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Full Length Research Paper

Generic relationship among *Cassia* L., *Senna* Mill. and *Chamaecrista* Moench using RAPD markers

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Generic relationships were examined among twenty-four species belonging to genus *Cassia* L., *Senna* Mill. and *Chamaecrista* Moench using RAPD marker. Total 80 primers were initially screened, 514 amplification products obtained with 38 informative primers, of which 514 were polymorphic. A vary high degree of polymorphism (100%) was observed among them. UPGMA cluster analysis of genetic similarity indices grouped all the species into three major clusters. Cluster I included four species of *Cassia* L., Cluster II included eighteen species of *Senna* Mill. and Cluster III included two species of *C.* Moench. Highest similarity (0.9%) was observed between *Cassia fistula* L. and *Cassia fistula* with nodded filaments and least (0.001%) between *Cassia fistula* L. and *Senna splendida*. The Polymorphic information contents (PIC) of the twenty-four species with RAPD marker varied from 0.08 to 0.49 with an average of 0.005. The result confirms the statement of Irwin and Barneby, they divided the genus *Cassia* L. into three subgenera; *Cassia* L., *Senna* Mill. and *C.* Moench on the basis of morphological characters. The results obtained from the present study support the previous taxonomic classification of the genus *Cassia* L. and showed large diversity among the species of three newly created genera. Our results suggested that RAPD marker is a sensitive, precise and efficient tool for genomic analysis of Cassia L. that may be useful in future studies by assigning new unclassified germplasm to specific taxonomic groups and reclassify previously classified species and genera.

Key words: *Cassia, Senna*, *Chamaecrista*, genetic relationship, RAPD**.**

INTRODUCTION

Cassia L. is the largest genus in the subfamily Caesalpiniodeae of the Caesalpinioceae. It contains about 600 flowering species which are distributed in most continents (Singh, 2001). *Cassia* L. species show large diversity related to habit, ranging from delicate, annual herbs to tall trees. Bentham (1871) divided the genus *Cassia* into three genera and nine sections. Britton and Rose (1930) splited the genus *Cassia* into twenty-eight genera. Recently Irwin and Barneby (1982) splited the genus *Cassia* L. into three subgenera; *Cassia* L., *Senna* Mill. and *Chamaecrista* Moench. Irwin and Baneby (1981 to 1882) realizing diversity and complexity and proposed an improved classification proposing new delimitation based on persistent suit of characters. They have raised the genus *Cassia* L. to the levels of subtribe (Cassiinae) and raised the subgenera *Senna* Mill. and *C.* Moench to genetic level.

The plants of *Cassia* L. are used as fodder, purgatives, timber and medicine (Tomlinson, 1981; Tiwari, 1983). The taxonomy and nomenclature of *Cassia* L. species are quite complex and intriguing. They are not easily differentiated from closely related species due to the large variation in similarities range causing mis-identification and misinterpretation of the components. The cultivars identification and assessing of diversity using phenotypic markers have several limitations especially in perennial crops (reference). In plants as general, due to the overlapping of morphological characters, a great amount of confusion persists for the selection of ideal plant. Generally morphological characters are influenced by environmental changes, and the changes are not constant in the species to species and differ from place to place.

Structural changes in DNA, that is, translocation, deletion, inversion mutation have been able to

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Table 1. Collected *Cassia, Senna* and *Chamaecrista* species, accessions no. and their location.

S/N	Accessions no.	Accessions code.	Accessions name	Location
1	200382	Cf1	*C. fistula*	Utterkashi (Uttranchal)
2	200390	Cf2	*C. fistula* (white)	NBRI, Lucknow India
3	200435	Cj	*C. javanica*	NBRI, Lucknow India
4	200436	Cr	*C. roxburghii*	BSI Jodhpur (Rajasthan) India
5	200437	St	*S. tora*	Kanpur (U.P) India
6	200438	So	*S. obtusifolia*	Unnao (U.P) India
7	200439	Su	*S. uniflora*	Karnataka university Dharwad India
8	200440	Sb	*S. biflora*	Lucknow (U.P) India
9	200441	Ss	*S. sophera*	Karnataka university Dharwad India
10	200442	Sp	*S. purpurea*	Metha (U.P) India
11	200443	Ss1	*S. spetabilis*	Karnataka collage Dharwad India
12	200444	Se	*S. excelsa*	NBRI, Lucknow India
13	200415	Ss2	*S. sulfurea*	Tehari garhwal (Uttranchal) India
14	200432	Ss3	*S. surattensis*	Aligarh (U.P) India
15	200447	Ss4	*S. splendida*	Madgavo Karnataka India
16	200448	So1	*S. occidantalis*	Bhopal (M.P) India
17	200449	Sa	*S. auriculata*	BSI Jodhpur (Rajasthan) India
18	200450	Ss5	*S. siamea*	Faizabad (U.P) India
19	200451	Si	*S. italica*	BSI Jodhpur (Rajasthan) India
20	200452	Sa1	*S. alexandrina*	CIMAP Lucknow
21	200453	Sh	*S. hirsuta*	Karnataka collage Dharwad India
22	200454	Sa2	*S. alata*	Lucknow (U.P)
23	200455	Chm	*C. mimosoides*	K.U Dharwad India
24	200456	Cha	*C. absus*	K.U Dharwad India

