Plant Science Research 34 (1&2) : 79-92, 2012

Plant Science Research

ISSN 0972-8546

Phylogenetic relationships among pigeon pea (*Cajanus cajan*) and its wild relatives as revealed by RAPD and ISSR markers

A. K. Debata[1], A. K. Mukherjee[2] and P. C. Panda[1Ψ]

[1] Taxonomy & Conservation Division, Regional Plant Resource Centre, Bhubaneswar 751 015, Odisha, India

[2] Central Institute for Cotton Research, Nagpur 440010, Maharashtra, India

ARTICLE INFO

Article history:
Received : 14 November 2012
Received in revised form : 12 December 2012
Accepted : 14 December 2012

Keywords:
Pigeonpea
genetic diversity
molecular markers

ABSTRACT

The genetic relationships among 10 species of *Cajanus* (Fabaceae) and 11 accessions of *Cajanus cajan* (pigeonpea) were assessed using RAPD and ISSR markers. All the species and accessions had an average genetic similarity of 63% and several accessions of *Cajanus cajan* had more than 90% similarity among themselves. In the genus *Cajanus*, the clustering of species in the dendrogram based on molecular data supported the sectional classification of the genus proposed by van der Maesen (1986) to a large extent. While *C. cajan* and its wild progenitor *C. cajanifolius* belonging to the sect. Cajanus came in a cluster, *C. lineatus, C. sericeus* and *C. reticulatus* of the sect. Atylia formed a separate clade. Similarly, members of the sect. Volubilis (*C. crassus* and *C. mollis*) and sect. Cantharospermum (*C. scarabaeoides* and *C. albicans*) also formed distinct groups justifying the established infra-generic classification. The pigeonpea (*Cajanus cajan*) accessions of Indian and African origin got separated in the dendrogram and Indian genotypes formed clusters according to their geographical area of occurrence and cultivation. The genetic diversity and molecular phylogeny of the genus *Cajanus* and pigeonpea cultivars have been discussed in the paper.

1. Introduction:

The subtribe Cajaninae (tribe Phaseoleae) of the family Fabaceae contains a large number of agriculturally important crops and currently, 11 genera come under Cajaninae, including *Cajanus, Flemingia, Rhynchosia, Eriosema, Dunbaria* and *Paracalyx*. Though the species of *Atylosia* and *Cajanus* were relegated to two separate genera mainly on the basis of the presence or absence of a seed strophiole, van der Maesen (1986) while revising the group, merged the two genera under *Cajanus* following systematic analysis of morphological, cytological and chemo-taxanomical data. The revised genus *Cajanus* now comprises 32 species distributed in Asia, Australia and West Africa. These species of *Cajanus* were grouped into six sections namely, Cajanus, Atylia, Fruticosa, Cantharospermum, Volubilis and Rhynchosoides based on growth habit, leaf shape, hairiness, nature of corolla, pod size and strophiole characteristics (van der Maesen, 1986).

Ψ *Corresponding author; Email*: pcpanda2001@yahoo.co.in

Numerous morphological and alpha taxonomic studies of *Cajanus* and related genera have been undertaken (Grear, 1978; Lackey, 1978; Stirton, 1981; Pundir & Singh, 1985a, b & c; van der Maesen, 1986, 1990). The isozymes (Krishna and Reddy, 1982) and seed proteins (Jha and Ohri, 1996; Panigrahi *et al*., 2007) have also been used to establish phylogeny of different taxa. A number of workers studied the cytogenetics and breeding behaviour of *Cajanus* and related genera (Deodikar and Thakar, 1956; Reddy, 1981a & b; Ohri & Singh, 2002; Mallikarjuna *et al*., 2011). During the last two decades, molecular marker techniques such as RFLP (Nadimpalli *et al*., 1992; Sivaramakrishnan *et al*., 2002), RAPD (Ratnaparkhe *et al*., 1995; AFLP (Parani *et al*., 2000; Panguluri *et al*., 2006; Ganapathy *et al*., 2011), SSR (Odeny *et al*., 2009; Dutta *et al*., 2011) have been used for estimating genetic diversity and phylogenetic relationship among different genera of Cajaninae.

Biochemical, cytological, molecular, crossability experiments and phytogeographical studies have established that India is the country of origin of cultivated pigeonpea

and *Cajanus cajanifoius* (=*Atylosia cajanifolia*) as its wild progenitor. The later was described by Haines (1919) from Odisha and subsequently, besides it type locality, the species has been reported to occur wild in a number of localities. It is found to exhibit morphological variations in its type locality which need to be examined using morphological and molecular tools. Several traditional landraces of pigeonpea cultivated in tribal districts of Odisha are important germplasm materials for crop improvement and require in depth molecular genetic studied. In the present study, the genetic diversity and molecular phylogeny of 10 species of *Cajanus* and 11 accessions of *Cajanus cajan* (pigeonpea) have been assessed using RAPD and ISSR markers. The findings of the study will prove useful in the process of selection of species and accessions for breeding and crop improvement in pigeonpea using its wild relatives.

2. Materials and Methods

2.1 Plant materials

Seed samples of 30 accessions belonging to 10 species of *Cajanus* of the sub-tribe Cajaninae were collected from the germplasm collection of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patencheru, Hyderabad and from different districts of Odisha. The accession number, locality of collection and abbreviation used for each of the taxon is shown in Table 1. The seed materials were geminated in pro-trays under greenhouse conditions at Regional Plant Resource Centre, Bhubaneswar and the tender leaves were used for DNA extraction for molecular analyses.

2.2 Genomic DNA extraction

Genomic DNA was extracted from the leaf tissues using the modified CTAB protocol (Doyle and Doyle, 1990) with modification. Two grams of leaf tissues from young seedlings were ground with grinding buffer composed of 100 mM sodium acetate (pH 4.8), 500 mM NaCl, 50 mM EDTA (pH 8.0); 50 mM Tris (pH 8.0); 2% polyvinyl pyrollidone (PVP) and 2% CTAB. Purification of DNA was done twice with extraction of phenol: chloroform: isoamyl alcohol (25:24:1). RNAse @ 40 µl from 1 mg/ ml was applied in the supernatant to get rid of RNA. The quality and quantity of DNA were checked through 0.8% agarose electrophoresis with standard DNA before PCR amplification.

