primers yielding unambiguous markers detectable as distinct bands. In the subsequent analysis, all the 16 primers produced polymorphic bands, a total of 117 distinct bands from 37 DNA templates of different genotypes, among which 60 (51.28%) bands were polymorphic (Table 2, Fig 1 and 2). The range of polymorphic bands in *C. forskohlii* genotypes was 1-7. The average number of bands per primer was 3.75. There was no genotype specific product. RAPD data from the 16 primers was used for cluster analysis. The PIC values ranged between 0.06 and 0.42 (Table 2). The mean PIC score for all loci was 0.24. The mean PIC score was greater than 0.24 for 50% of the RAPD primers. The PIC value provides an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at a locus but also the relative frequencies of these alleles.

Cluster analysis was performed on Jaccard's similarity coefficient matrices calculated from RAPD markers to generate a dendrogram of 37 genotypes of *C.*

Table 2. Primers used for RAPD analysis and outcome from PCR amplification

S.No.	Primer Name	Primer sequence	Polymorphic bands	Total number of bands	% polymorphism	PIC
1	OPZ 01	5'-TCTGTGCCAC-3'	4	10	40.00	0.38
2	OPZ 02	5'-CCTACGGGGA-3'	2	6	33.33	0.16
3	OPZ 03	5'-CAGCACCGCA-3'	6	8	75.00	0.24
4	OPZ 04	5'-AGGCTGTGCT-3'	3	8	37.50	0.14
5	OPZ 05	5'-TCCCATGCTG-3'	4	5	80.00	0.25
6	OPZ 06	5'-GTGCCGTTCA-3'	2	7	28.57	0.41
7	OPZ 07	5'-CCAGGAGGAC-3'	4	11	36.36	0.28
8	OPZ 08	5'-GGGTGGGTAA-3'	1	5	20.00	0.06
9	OPZ 09	5'-CACCCCAGTC-3'	4	7	57.14	0.42
10	OPZ 10	5'-CCGACAAACC-3'	3	6	50.00	0.17
11	OPZ 11	5'-CTCAGTCGCA-3'	7	12	58.33	0.29
12	OPZ 12	5'-TCAACGGGAC-3'	5	8	62.50	0.33
13	OPZ 13	5'-GACTAAGCCC-3'	7	9	77.77	0.18
14	OPZ 15	5'-CAGGGCTTTC-3'	2	5	40.00	0.07
15	OPZ 16	5'-TCCCCATCAC-3'	1	4	25.00	0.10
16	OPAW03	5'-CCATGCGGAG-3'	5	6	83.33	0.37
Mean			60	117	51.28	0.24

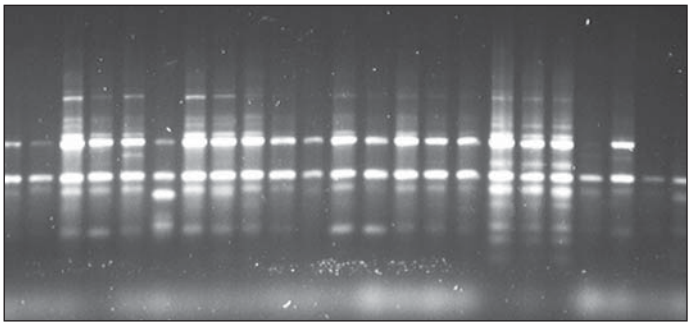

Fig 1. A section of RAPD profile of *C. forskohlii* genotypes for primer OPZ 11

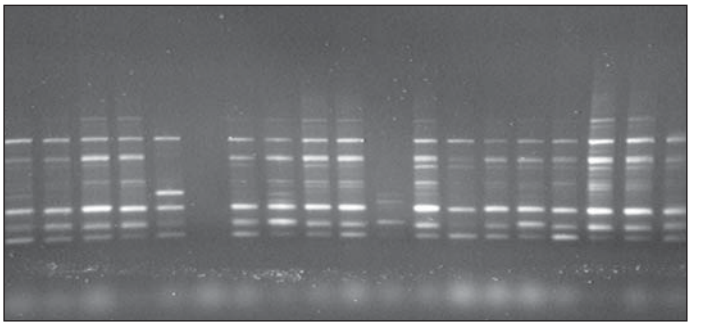

Fig 2. A section of RAPD profile of *C. forskohlii* genotypes for primer OPZ 13

forskohlii (Fig. 3). All the genotypes could be placed in the range of 0.07 to 0.97 of similarity indices.