change the genomic constitution of species. In decades, these changes in the genome of species, separate the particular species from their ancestors. To assess these changes, parameters based on morphological characters are not sufficient. Now it is possible to single out differences on the basis of molecular markers, which is authentic and less affected by environmental factors. Hence characterization of species at the genetic level supplemented efficient conservation, maintenance and utilization of the existing genetic diversity.

In the present study, RAPD markers were used to assess genetic relationship among the species of *Cassia*, *Senna* and *Chamaecrista* by using a single arbitrary primer (10-mer) and amplifying DNA by polymerase chain reaction (PCR) as the resulting DNA marker can be easily separated on an agarose gel by electrophoresis (Williams et al., 1990). The advantage of RAPD is its simplicity, rapidity and requirement for only a small quantity of DNA, and the ability to generate numerous polymorphism (Stebbins, 1957; Cheng et al., 1997; Khanuja et al., 1998).

MATERIALS AND METHODS

Plant materials

Twenty-four species belongs to three genera; *Cassia*, *Senna* and *Chamaecrista* were collected from different ecological locations of Uttrakhand, Uttar Pradesh, Madhya Pradesh, Karnataka, Rajasthan and Himachal Pradesh, India (Table 1). Out of which some species are cultivated due to the medicinal and ornamental properties and some are wild.

Preparation of genomic DNA

Total genomic DNA from the leaves of *Cassia, Senna* and *Chamaecrista* species was extracted using the protocol proposed by Doyle and Doyle (1987) with slight modification. 1 g of leaves around to powder with liquid nitrogen in a morter and pestle, then transferred to a 30 ml centrifuge tube containing 12 ml of CTAB buffer (2% CTAB, 100 mM Tris-Cl, 20 mM EDTA and 1.4 M NaCl) and 120 µl β-mercaptoethanol mixed thoroughly and incubated in water bath at 68°C for 3 h. The tube was cool at room temperature, mix with equal volume of chloroform and tube was gently inverted repeatedly. The tube was centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to a corex tube and 0.7% volume of 2-propanol was added. Tube was put at -20°C for 2 h. The tube was centrifuged at 12000 rpm 10 min at 4°C to collect precipitated DNA. The pellet was resuspended with 700 µl TE (10 mM, Tris-Cl pH-8.0, 1 mM EDTA) and incubated with 2 µl (10 mM DNase free RNase A) for 40 min at 37°C. The RNase A and the remaining protein were extracted with equal volume of phenol: chloroform 1:1 and centrifuged at 12000 rpm for 15 min at room temperature. The supernatant was transferred to a new tube and the DNA was precipitated by the addition of 0.7 volume of 2-propanol. Precipitated DNA was collected by centrifugation at 12000 rpm for 15 min at 4°C washed with 70% ethanol twice and dried before redissolving in 100 µl of TE. DNA yield were calculated by

fluorometer (DyNa Quant ^{200}) (Amersham pharmacia Biotech, USA).

RAPD reactions

Sixty-eight decamer Oligonucleotide primers (Operon, USA) were screened by polymerase chain reaction (PCR). PCR reactions were performed by using a 20 µl mixture, conataing 50 ng template (genomic DNA), dNTPs (0.2 mM each), 0.5 mM primer, 1.5 U Taq DNA polymerase, 25 mM $MgCl_2$ and remain Mili Q water. The DNA thermocycler (Applied Biosystem, 9700) was programmed as follow: Incubation at 94°C for 2 min, 44 cycles at 94°C for 1 min. 36°C for 1.30 min and 72°C for 1.30 min followed by electrophoresis in 1.4 % (w/v) agarose gel with 0.5 × TBE stained with diluted ethidium bromide (10 mg/ml) and photographed in gel documentation unit (AlphaImager^{TM} 3400).