2.3 RAPD and ISSR analyses

A total of 36 RAPD and ISSR primers (Operon Technologies, Alameda, USA) were selected for PCR analysis based upon their performance and reproducibility (Table 2, 3). PCR mixture of 25 µl contained 25 ng of genomic DNA template, 0.6 U of Taq DNA polymerase (Bangalore Genei, Bangalore, India), 0.3 µM of decamer primers, 2.5 µl of 10 x PCR assay buffer (50 mM KCI, 10 mM Tris-HCl), 1.5 mM $MgCl_2$) and 0.25 µl of pooled dNTPs. PCR condition used for ISSR amplification was: Initial denaturing step at 94^0C for 5 min followed by 42 cycles of 94^0C for 1 min, 45^0 - 55^0C for 1 min and 72^0C for 2 min, the last cycle, primer extension at 72^0C for 7 min. The PCR condition used for RAPD was: Initial denaturing step at 94^0C for 5 min followed by 42 cycles of 94^0C for 1 min, 37^0C for 1 min and 72^0C for 2 min, the last cycle, primer extension at 72^0C for 7 min. The amplified products were separated by agarose (1.5%) gel electrophoresis and documented in gel documentation system (Bio Rad XR, Biorad, USA). O'Gene Ruler™ 100 bp DNA Ladder plus (ladder range 3000 bp to 100 bp from Fermentas Life Sciences, USA) was used as molecular weight marker. Bands were scored for its presence/absence (1/0) for each primer-genotype combination. The NTSYS-pc, version 2.1 software (Rolf, 2000) was used for estimation of genetic relatedness among the genotypes using Jaccard's similarity coefficient and clustering was done with UPGMA (unweighted pair group method using arithmetic averages).

3. Results

3.1 Randomly Amplified Polymorphic DNA (RAPD) analysis

Out of 40 RAPD primers screened, 18 primers produced distinct reproducible amplifications in all the 10 species and 30 accessions of *Cajanus*. The RAPD banding pattern is shown in Fig 1. The DNA profiles obtained from RAPD analysis are presented in Table 2. A total of 128 amplified fragments were generated, which includes 87 polymorphic; 29 monomorphic and 7 unique bands. The resolving power of primers ranged from 0.58 (OPP02) to 1.84 (OPA10), whereas the primer index varied from 0.13 to 0.41 with the primers OPN15 and OPD08 respectively. OPN06 and OPD08 produced highest number of amplified bands (13 & 12 respectively), whereas OPA10 and OPP02 produced least number of loci (2). Two primers OPD08 and OPP02 showed 100% polymorphism and the polymorphism obtained using OPN06 primer was as low as 30.8%. The average number of bands and polymorphic bands per primer was 7.11 and 4.83 respectively. Jaccard's similarity coefficient analysis revealed that all the taxa were related to each other with an average similarity of 70%. The highest similarity (100%) was observed between *Cajanus cajan* (Ca-c2/1) and *Cajanus cajan* (Ca-c1) and lowest (48%) between *Cajanus platycarpus* (Ca-pl6/2) and *Cajanus albicans* (Ca-a1/1) (Table 3). The highest numbers of bands (93) were amplified in case of *Cajanus reticulatus* (Ca-rt7) and lowest (71) in *Cajanus platycarpus* (Ca-pl6/2), *Cajanus crassus* (Ca-sc3/1) and *Cajanus mollis* (Ca-mo5/2).

Table 1
Details of plant samples used for study of genetic diversity and phylogeny

Sl.No.	Accession No.	Origin	Species	Code used
1	RPRC-C/4	India (Odisha)	*Cajanus scarabaeoides*	Caj-sca
2	RPRC-C/3	India (Odisha)	*Cajanus cajanifolius*	Caj-cajanifolius
3	RPRC-C/1	India(Odisha-Kandhamal)	*Cajanus cajan*	Caj-c1
4	RPRC-C/2	India(Odisha-Nayagarh)	*Cajanus cajan*	Ca-c 2/1
5	ICP-7035	India (MP)	*Cajanus cajan*	Ca-c 2/2
6	ICP-7182	India (MP)	*Cajanus cajan*	Ca-c 2/3
7	ICP-7613	India (MP)	*Cajanus cajan*	Ca-c 2/4
8	ICP-9150	Kenya	*Cajanus cajan*	Ca-c 2/5
9	ICP-9880	India (AP)	*Cajanus cajan*	Ca-c 2/6
10	ICP-11975	India (AP)	*Cajanus cajan*	Ca-c 2/7
11	ICP-12746	India (AP)	*Cajanus cajan*	Ca-c 2/8
12	ICP-12825	Tanzania	*Cajanus cajan*	Ca-c 2/9
13	ICP-13434	Malawi	*Cajanus cajan*	Ca-c 2/10
14	ICP-15620	SriLanka	*Cajanus albicans*	Ca-al 1/1
15	ICP-15621	India	*Cajanus albicans*	Ca-al 1/ 2
16	ICP-15622	India	*Cajanus albicans*	Ca-al 1/3
17	ICP-15634	Australia	*Cajanus reticulatus*	Ca-ret 7
18	ICP-15641	India	*Cajanus lineatus*	Ca-lin 4/1
19	ICP-15642	India	*Cajanus lineatus*	Ca-lin 4/2
20	ICP-15643	India	*Cajanus lineatus*	Ca-lin 4/3
21	ICP-15653	India	*Cajanus mollis*	Ca-mo 5/1
22	ICP-15654	India	*Cajanus mollis*	Ca-mo 5/2
23	ICP-15657	India	*Cajanus mollis*	Ca-mo 5/3
24	ICP-15661	India	*Cajanus platycarpus*	Ca-pl 6/1
25	ICP-15664	India	*Cajanus platycarpus*	Ca-pl 6/2
26	ICP-15665	India	*Cajanus platycarpus*	Ca-pl 6/3
27	ICP-15760	India	*Cajanus sericeus*	Ca-se 8/1
28	ICP-15762	Australia	*Cajanus sericeus*	Ca-se 8/2
29	ICP-15767	India	*Cajanus crassus*	Ca-cs 3/1
30	ICP-15770	India	*Cajanus crassus*	Ca-cs 3/2

Note: ICP is an acronym for ICRISAT accession number

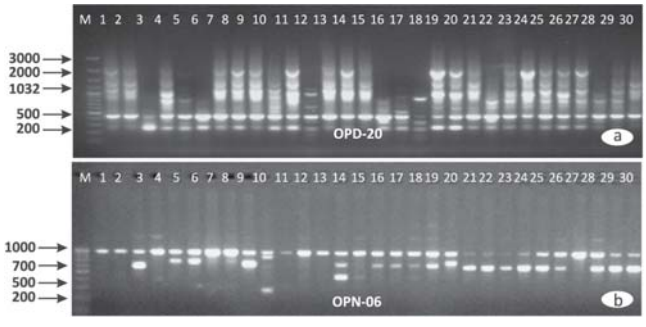

Fig.1. RAPD banding pattern of different species and accessions of *Cajanus* with the use of primers (a) OPD20 (b) OPN-6