The dendrogram, based on the similarity index, is indicative of considerable level of polymorphism among the genotypes at DNA level. The dendrogram obtained through cluster analysis revealed two major clusters. The dendrogram clearly depicted the variability present in the *C. forskohlii* germplasm and it separated 37 genotypes into two major clusters. The first cluster consisted of two genotypes CF 1 and CF 2 which were distinctly different from the rest of genotypes by possessing round shaped leaves and being non tuberous in nature. The second cluster enclosed the remaining 35 genotypes and was again grouped into two sub clusters. The sub cluster I consisted of tuberous and non tuberous genotypes whereas the sub cluster II consisted of only tuberous genotypes. The genotypes in these sub clusters were also again divided into sub sub clusters and there was not a clear discrimination between the genotypes. The reason might be the gene responsible for the tuber bearing habit might not have been amplified with the primers used for the study. Moreover, higher percentage of similarity of almost 50 per cent among the genotypes could be attributed to the lot of missing data points

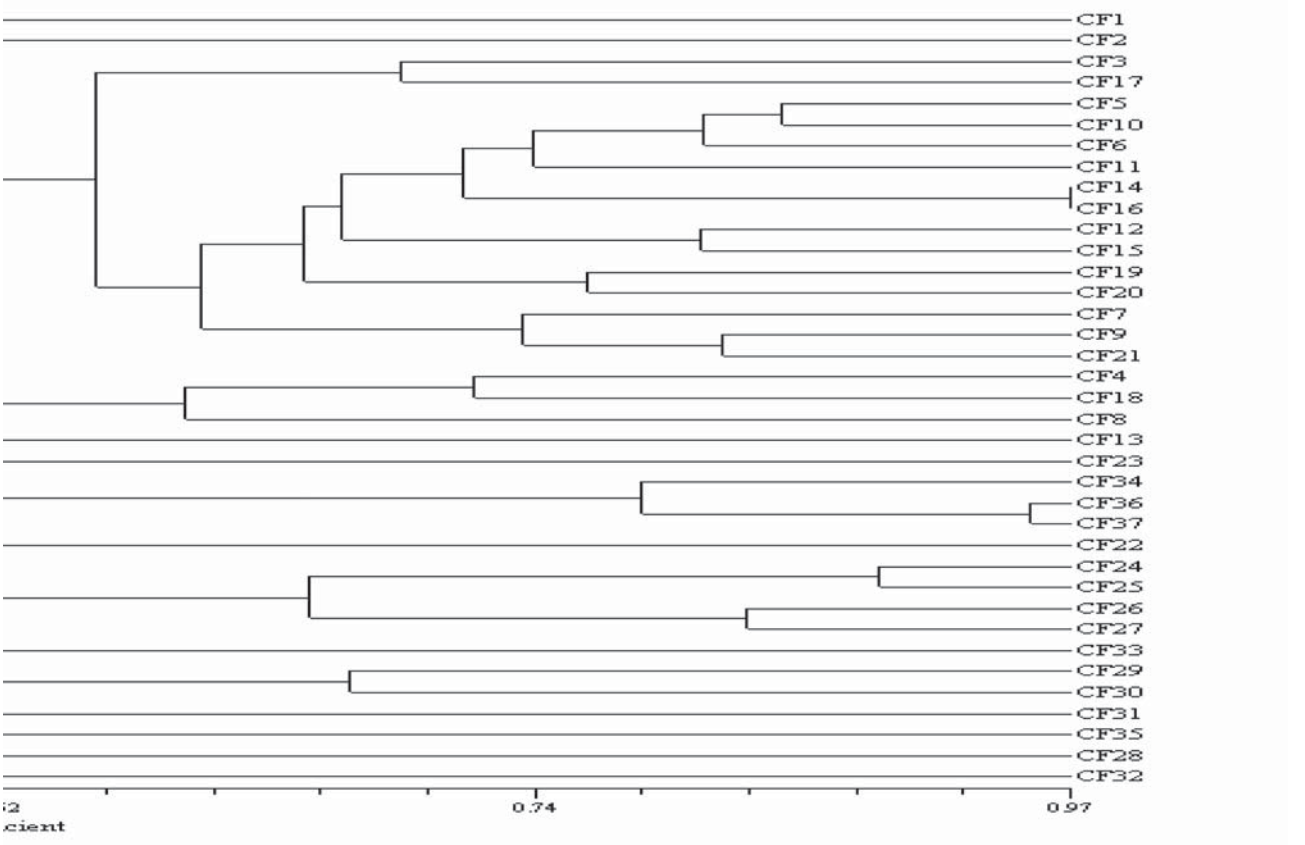

Fig 3. Dendrogram of 37 *Coleus forskohlii* genotypes based on 60 RAPD markers constructed using UPGMA based on Jaccard's coefficient

as the number of primers and amplified products analysed were relatively less. Similar report in *Andrographis paniculata* was also reported (Padmesh *et al,* 1999).

Pejie *et al* (1998) reported that 150 polymorphic bands make possibly a reliable estimate of genetic similarities among genotypes within the same species. In the present study, a total of 60 distinct bands were produced by the 16 primers out of 25 primers used. Hence, it is suggested that further genetic diversity analysis in *C. forskohlii* with more number of primers for RAPD or the advanced marker system producing a large number of informative polymorphic markers per primer pair that are highly reliable and reproducible (Mueller and Wolfenbarger, 1999 ; Mullis *et al,* 1986) has to be attempted.

Although the data presented here are not conclusive to infer the phenetic relationship between the various genotypes, they reflect the utility of RAPD in the analysis of genetic variability distribution within this important indigenous medicinal plant. This is probably the first report which deals with the analysis of genetic diversity at the molecular level in *C. forskohlii*.

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