Data analysis

Amplification of DNA was repeated at least three to six only reproducible and unambiguous fragment were scored as (1) for its presence or (0) for its absence. A fragment was considered polymorphic if both the presence and absence of that fragment were observed in the same species and monomorphic if it was present among all individual within a species. To reduce the possibility of comparing non-homologous bands, a positive control (an individual possessing the band to be scored) was included on each agarose. Analysis of RAPD markers was based on the following three assumptions: (1) Each RAPD marker represented a single locus comprising two alleles, a marker allele (amplified product present) and a non-marker alleles (amplified product absent). (2) RAPD marker is inherited in a dominant fashion with the marker allele dominant to the non-marker allele. (3) Co-migrating bands from different populations present homologous amplified products (Allan et al., 1997; Hadrys et al., 1992).

The genetic associations among species were evaluated by calculating the Jaccard similarity coefficient for pair-wise comparisons based on the proportion of shared bands (alleles) produced by primer. Similarity matrices were generated using 'Simqual subprogram, similarity coefficients were used for cluster analysis of accessions performed using the 'SHAN' sub program, dendrogram was built by the un-weighted pair group method with arithmetic average (UPGMA). The computer program used was NTSYS-pc Version 2.02 (Rohlf, 1998).

The polymorphic information content (PIC) was calculated by applying the formula given by Powell et al. (1996) and Smith et al. (1997).

$$PIC = 1-\sum_{i=1}^{n} f_i^2$$

where f i is the frequency of the ith alleles and the summation extends over n alleles.

RESULTS

A total 80 primers were initially screened among twenty-four species belonging to *Cassia*, *Senna* and *Chamaecrista*. Out of these 80 primers, 38 primers exhibited amplification pattern with all the species. Examples of amplification pattern of these species with primer OPAP-17, OPAP-16 and C-19 are shown in Figure 1. A total 514 bands were scored from PCR amplification of genomic DNA with all the species. The RAPD markers showed 100% polymorphism (Table 2). Homology of the RAPD bands with the same molecular weight was confirmed using EcoRI and Hind III digested marker. Average number of 14 bands was obtained per primer and amplification product ranged in size from 100 bp to 4.2 kb. Maximum numbers of 21 amplification products were obtained with primer OPC-19 followed by 20 products with primer OPAP-1 and OPAP-20. Minimum numbers of RAPD products were generated with primers OPAP-1, OPAP-17 and OPAP-16. The polymorphic information contents (PIC) ranged from 0.08 to 0.49 with an average of 0.005.

Genetic relationship measured through analysis of RAPD data of twenty-four species of *Cassia*, *Senna* and *Chamaecrista*. Highest similarity (0.9%) was observed between *Cassia fistula* L. and *Cassia fistula* with nodded filaments and least (0.001%) between *C. fistula* L. and *Senna splendida* (Table 3). Dendrogram showed that RAPD markers distinguished all species in three major groups (Figure 2). Cluster I included four species of the genus *Cassia* (*C. fistula* L., with nodded filament, *Cassia javanica* L, *Cassia roxburghii*). Cluster II included eighteen species belong to genus *Senna* (*Senna tora* L., *Senna obtusifolia* L., *Senna uniflora* mill., *Senna biflora, Senna sophera* L., *Senna purpurea*, *Senna spetabilis*, *Senna spetabilis* var. *excelsa*, *Senna sulfurea* DC, *Senna surattensis* Burm.f., *Senna splendida*, *Senna occidantalis* L., *Senna auriculata*, *Senna siamea* L., *Senna hirsuta* L., *Senna italica*, *Senna alata* and *Senna alexandrina*, and cluster III included two species of the genus *Chamaecrista* (*Chamaecrista absus* and *Chamaecrista mimosoides*).

DISCUSSION

When dealing with morphologically similar taxa, study of molecular characters has exemplified a more definitive approach than morphological observations. Among the methods used in such studies, DNA markers have proved to be an excellent parameter to resolve the problems of identification of critical taxa and to understand their relationships and taxonomic status (Esen and Hilu, 1991; Khan, 1992). The groups of natural populations produced distinct group than other groups (Mayer, 1970). The reorganization and appreciation of the dynamic variations in the genus inter and intra-specific levels necessitate thecharacterization of genetic variation in order to determine the genetic base and phylogenetic status of its species.

Genus *Cassia* was considered to consist of three subgenera (Linnaeus, 1754) but recently, each of these has been raised to the generic level. Classification into these groups had often been confused, because of the absence of a clearly defined set of taxonomic characters.