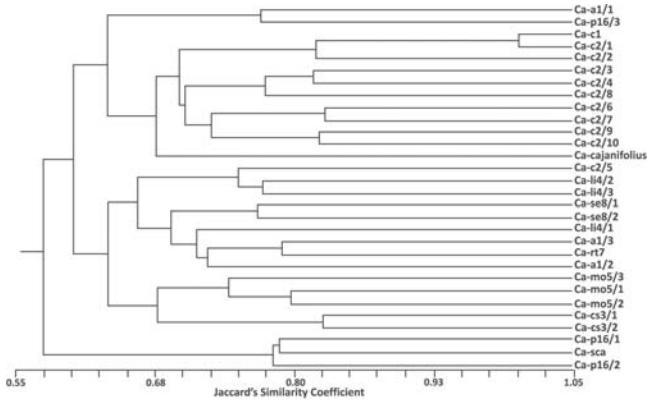

Fig. 2. Dendrogram showing genetic relationship among different species of *Cajanus* and accessions of *C. cajan* as revealed from RAPD

Table 2
Analysis of RAPD primers and bands details

Primer/Primer Combination	Sequences	Range of amplicons	Total bands	Polymorphic bands	Monomorphic bands	Unique bands	% of Polymorphic Band (PPB)	Resolving Power	Primer Index
OPD-08	TGCCGAGCTG	2800-350	12	12	0	0	100	0.67	0.41
OPA-03	AGTCAGCCAC	2900-650	7	3	3	1	42.9	1.14	0.18
OPD-18	AATCGGGCTG	2000-650	5	4	1	0	80	1.75	0.2
OPA-04	GGGTAACGCC	2500-460	4	2	1	1	50	1.16	0.16
OPA-10	GTTGCGATCC	2100-1032	2	1	1	0	50	1.84	0.13
OPD-20	GGACCCAACC	2600-660	7	5	2	0	71.4	1.43	0.26
OPN-06	TTGGCACGGG	2600-100	13	4	2	2	30.8	0.91	0.27
OPAF-14	GTGTGCCCCA	3000-610	10	5	4	1	50	1.15	0.2
OPN-10	CACCGTATCC	2000-220	10	7	3	0	70	1.66	0.24
OPP-02	GAGAGCCAAC	1500-900	2	2	0	0	100	0.58	0.41
OPS-07	GACCGACCCA	1900-250	8	6	1	1	75	1.16	0.26
OPT-04	GAGACGCACA	1850-700	6	4	2	0	66.7	0.98	0.19
OPN-15	AAGCGACCTG	1200-490	4	2	2	0	50	1.16	0.13
OPP-05	GGTGAGGTCA	2900-360	7	6	1	0	85.7	1.14	0.36
OPN-18	TTGCGGCTGA	1900-240	8	5	3	0	62.5	1.61	0.24
OPN-20	GGTGCGCACT	1500-220	6	5	1	0	83.3	1.77	0.19
OPN-14	TGATGCTGTC	2300-250	8	6	1	1	75	1.11	0.26
OPN-16	CCGAACACGG	2850-500	9	8	1	0	88.9	1.19	0.38
Total			128	87	29	7			

Table 3
Jaccard's similarity table for different taxa of *Cajanus* as revealed through RAPD analysis

	Ca-a1/1	Ca-pl6/3	Ca-c1	Ca-pl6/1	Ca-pl6/2	Ca-sca	Ca-c2/1	Ca-c2/2	Ca-c2/3	Ca-c2/4	Ca-cajanifolius	Ca-c2/6	Ca-c2/7	Ca-c2/8	Ca-c2/9	Ca-c2/10	Ca-c2/5	Ca-li4/2	Ca-li4/3	Ca-se8/1	Ca-se8/2	Ca-li4/1	Ca-a1/3	Ca-rt7	Ca-a1/2	Ca-mo5/3	Ca-mo5/1	Ca-mo5/2	Ca-cs3/1	Ca-cs3/2
Ca-a1/1	1.00																												
Ca-pl6/3	0.77	1.00																											
Ca-c1	0.64	0.67	1.00																										
Ca-pl6/1	0.58	0.63	0.61	1.00																									
Ca-pl6/2	0.48	0.54	0.52	0.78	1.00																								
Ca-sca	0.49	0.56	0.56	0.79	0.79	1.00																							
Ca-c2/1	0.64	0.67	**1.00**	0.61	0.52	0.56	1.00																						
Ca-c2/2	0.66	0.69	0.82	0.61	0.54	0.56	0.82	1.00																					
Ca-c2/3	0.65	0.68	0.68	0.64	0.53	0.56	0.68	0.74	1.00																				
Ca-c2/4	0.66	0.64	0.64	0.66	0.57	0.58	0.64	0.71	0.82	1.00																			
Ca-cajanifolius	0.53	0.61	0.67	0.57	0.50	0.50	0.67	0.70	0.64	0.65	1.00																		
Ca-c2/6	0.54	0.59	0.65	0.58	0.52	0.49	0.65	0.67	0.66	0.64	0.68	1.00																	
Ca-c2/7	0.61	0.67	0.76	0.61	0.52	0.52	0.76	0.79	0.77	0.71	0.73	0.83	1.00																
Ca-c2/8	0.62	0.71	0.69	0.63	0.57	0.56	0.69	0.77	0.82	0.73	0.70	0.71	0.79	1.00															
Ca-c2/9	0.65	0.70	0.73	0.64	0.51	0.54	0.73	0.76	0.73	0.70	0.72	0.74	0.79	0.78	1.00														
Ca-c2/10	0.57	0.60	0.64	0.57	0.51	0.52	0.64	0.67	0.66	0.63	0.62	0.68	0.70	0.67	0.82	1.00													
Ca-c2/5	0.63	0.68	0.71	0.60	0.50	0.52	0.71	0.76	0.71	0.70	0.78	0.71	0.77	0.76	0.93	0.76	1.00												
Ca-li4/2	0.53	0.54	0.54	0.65	0.59	0.60	0.54	0.58	0.62	0.61	0.58	0.53	0.58	0.61	0.62	0.57	0.60	1.00											
Ca-li4/3	0.59	0.60	0.60	0.66	0.57	0.55	0.60	0.61	0.68	0.64	0.64	0.57	0.64	0.64	0.65	0.65	0.63	0.77	1.00										
Ca-se8/1	0.55	0.61	0.61	0.65	0.55	0.56	0.61	0.58	0.61	0.58	0.65	0.63	0.65	0.67	0.69	0.64	0.67	0.65	0.72	1.00									
Ca-se8/2	0.59	0.62	0.67	0.66	0.55	0.56	0.67	0.61	0.68	0.67	0.66	0.67	0.69	0.66	0.75	0.65	0.73	0.64	0.72	0.77	1.00								
Ca-li4/1	0.50	0.56	0.61	0.67	0.64	0.60	0.61	0.55	0.57	0.63	0.62	0.58	0.58	0.58	0.60	0.57	0.62	0.67	0.66	0.64	0.73	1.00							
Ca-a1/3	0.58	0.60	0.64	0.65	0.57	0.59	0.64	0.65	0.66	0.63	0.69	0.59	0.63	0.67	0.73	0.64	0.73	0.63	0.66	0.67	0.70	0.73	1.00						
Ca-rt7	0.57	0.60	0.67	0.65	0.55	0.57	0.67	0.64	0.65	0.64	0.73	0.61	0.68	0.66	0.72	0.62	0.70	0.66	0.71	0.73	0.72	0.70	0.79	1.00					
Ca-a1/2	0.54	0.56	0.64	0.68	0.60	0.60	0.64	0.66	0.63	0.66	0.66	0.60	0.63	0.64	0.68	0.60	0.66	0.65	0.66	0.63	0.71	0.72	0.72	0.73	1.00				
Ca-mo5/3	0.51	0.57	0.66	0.69	0.60	0.63	0.66	0.66	0.69	0.66	0.61	0.55	0.63	0.70	0.67	0.63	0.65	0.63	0.66	0.69	0.66	0.69	0.71	0.66	0.72	1.00			
Ca-mo5/1	0.53	0.58	0.61	0.70	0.57	0.62	0.61	0.61	0.62	0.61	0.57	0.53	0.58	0.66	0.62	0.59	0.60	0.70	0.66	0.67	0.66	0.68	0.65	0.66	0.74	0.79	1.00		
Ca-mo5/2	0.53	0.58	0.57	0.64	0.58	0.59	0.57	0.59	0.58	0.60	0.57	0.52	0.54	0.62	0.59	0.58	0.59	0.72	0.65	0.59	0.62	0.68	0.61	0.62	0.66	0.70	0.80	1.00	
Ca-cs3/1	0.53	0.55	0.58	0.61	0.54	0.55	0.58	0.59	0.61	0.60	0.54	0.52	0.52	0.60	0.58	0.58	0.57	0.57	0.60	0.56	0.60	0.62	0.64	0.59	0.64	0.66	0.74	0.69	1.00
Ca-cs3/2	0.49	0.53	0.54	0.60	0.55	0.52	0.54	0.56	0.55	0.56	0.53	0.50	0.52	0.56	0.57	0.54	0.55	0.56	0.59	0.60	0.63	0.60	0.65	0.62	0.59	0.65	0.68	0.66	0.83

A dendrogram was constructed to derive the relationship among 30 different taxa of the genus *Cajanus* (Fig 2)**,** which separated all of them into two distinct clusters of 27 and 3, sharing a common node at 57% similarity level. The smaller cluster had two accessions of *Cajanus platycarpus* (Ca-pl6/1 and Ca-pl6/2) and *Cajanus scarabaeoides* (Ca-sca) and the similarity among them was about 79% but *Cajanus platycarpus* (Ca-pl6/1) was closer to *Cajanus scarabaeoides* (Ca-sca) than *Cajanus platycarpus* (Ca-pl6/2). The larger sub-group was further divided into 2 sub-clusters (14+13) where cultivated taxa got separated from wild species and accessions. The first sub-cluster again had two divisions of 5 and 9 taxa. Of the five taxa namely *Cajanus crassus* (Ca-cs3/1 and Ca-sc3/2), *Cajanus mollis* (Ca-mo5/1, Ca-mo5/2 and Ca-mo5/3), *Cajanus crassus* (Ca-cs3/1) and *Cajanus crassus* (Ca-cs3/2) formed one group with 81% similarity and got separated from the rest, sharing a common node at 68% level of similarity. *Cajanus mollis* (Ca-mo5/1, Ca-mo5/2 and Ca-mo5/3) came in a sub-cluster with similarity of 72% and *Cajanus mollis* (Ca-mo5/1) and *Cajanus mollis* (Ca-mo5/2) were found to be genetically closely related. The second sub-group included 8 genotypes of wild *Cajanus* species which subsequently segregated into two groups of 6 [*Cajanus albicans* (Ca-a1/2), *Cajanus reticulatus* (Ca-rt7), *Cajanus albicans* (Ca-a1/3), *Cajanus lineatus* (Ca-li4/1), *Cajanus sericeus* (Ca-se8/1) and *Cajanus sericeus* (Ca-se8/2)] having similarity of 68% among them and rest two accessions of *Cajanus lineatus* (Ca-li4/2 and Ca-li4/3) had a similarity of 77%.

The other sub-group included most of the cultivated accession of *Cajanus cajan* which got separated from wild species (e.g. *C. cajanifolius* and *C.sericeus*) sharing a common node at 60% similarity level. This group included the accession of *Cajanus cajan* (Ca-c1, Ca-c2/1, Ca-c2/2, Ca-c2/3, Ca-c2/4, Ca-c2/5, Ca-c2/6, Ca-c2/7, Ca-c2/8, Ca-c2/9, Ca-c2/10), *Cajanus cajanifolius* (Caj-cajanifolius), *Cajanus platycarpus* (Ca-pl6/3) and *Cajanus albicans* (Ca-a1/1). *Cajanus cajan* (Ca-c1) and *Cajanus cajan* (Ca-c2/1) showed maximum similarity of 100%. *Cajanus albicans* (Ca-a1/1) and *Cajanus platycarpus* (Ca-pl6/3) were observed to exhibit a similarity of 78% between them and this group was segregated from *Cajanus cajan* with whom it shared 65% genetic similarity.

3.2 Inter simple sequence repeat (ISSR) analysis

The results obtained from the molecular fingerprinting by ISSR primers in 30 accessions of *Cajanus* representing 10 species are presented in Table 4. Out of the 35 ISSR primers tested, only 18 primers produced good and reproducible amplified product. A total of 147 bands were amplified which include 125 polymorphic, 10 monomorphic and 12 unique bands. The size of amplicons ranged from 200bp to 3000bp. The resolving power of primers ranged from 0.51 [G $(CTGT)_4$] to 1.53 [$(CA)_8$ AG] and the primer index varied in the ranges of 0.16 - 0.44 for the $(CA)_8$ AG and $(CT)_8$G respectively. The ISSR banding pattern is shown in the (Fig. 3). $(AG)_{10}$ produced highest number of amplified loci (14) whereas $(CT)_8$ G and $(GACA)_4$ T produced least number (5) of bands. Nine primers yielded 100% polymorphic bands but the polymorphism observed with $(CA)_8$ AG and $(GGGGT)_3$ primers was only 50%. The average no of amplified bands and polymorphic bands per primer was 8.16 and 6.94 respectively. $(CA)_8$ AG and $(AG)_{10}$ amplified maximum no. of monomorphic loci (3 and 4), and the primer $(GGAGA)_3$ and $(GGGGT)_3$ produced 3 and 4 unique bands respectively.