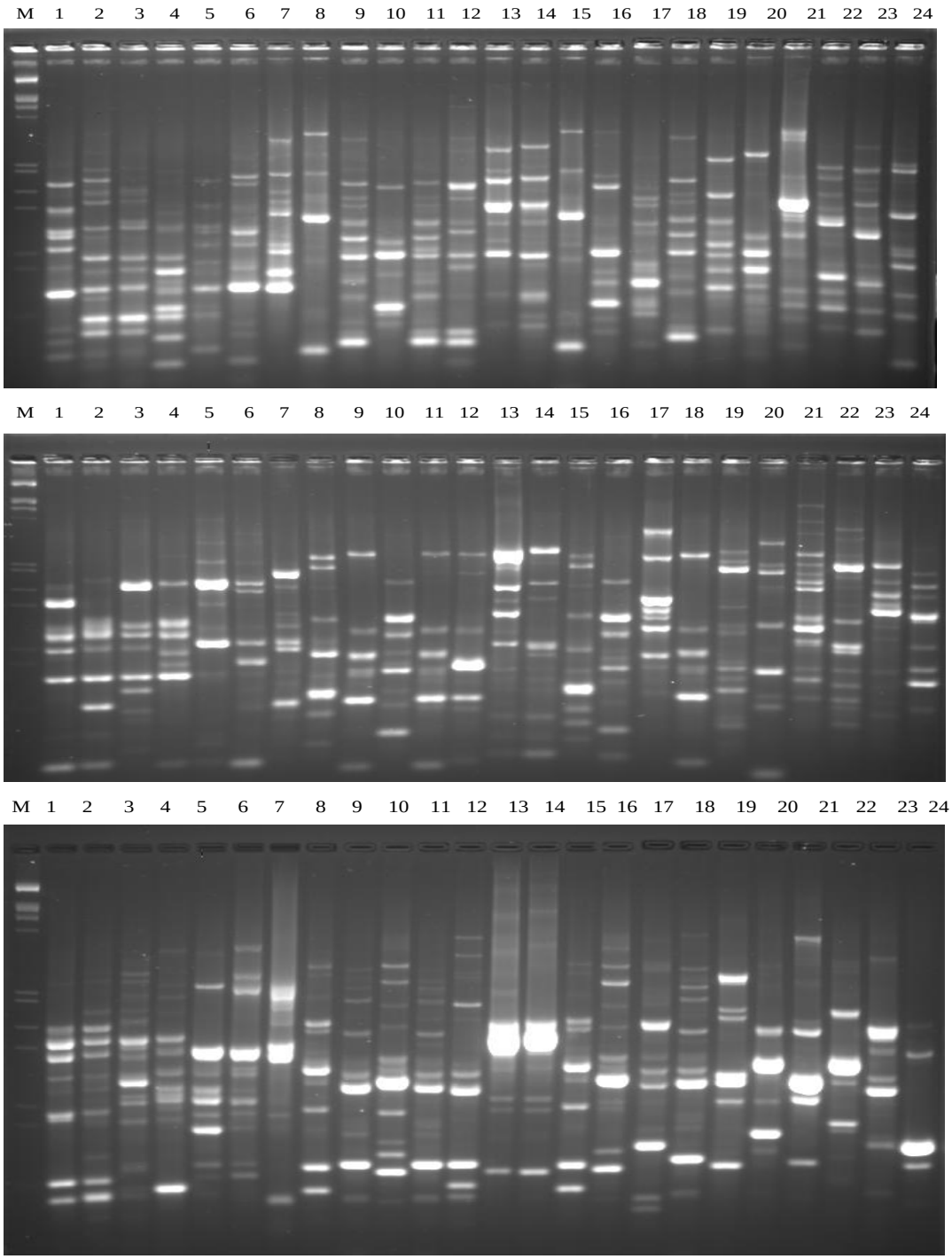

Figure 1. Lane-M Eco RI and Hind III digested λ DNA. Lane-1-24: RAPD profile of *Cassia, Senna* and *Chamaecrista* with primers OP AP-17 (Upper), OP AP-16 (Middle) and OPC-19 (Lower).

Irwin and Barneby (1982) described a suite of characteristics which members of the Cassiinae, could be consistently classified into one of the three genera; *Cassia* L., *Senna* sensu stricto and *C.* Moench. Initially, *Chamaecrista* separated from *Senna* and *Cassia* using characters relating to the androcium. Subsequently separation of *Cassia* and *Senna* involves consideration of a number of floral characters.