All the 30 taxa genetically analysed had an average similarity of 56% as per the Jaccard's similarity coefficient analysis Table 5. The highest similarity (0.96) was observed between two accessions of cultivated pigeonpea (Ca-c2/6 and Ca-c2/7) and the lowest (0.22) between *Cajanus platycarpus* (Caj-pl6/2) and *Cajanus mollis* (Ca-mo5/1). The highest numbers of bands (90) were amplified in case of *Cajanus cajan* (Ca-c2/10) and the lowest (33) in *Cajanus mollis* (Ca-mo5/1). The dendrogram (Fig 4) divided the taxa into two distinct clusters of 1 and 29. The first and smallest cluster contained a single accession of *Cajanus mollis* (Ca-mo5/1) and both the groups shared a common node at 25% similarity. The large cluster was further divided into two subclusters (3+26) and the small sub-cluster contained *Cajanus crassus* (Ca-cs3/1 and Ca-cs3/2) and *Cajanus mollis* (Ca-mo5/2). Of these three, *Cajanus crassus* (Ca-cs3/1) and *Cajanus crassus* (Ca-cs3/2) had 78% similarity between them.

The second and larger cluster was divided into two sub-clusters (1+25). The lone accession *Cajanus mollis* (Ca-mo5/3) formed a separate cluster, sharing 45% similarity with the cluster of 25 taxa. The smaller sub-cluster contained

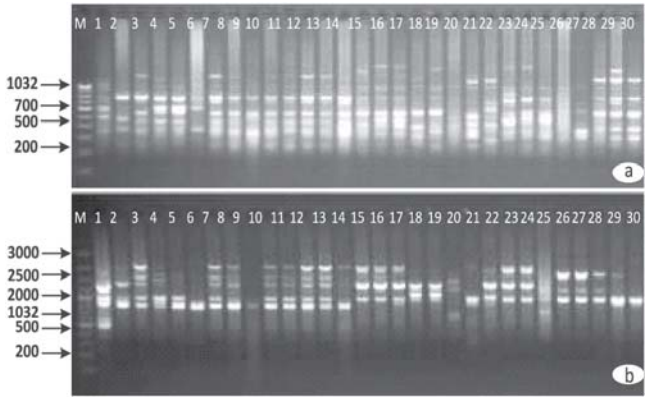

Fig. 3. ISSR banding pattern of different species and accessions of *Cajanus* with the use of primers: a. (AG)10, b. (CT)8A

Table 4
Details of ISSR primers used and bands amplified

Primer/Primer Combination	Sequences	Range of amplicons	Total bands	Polymorphic bands	Monomorphic bands	Unique bands	% of Polymorphic Band (PPB)	Resolving Power	Primer Index
(GA)8G	GAGAGAGAGAGAGAGAG	2200-370	8	8	0	0	100	0.87	0.42
(CA)8AG	CACACACACACACACAAG	1700-300	6	3	3	0	50	1.53	0.16
(AG)10	AGAGAGAGAGAGAGAGAGAG	2000-250	14	9	4	1	64.3	1.09	0.25
(CT)8A	CTCTCTCTCTCTCTCTA	2100-480	10	10	0	0	100	0.96	0.4
(AG)8C	AGAGAGAGAGAGAGAGC	1900-350	8	6	1	1	75	0.97	0.29
(GGAGA)3	GGAGAGGAGAGGAGA	1500-250	11	8	0	3	72.7	0.7	0.27
(GGGGT)3	GGGGTGGGGTGGGGT	1500-300	10	5	1	4	50	0.64	0.22
(AG)8	AGAGAGAGAGAGAGAG	1700-460	9	8	0	1	88.9	0.8	0.3
(GAA)6	GAAGAAGAAGAAGAAGAA	2700-550	7	7	0	0	100	1.16	0.4
(AGG)6	AGGAGGAGGAGGAGGAGG	1900-380	6	6	0	0	100	1	0.3
(CT)8G	CTCTCTCTCTCTCTCTG	1500-700	5	5	0	0	100	1.05	0.44
T(GACA)4	TGACAGACAGACAGACA	1900-300	8	7	1	0	87.5	1.11	0.33
(GA)9T	GAGAGAGAGAGAGAGAGAT	1800-400	7	7	0	0	100	1.11	0.36
G(CT)8	GCTCTCTCTCTCTCTCT	2500-450	9	9	0	0	100	0.74	0.33
(GATA)4C	GATAGATAGATAGATAC	2800-300	10	10	0	0	100	0.83	0.43
(GACA)4G	GACAGACAGACAGACAG	1200-500	7	6	0	1	85.7	0.74	0.39
G(CTGT)4	GCTGTCTGTCTGTCTGT	1500-600	7	6	0	1	85.7	0.51	0.3
(GACA)4T	GACAGACAGACAGACAT	2200-400	5	5	0	0	100	0.91	0.29
Total			147	125	10	12	86.7		

one accession of each of *Cajanus platycarpus* (Ca-pl6/3), *Cajanus lineatus* (Ca-li4/1), *Cajanus albicans* (Ca-a1/3), *Cajanus reticulatus* (Ca-ret7) and *Cajanus sericeus* (Ca-se8/2) sharing varying levels of similarity among them. The larger sub-cluster was comprised of all accessions of cultivated *Cajanus cajan*, *Cajanus cajanifolius* and few accessions of *Cajanus albicans, Cajanus scarabaeoides, Cajanus lineatus* and *Cajanus sericeus*.

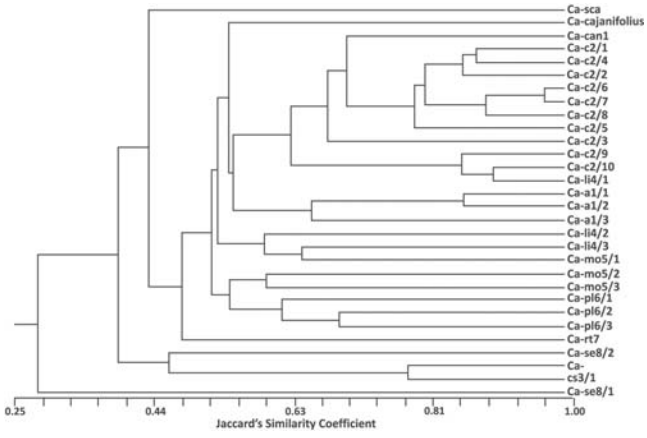

Fig.4. Relationship among different species of *Cajanus* and accessions of *C. cajan* through ISSR analysis

Most of the wild species formed one sub-group having an average similarity of more than 58% among them. The cultivated accessions formed another sub-cluster. The maximum similarity of 96% was obtained between two *Cajanus cajan* accessions (Ca-c2/6 and Ca-c2/7). Similarly, three *Cajanus cajan* accessions namely Ca-c2/1, Ca-c2/4 and Ca-c2/2 got separated in the cluster with more than 81% similarity among them. *Cajanus cajanifolius* (Caj-cajanifolius) showed close affinity with cultivated *Cajanus cajan* (Ca-c2/10 and Ca-c2/9), whereas *Cajanus cajanifolius* (Caj-cajanifolius) was closer to *Cajanus cajan* (Ca-c2/10) than *Cajanus cajan* (Ca-c2/9). *Cajanus platycarpus* (Ca-pl6/2) showed similarity of 50% with cultivated pigeonpea cluster.