Todaria et al. (1983) had investigated the electrophoresis protein profile of nodulated and non-nodulated *Cassia* species (*C. fistula*, *C. occidentalis*, *C. tora*, *C. laevigata, C. glauca, C. absus* and C. *dimidiala*)

Table 2. Analysis of polymorphism among species of *Cassia, Senna* and *Chamaecrista* obtained with random primers

Primer no.	Total no. of amplicon	Total no. of bands	Polymorphic bands	Monomorphic bands	PIC value	Average	Average no of bands	Size range of amplified product (bp)
AP-1	146	20	20	0	0.30	0.16 -0.49	6	200-2500
AP-2	53	11	11	0	0.13	0.16-0.48	2.2	100-990
AP-8	80	17	17	0	0.20	0.08-0.47	3.3	100-2700
AP-9	79	14	14	0	0.21	0.16-0.48	3.2	100-3000
AP-12	56	12	12	0	0.14	0.08-0.4	2.3	200-1900
AP-14	95	19	19	0	0.26	0.16-0.49	3.9	250-3530
AP-15	40	13	13	0	0.11	0.08-0.37	1.6	200-1500
AP-16	118	17	17	0	0.23	0.08-0.49	4.9	300-1900
AP-17	126	16	16	0	0.49	0-0.26	5.2	250-4268
AP-20	119	20	20	0	0.26	0.08-0.48	4.9	100-3500
C-6	56	13	13	0	0.13	0.08-0.47	2.3	100-800
C-8	81	17	17	0	0.21	.08-0.45	3.3	300-1000
C-9	62	13	13	0	0.13	0.08-0.48	2.5	200-1500
C-11	47	15	15	0	0.13	.08-0.47	1.9	250-1500
C-12	62	14	14	0	0.16	0.08-0.48	2.5	100-3100
C-13	57	11	11	0	0.09	.08-0.33	2.3	100-1900
C-14	93	16	16	0	0.22	0.08-0.48	3.8	250-3100
C-16	53	16	16	0	0.13	0.08-0.49	2.2	100-1700
C-18	78	18	18	0	0.20	0.16-0.48	3.2	100-2400
C-19	145	21	21	0	0.3	0.08-0.49	6	150-2100
B-5	69	14	14	0	0.13	0.08-0.48	2.8	210-2000
B-8	88	17	17	0	0.21	0.08-0.48	3.6	210-2000
B-15	68	15	15	0	0.16	0.08-0.47	2.8	180-1300
B-18	59	14	14	0	0.13	0.08-0.45	2.4	250-1500
B-19	86	17	17	0	0.21	0.4-0.5	3.5	100-3530
B-20	96	15	15	0	0.23	0.22-0.48	4	150-3530
U-1	52	15	15	0	0.24	0.08-0.48	2.1	100-1900
U-2	52	15	15	0	0.19	0.08-0.49	3.1	200-2000
U-3	80	16	16	0	0.19	0.08-0.47	3.3	100-3500
U-4	43	14	14	0	0.11	0.08-0.45	1.7	250-1500
U-7	50	14	14	0	0.12	0.08-0.47	2	150-1200
U-15	84	17	17	0	0.18	0.08-0.47	3.5	200-2500
U-16	54	12	12	0	0.14	0.16-0.49	2.2	250-2500
U-17	98	16	16	0	0.08	0.03-0.47	4	150-1700
35	2648	514	514	0	0.188		Min. 1.6 Max.6	

and agreed their placement according to the Bentham and Hooker system. Whitty et al. (1994) used RAPD as molecular marker for examining four *Cassia* species, 11 *Chamaecrista* species and 14 *Senna* species including *C. glauca* for separation of the nodulated nitrogen fixing genus *Chamaecrista* from the previously congeneric group *Cassia* and *Senna.* Gareeb et al. (1999) discussed seed protein profile, chromosome number and morphological characters between ten species of genus *Cassia*. According to numerical cluster analysis, the studied taxa were splited into two groups. Group I (belonging to subgenus *fistula*) includes three *Cassia* spp. (*C. fistula, C. javanica,* and *C. nodosa*), while *C. occidentalis, C. sophera*, *C. siamea*, *C. didymobotrya, C. italica*, *C. senna* and *C. surattensis* are included in group II (belonging to subgenus *Senna*). Mondal et al. (2000) evaluated interspecific variation among eight species of *Cassia* L. on the basis of the free amino acid composition, SDS-polyacrylamide gel electrophoresis of total seed protein and mitochondrial DNA restriction fragment length polymorphism to understand their phylogenetic relationships and grouped the eight species into two clusters; clusters 1 consist *C. occidentalis, C. sophera*, *C. mimosoides* and *C. tora,* and clusters 2