3.3 RAPD and ISSR combined markers

The molecular phylogeny of the species of *Cajanus* inferred from data obtained from a combination of RAPD and ISSR markers has been discussed here. A total of 36 RAPD and ISSR primers produced good and reproducible amplification products. The highest (172) number of bands were amplified in case of an accessions of *Cajanus cajan* (Ca-caj2/10) and lowest (113) in *Cajanus mollis* (Ca-mo5/1)**.** Relationships among the 30 taxa containing 10 species of *Cajanus* were determined through analysis of Jaccard's similarities coefficient (Table 6). From the Jaccard's table it was observed that all the species were related to each other with an average similarity of 0.63. Highest similarity (0.89) was observed between two accession of *Cajanus cajan* (Ca-c2/6 and Ca-c2/7) and lowest (39%) between *Cajanus mollis* (Ca-mo5/1) and *Cajanus platycarpus* (Ca-pl6/1) [Table 6].

The cladogram (Fig 5) constructed taking both RAPD and ISSR data in respect of all the 30 taxa of *Cajanus* showed grouping of them into 2 distinct clusters of 26 and 4. Both these clades shared a node at 46% similarity level. The small cluster of 4 taxa was comprised of two accessions from each of *Cajanus crassus* and (Ca-cs3/1 and Ca-cs3/2) and *Cajanus mollis* (Caj-mo5/1 and Caj-mo5/2) and these two species had 55% similarity between them. The large cluster which included 26 taxa of *Cajanus* had two groups of very unequal sizes of 24 and 2. The small group had species like *Cajanus platycarpus* (Ca-pl6/1 and Ca-pl6/2), which got separated in the first place and shared 61% similarity with the other 24 species. The next group was formed of 2 accession of *Cajanus albicans* (Ca-al 1/1, Ca-al1/ 2) and *Cajanus scarabaeoides* (Ca-sca) which had more than 70% similarity among them and 55% with rest of the species in the dendrogram. Five accessions one from each species namely *Cajanus mollis* (Ca-mo5/3), *Cajanus platycarpus* (Ca-pl6/3), *Cajanus lineatus* (Ca-li4/1), *Cajanus albicans* (Ca-al1/3) and *Cajanus reticulatus* (Ca-ret7) got separated in the next level leaving the rest 16 taxa in a bigger cluster. Of these 16 taxa, 2 accessions of *Cajanus lineatus* (Ca-li 4/2 and Ca-li 4/3) and 2 accessions of *Cajanus sericeus* (Ca-se8/1 & Ca-se8/2) got out of the cluster justifying their species status and this clade had 65% similarity with rest others. Further, 11 accessions of cultivated pigeonpea (*Cajanus cajan*) along with one accession of *Cajanus cajanifolius* (Ca-cajanifolius) come together in a bigger clade with varying level of similarities among them. Of the pigeonpea accessions, Ca-c1, Ca-c2/1, Ca-c2/6) and Ca-c2/7 had more than 90% similarity among them.

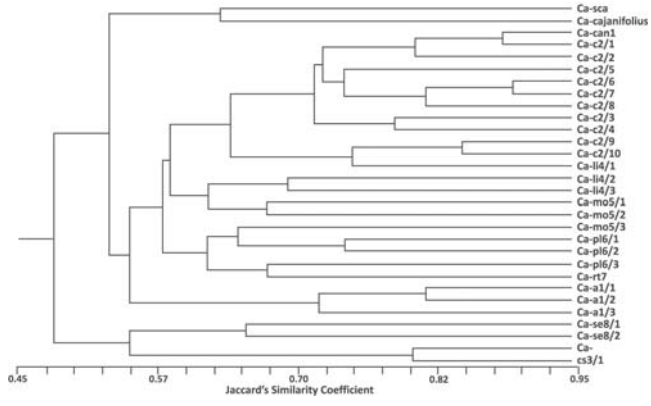

Fig. 5: Phylogeny of different species of *Cajanus* and accessions of *C. cajan* as inferred revealed from combined RAPD and ISSR markers

Table 5
Jaccard's similarity table for different species of *Cajanus* as inferred from ISSR analysis