Table 3. similarity indices (Jaccards coefficient of 24 species of genus *Cassia, Senna* and *Chamaecrista* obtained with 38 primers)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1																							
2	0.9	1																						
3	0.262	0.042	1																					
4	0.315	0.019	0.163	1																				
5	0.082	0.121	0.156	0.141	1																			
6	0.131	0.111	0.146	0.165	0.065	1																		
7	0.361	0.013	0.161	0.198	0.192	0.214	1																	
8	0.011	0.091	0.057	0.152	0.097	0.235	0.15	1																
9	0.082	0.022	0.131	0.112	0.521	0.174	0.1	0.097	1															
10	0.041	0.091	0.048	0.005	0.061	0.077	0.14	0.114	0.052	1														
11	0.011	0.086	0.125	0.225	0.028	0.036	0.09	0.124	0.981	0.088	1													
12	0.096	0.023	0.005	0.068	0.022	0.029	0.08	0.092	0.023	0.063	0.18	1												
13	0.069	0.059	0.215	0.153	0.091	0.071	0.12	0.034	0.052	0.029	0.17	0.165	1											
14	0.126	0.098	0.031	0.181	0.048	0.142	0.14	0.042	0.109	0.127	0.18	0.178	0.072	1										
15	0.001	0.001	0.051	0.071	0.052	0.097	0.16	0.092	0.071	0.022	0.18	0.147	0.042	0.062	1									
16	0.031	0.047	0.108	0.024	0.048	0.232	0.66	0.035	0.074	0.121	0.08	0.141	0.035	0.016	0.094	1								
17	0.136	0.022	0.125	0.097	0.102	0.209	0.12	0.105	0.019	0.081	0.12	0.051	0.017	0.072	0.134	0.086	1							
18	0.091	0.042	0.162	0.098	0.071	0.128	0.63	0.168	0.061	0.616	0.93	0.155	0.021	0.071	0.101	0.126	0.03	1						
19	0.178	0.037	0.178	0.071	0.972	0.222	0.44	0.156	0.011	0.141	0.12	0.121	0.037	0.055	0.156	0.049	0.017	0.013	1					
20	0.155	0.001	0.149	0.117	0.032	0.292	0.07	0.121	0.009	0.109	0.14	0.151	0.109	0.047	0.074	0.021	0.028	0.062	0.013	0.054	1			
21	0.117	0.108	0.031	0.015	0.036	0.123	0.23	0.063	0.015	0.141	0.11	0.091	0.053	0.116	0.105	0.023	0.091	0.094	0.028	0.052	0.026	1		
22	0.082	0.032	0.188	0.176	0.053	0.194	0.14	0.081	0.137	0.038	0.16	0.104	0.481	0.112	0.085	0.047	0.081	0.034	0.038	0.061	0.291	0.273	1	
23	0.271	0.081	0.062	0.159	0.081	0.112	0.06	0.025	0.109	0.189	0.12	0.041	0.215	0.673	0.053	0.017	0.084	0.045	0.031	0.114	0.262	0.319	0.069	1
24	0.091	0.037	0.163	0.132	0.045	0.237	0.13	0.109	0.074	0.138	0.62	0.011	0.583	0.044	0.071	0.036	0.115	0.188	0.351	0.512	0.254	0.283	0.091	0.183

consist *C. alata, C. siamea, C. fistula* and *C. renigera.* RAPD markers have found wide application due to the easiness of performing the assay and also for the reason of being less time/labor consuming and less expensive. However, some doubts have also been raised