	Ca-pl6/1	Ca-pl6/2	Ca-c1	Ca-a1/1	Ca-a1/2	Ca-sca	Ca-c2/1	Ca-c2/2	Ca-c2/3	Ca-c2/4	Ca-c2/5	Ca-c2/6	Ca-c2/7	Ca-c2/8	Ca-c2/9	Ca-c2/10	Ca-cajanifolius	Ca-li4/2	Ca-li4/3	Ca-se8/1	Ca-se8/2	Ca-rt7	Ca-a1/3	Ca-li4/1	Ca-pl6/3	Ca-mo5/3	Ca-mo5/1	Ca-mo5/2	Ca-cs3/1	Ca-cs3/2
Ca-pl6/1	1.00																												
Ca-pl6/2	0.51	1.00																											
Ca-c1	0.46	0.61	1.00																										
Ca-a1/1	0.46	0.57	0.64	1.00																									
Ca-a1/2	0.47	0.55	0.66	0.85	1.00																								
Ca-sca	0.52	0.49	0.56	0.63	0.67	1.00																							
Ca-c2/1	0.44	0.59	0.77	0.63	0.63	0.55	1.00																						
Ca-c2/2	0.45	0.57	0.72	0.59	0.59	0.56	0.86	1.00																					
Ca-c2/3	0.36	0.44	0.59	0.48	0.49	0.51	0.68	0.72	1.00																				
Ca-c2/4	0.47	0.58	0.72	0.61	0.59	0.56	0.87	0.85	0.75	1.00																			
Ca-c2/5	0.42	0.53	0.66	0.53	0.51	0.51	0.75	0.77	0.71	0.84	1.00																		
Ca-c2/6	0.42	0.57	0.67	0.60	0.55	0.51	0.85	0.79	0.67	0.87	0.80	1.00																	
Ca-c2/7	0.40	0.55	0.68	0.58	0.54	0.49	0.84	0.78	0.65	0.84	0.81	0.96	1.00																
Ca-c2/8	0.39	0.52	0.65	0.56	0.53	0.47	0.76	0.71	0.60	0.77	0.75	0.89	0.88	1.00															
Ca-c2/9	0.41	0.46	0.50	0.53	0.47	0.47	0.61	0.60	0.51	0.65	0.59	0.66	0.65	0.69	1.00														
Ca-c2/10	0.43	0.50	0.55	0.56	0.50	0.46	0.63	0.61	0.51	0.66	0.62	0.69	0.68	0.71	0.87	1.00													
Ca-cajanifolius	0.45	0.51	0.56	0.57	0.52	0.49	0.65	0.62	0.53	0.69	0.65	0.67	0.66	0.68	0.83	0.89	1.00												
Ca-li4/2	0.47	0.49	0.49	0.49	0.47	0.48	0.52	0.52	0.53	0.57	0.58	0.52	0.49	0.52	0.55	0.58	0.60	1.00											
Ca-li4/3	0.42	0.46	0.47	0.49	0.47	0.48	0.54	0.51	0.50	0.58	0.57	0.53	0.52	0.54	0.58	0.64	0.67	0.61	1.00										
Ca-se8/1	0.41	0.48	0.46	0.44	0.46	0.49	0.49	0.52	0.51	0.56	0.53	0.51	0.50	0.49	0.55	0.53	0.58	0.56	0.64	1.00									
Ca-se8/2	0.44	0.49	0.47	0.51	0.48	0.52	0.52	0.55	0.47	0.56	0.53	0.52	0.51	0.53	0.55	0.50	0.54	0.48	0.53	0.56	1.00								
Ca-rt7	0.40	0.43	0.49	0.54	0.48	0.50	0.55	0.50	0.49	0.55	0.53	0.54	0.53	0.52	0.54	0.56	0.58	0.44	0.59	0.49	0.59	1.00							
Ca-a1/3	0.40	0.49	0.51	0.48	0.50	0.52	0.57	0.57	0.54	0.59	0.55	0.57	0.58	0.53	0.56	0.57	0.60	0.50	0.61	0.54	0.57	0.58	1.00						
Ca-li4/1	0.39	0.46	0.47	0.51	0.51	0.47	0.55	0.54	0.48	0.56	0.52	0.54	0.55	0.51	0.52	0.55	0.56	0.43	0.53	0.46	0.51	0.57	0.69	1.00					
Ca-pl6/3	0.41	0.49	0.46	0.51	0.52	0.48	0.53	0.52	0.47	0.54	0.48	0.49	0.49	0.47	0.50	0.49	0.52	0.41	0.45	0.46	0.50	0.52	0.53	0.69	1.00				
Ca-mo5/3	0.36	0.47	0.42	0.48	0.45	0.44	0.46	0.44	0.44	0.48	0.49	0.47	0.46	0.46	0.49	0.48	0.50	0.44	0.51	0.47	0.41	0.48	0.48	0.52	0.62	1.00			
Ca-mo5/1	0.24	0.22	0.26	0.30	0.31	0.33	0.24	0.25	0.32	0.27	0.27	0.25	0.24	0.28	0.27	0.27	0.29	0.30	0.29	0.34	0.25	0.30	0.26	0.22	0.24	0.29	1.00		
Ca-mo5/2	0.29	0.29	0.33	0.32	0.29	0.30	0.31	0.32	0.38	0.35	0.33	0.34	0.32	0.32	0.34	0.34	0.33	0.35	0.33	0.37	0.32	0.33	0.30	0.30	0.31	0.33	0.44	1.00	
Ca-cs3/1	0.33	0.44	0.43	0.43	0.41	0.43	0.48	0.47	0.42	0.47	0.43	0.47	0.45	0.41	0.42	0.39	0.41	0.43	0.41	0.47	0.43	0.44	0.42	0.43	0.50	0.49	0.30	0.46	1.00
Ca-cs3/2	0.31	0.43	0.38	0.41	0.38	0.39	0.44	0.42	0.39	0.43	0.40	0.45	0.42	0.41	0.40	0.39	0.40	0.41	0.40	0.41	0.40	0.41	0.39	0.40	0.46	0.45	0.32	0.46	0.78	1.00

Table 6
Coefficient of similarity among species of *Cajanus* revealed from combined markers (RAPD + ISSR) analysis.

	Ca-pl6/1	Ca-pl6/2	Ca-c1	Ca-a1/1	Ca-a1/2	Ca-sca	Ca-c2/1	Ca-c2/2	Ca-c2/3	Ca-c2/4	Ca-c2/5	Ca-c2/6	Ca-c2/7	Ca-c2/8	Ca-c2/9	Ca-c2/10	Ca-cajanifolius	Ca-li4/2	Ca-li4/3	Ca-se8/1	Ca-se8/2	Ca-rt7	Ca-a1/3	Ca-li4/1	Ca-pl6/3	Ca-mo5/3	Ca-mo5/1	Ca-mo5/2	Ca-cs3/1	Ca-cs3/2
Ca-pl6/1	1.00																												
Ca-pl6/2	0.63	1.00																											
Ca-c1	0.55	0.64	1.00																										
Ca-a1/1	0.52	0.60	0.62	1.00																									
Ca-a1/2	0.48	0.54	0.58	0.81	1.00																								
Ca-sca	0.51	0.53	0.56	0.71	0.73	1.00																							
Ca-c2/1	0.54	0.63	0.88	0.62	0.57	0.56	1.00																						
Ca-c2/2	0.55	0.63	0.77	0.60	0.56	0.56	0.84	1.00																					
Ca-c2/3	0.51	0.57	0.64	0.56	0.51	0.53	0.68	0.73	1.00																				
Ca-c2/4	0.56	0.61	0.68	0.63	0.58	0.57	0.74	0.78	0.79	1.00																			
Ca-c2/5	0.47	0.57	0.66	0.55	0.50	0.51	0.71	0.73	0.67	0.73	1.00																		
Ca-c2/6	0.48	0.58	0.66	0.59	0.53	0.50	0.74	0.73	0.66	0.75	0.73	1.00																	
Ca-c2/7	0.50	0.61	0.72	0.60	0.53	0.51	0.80	0.78	0.72	0.77	0.77	0.89	1.00																
Ca-c2/8	0.50	0.61	0.67	0.60	0.55	0.52	0.72	0.74	0.71	0.75	0.72	0.80	0.83	1.00															
Ca-c2/9	0.52	0.57	0.61	0.58	0.49	0.51	0.67	0.68	0.62	0.68	0.66	0.70	0.72	0.73	1.00														
Ca-c2/10	0.50	0.55	0.59	0.57	0.51	0.49	0.64	0.64	0.58	0.65	0.62	0.68	0.69	0.69	0.85	1.00													
Ca-cajanifolius	0.50	0.53	0.57	0.58	0.51	0.49	0.62	0.59	0.55	0.64	0.62	0.62	0.60	0.61	0.75	0.75	1.00												
Ca-li4/2	0.50	0.52	0.52	0.57	0.53	0.54	0.53	0.55	0.58	0.59	0.58	0.52	0.54	0.56	0.58	0.57	0.68	1.00											
Ca-li4/3	0.50	0.53	0.54	0.57	0.52	0.51	0.57	0.56	0.59	0.61	0.61	0.55	0.58	0.59	0.61	0.64	0.70	0.69	1.00										
Ca-se8/1	0.48	0.55	0.54	0.54	0.50	0.52	0.55	0.56	0.57	0.57	0.60	0.57	0.58	0.58	0.62	0.59	0.63	0.61	0.68	1.00									
Ca-se8/2	0.52	0.56	0.57	0.58	0.52	0.54	0.60	0.58	0.59	0.62	0.60	0.60	0.60	0.60	0.65	0.57	0.62	0.57	0.63	0.67	1.00								
Ca-rt7	0.45	0.50	0.55	0.60	0.56	0.55	0.58	0.53	0.53	0.59	0.57	0.56	0.56	0.55	0.57	0.57	0.59	0.55	0.63	0.57	0.66	1.00							
Ca-a1/3	0.48	0.54	0.57	0.56	0.54	0.55	0.61	0.61	0.61	0.61	0.62	0.58	0.61	0.60	0.64	0.61	0.61	0.57	0.64	0.61	0.64	0.66	1.00						
Ca-li4/1	0.48	0.53	0.57	0.58	0.53	0.53	0.62	0.60	0.57	0.61	0.63	0.58	0.62	0.58	0.62	0.58	0.60	0.55	0.62	0.60	0.62	0.64	0.74	1.00					
Ca-pl6/3	0.47	0.53	0.55	0.59	0.56	0.54	0.59	0.59	0.56	0.60	0.57	0.55	0.56	0.55	0.59	0.54	0.58	0.52	0.55	0.55	0.61	0.62	0.63	0.71	1.00				
Ca-mo5/3	0.43	0.52	0.54	0.58	0.52	0.54	0.56	0.55	0.57	0.57	0.55	0.51	0.54	0.58	0.58	0.55	0.54	0.54	0.58	0.58	0.54	0.58	0.59	0.59	0.67	1.00			
Ca-mo5/1	0.39	0.41	0.45	0.51	0.45	0.49	0.44	0.45	0.50	0.46	0.44	0.40	0.42	0.48	0.45	0.43	0.46	0.52	0.48	0.53	0.48	0.51	0.47	0.47	0.50	0.55	1.00		
Ca-mo5/2	0.41	0.44	0.45	0.47	0.43	0.45	0.44	0.46	0.49	0.48	0.46	0.43	0.43	0.47	0.47	0.45	0.45	0.54	0.49	0.49	0.48	0.50	0.45	0.47	0.48	0.51	0.65	1.00	
Ca-cs3/1	0.42	0.50	0.51	0.52	0.47	0.49	0.53	0.53	0.52	0.54	0.49	0.49	0.49	0.50	0.49	0.48	0.48	0.50	0.50	0.52	0.52	0.53	0.53	0.51	0.57	0.58	0.53	0.58	1.00
Ca-cs3/2	0.40	0.48	0.46	0.50	0.47	0.45	0.49	0.49	0.48	0.50	0.47	0.47	0.47	0.49	0.49	0.46	0.48	0.49	0.49	0.51	0.52	0.51	0.52	0.51	0.53	0.55	0.52	0.57	0.80