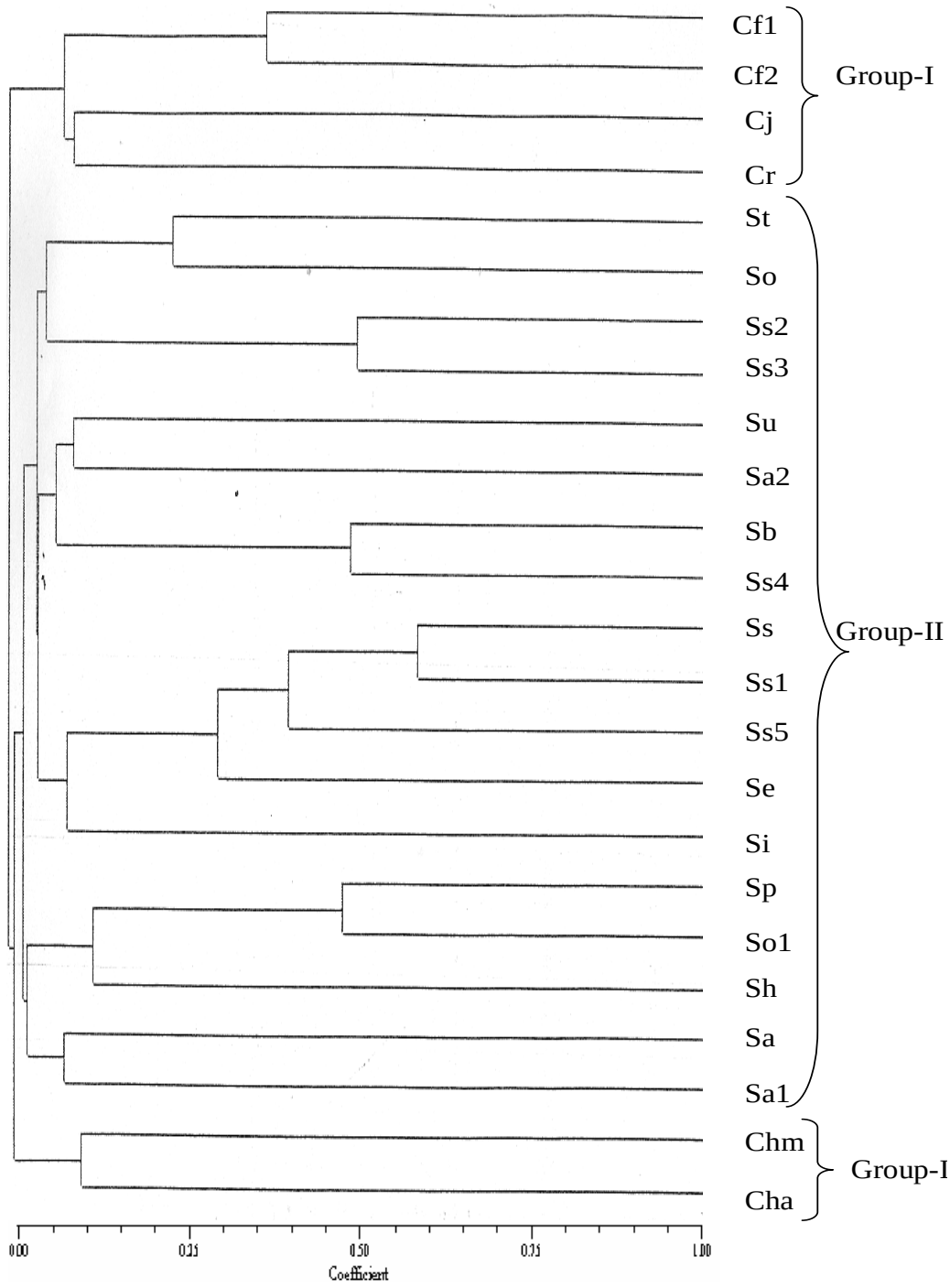

Figure 2. Dendrogram showing the relationship among different species of *Cassica, Senna and Chaemaecrisia* based on UPGMA AND sequential agglomerative hierarchical nested (SHAN) clustering.

regarding the suitability of RAPD for diversity analysis. It is debated mostly that co-migrating RAPD bands may not be allelic or composed of similar sequences (Bowditch et al., 2003). On the other hand, studies in some species of *Glycine* and *Allium* have demonstrated the homology of co-migrating RAPD bands (Williams et al., 1993; Wilkie et al., 1993). Also the use of large number of polymorphic markers minimizes the skewing of results due to non-allellism (Pujar et al., 1999). Another problem often encountered and questioned regarding RAPD analysis is the reproducibility of band patterns. This problem can be solved by thoroughly optimizing PCR reaction conditions and following the same protocol each time. For more accurate analysis, reaction should be performed thrice,

scoring only those bands that are reproducible in each reaction. The intra-population genetic variations observed in heterogeneous populations of out breeding plant species complicate the analysis of genetic diversity among populations.

In the present study, RAPD patterns were generated by using genomic DNA from twenty-four species of *Cassia*, *Senna*, and *Chamaecrista*. PCR was done with random primer followed according to Williams et al. (1990). RAPD marker is able to differentiate closely related species of *Cassia*, *Senna*, and *Chamaecrista*. The study has shown RAPD analysis to be a robust and reliable method to detect intra and inter specific genetic relationship among *Cassia*, *Senna*, and *Chamaecrista.* In the case of twenty-four species of *Cassia*, *Senna* and *Chamaecrista* 80 primers were screened, out of which 38 RAPD primers were considered for the data analysis. These primers resulted reproducible profiles in the twenty-four species of *Cassia*, *Senna* and *Chamaecrista*. The high interspecific genetic divergence in species demonstrates that the level of genetic variation with in species is also substantial and suggested that genetic base is quite broad.

Dendrogram showed grouping of species into three major clusters. Cluster I grouped four species (*C. fistula*, *C. fistula* with nodulated filament, *C. javanica* and *C. roxburghii*). Cluster III grouped two species (*C. mimosoides* and *C. absus*) and remaining eighteen species (*S. tora*, *S. obtusifolia*, *S. uniflora, S. biflora*, *S. sophera, S. purpurea*, *S. spectabilis*, *S. excelsa*, *S. sulfurea*, *S. surattensis, S. splendida*, *S. occidantalis*, *S. auriculata, S. siamea*, *S. italica*, *S. alexandrina*, *S. hirsuta* and *S. alata*) were grouped in cluster II. Cluster II has divided into seven sub groups and show high closeness among the *Senna* species, *S. tora* L. grouped with *S. obtisifolia* L. *S. sulfurea* DC. ex Collad. with *S. surattensis* Burm f., *S. uniflora* with *S. alata*, *S. biflora* with *S. splendida, S. sophera* with *S. spectabilis, S. siamea* with *S. excelsa* and *S. italica*, *S. purpurea* and *S. occidentalis* with *S. hirsuta* and *S. alexandrina*. Hook (1878) considered *Senna purpurea* (Roxb. ex Lindl) is a variety of *S. sophera* L. but the present study clearly indicated that there are no grouping between *S. purpurea* and *S. sophera*. Irwin and Barneby (1982) separated *S. excelsa* and considered as a variety of *S. spectabilis* but they are not grouped together, while *S. sophera* is very close to *S. spectabilis*. Dendrogram showed clear separation of the three genera *Cassia*, *Senna* and *Chamaecrista*. The polymorphic information content (PIC) of *Cassia*, *Senna* and *Chamaecrista* with RAPD showed high level of genetic diversity. The study has revealed clear groups of three genera *Cassia*, *Senna* and *Chamaecrista* but do not show any clear pattern according to the location in which they were collected. This results is in conforimty with Irwin and Barneby (1982); they divided the genus *Cassia* into three major groups; *Cassia*, *Senna* and *Chamaecrista* on the basis of morphological criteria. This result supported the previous taxonomic classification of the genus *Cassia* L. and show great diversity among the species of *Cassia*, *Senna* and *Chamaecrista*.

From the previous report, on basis of morphological and cytological studies, twenty-four species divided into three groups (Irwin and Barneby, 1982). RAPD maker revealed high degree of polymorphism (100%) among the twenty-four species belonging to genus *Cassia*, *Senna* and *Chamaecrista*. RAPD markers have been used earlier for taxonomic and phylogenetic relationships (Demcke et al., 1992; Millan et al., 1996). The use of molecular marker can help in establishing the limits among the defined groups on a more objective basis. In the case of genus *Scaevola*, which was initially misclassified by Linnaneus in 1753 and further rearranged several times by other scientist (Bentham, 1868; Krauze, 1912; Carolin, 1992) has now been reclassified, resolving the previous confusions through RAPD analysis (Swoboda and Bhalla, 1997).

The use of RAPD analysis has also been successful for resolving phylogenetic relationship in other plant groups. For example, phylogenetic relationship investigated using RAPD analysis among the *Rosa* species accessions, proved useful in assigning unclassified accessions to specific taxonomic groups. In another case Virk et al. (1995) have analysed the germplasm collection of rice accessions by RAPD markers and classified the unclassified rice accessions as *indica* or *japonica* types. Similarily, Pipe et al. (1995) supported the separation of two groups of *Opiostoma piceae* into two species based on the clear-cut divergence revealed by RAPD maker. In a typical defining a species, that is, whether the plant *Eucalptus granticola* is a relict species or a hybrid of existing species, the RAPD data show that *E. granticola* is 40% similar to *Eucalptus rudis* and *Eucalptus drummondii* are 25% similar among themselves. This is combination with morphological data revealed that the plant *E. granticola* is a hybrid and not a relict species (Rosseto et al., 1997). RAPD analysis of Tibetan wheat, common wheat and European spelt wheat supported the previous classification of the Tibetan wheat as a subspecies of common wheat (Sun et al., 1998).

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