4. Discussion

The revised genus *Cajanus* currently comprises of 18 species from Asia, 15 species from Australia, and one species from West Africa. Of these, 13 are found only in Australia, 8 in the Indian subcontinent, and 1 in West Africa, with the remaining 14 species occurring in more than one country. Based on growth habit, leaf shape, hairiness, structure of corolla, pod size, and presence of strophiole, van der Maesen (1980) grouped the genus *Cajan* into six sections. Eighteen erect species were placed under three sections: seven species in Atylia, nine species in section Fruticosa, and two species in section Cajanus that consists of the cultivated species along with its progenitor, *C. cajanifolius*. Eleven climbing and creeping species were arranged in two sections, Cantharospermum (5) and Volubilis (6) and the remaining three trailing species were classified under Rhynchosoides.

With a view to conserving germplasm of the diverse array of species of *Cajanus* and allied taxa and to incorporate desirable genes from these plants into cultivated *C. cajan,* emphasis has been laid on the need to understand the phylogenetic relationships of these species more completely (Reddy 1981a, b & c; Reddy and De, 1983; Pundir and Singh 1985a, b & c; Saxena & Sharma, 1990). Although related wild species are a rich reservoir of not only resistance genes against various biotic and abiotic stresses but also of genes responsible for yield components, use of closely related species in pigeonpea improvement have been limited. Ongoing efforts using molecular tools to examine taxonomic relationships within the subtribe Cajaninae would throw light on the phylogenetic relationships within the group, and may suggest parsimonious routes for trait introgression.

4.1 Analysis of genetic diversity and phylogeny among species of Cajanus

The members of the genus *Atylosia* closely resemble the genus *Cajanus* in vegetative and reproductive characters and were relegated to two separate genera mainly on the basis of the presence or absence of a seed strophiole. Although some earlier taxonomists pointed out the unsatisfactory placement of *Atylosia* and *Cajanus* under two different genera, the irrefutable experimental evidence from the studies on inter-specific hybridization, cytotaxonomy and chemotaxonomy led to merger of the two under *Cajanus* (van der Maesen, 1986).

Baker (1876), besides considering *Cajanus* and *Atylosia* as two separate genera, divided the genus Atylosia into two sub-genera i. e. Atylia (containing species like *A. lineate*, A. *trinervia, A. sericea, A. mollis, A. heynei*) and Cantharospermum (with species like *A. scarabaeoides, A. albicans, A. platycarpa. A. goensis*). Taking into account a few key characters like growth habit, leaf shape, hairiness, nature of corolla, pod size, and strophiole characteristics, van der Maesen (1985) divided the genus into six sections namely, Cajanus (2 spp.), Atylia (7 spp.), Fruticosa (9 spp.), Cantharospermum (5 spp), Volubilis (6 spp.) and Rhynchosoides (3 spp). While suggesting sectional arrangement within the genus, van der Maesen (1985) himself admitted that this classification into sections would not always exhibit natural relationships and members of one section share a number of characters with the species of another section.

In the present investigation, the genetic relationships among 10 species of *Cajanus* were assessed using RAPD and ISSR markers. The dendrogram constructed using UPGMA method based on molecular data revealed the grouping of species under different sections of the genus (Fig 5) as proposed by van der Maesen (1986). All the accessions of cultivated pigeonpea (*Cajanus cajan*) and its wild progenitor *Cajanus cajanifolius* formed a single cluster conforming their placement under the sect. Cajanus. While the two species (*C. crassus* and *C. mollis*) of the sect. Volubilis came together in the phylognetic tree, two members of sect. Cantharospermum namely, *C. scarabaeoides and C. albicans* formed another sub-cluster. Species like *C. lineatus, C. sericeus, C. reticulatus*, classified under the sect. Atylia by van der Maesen (1986) also formed a cluster with few accessions of other species from different sections. The findings of the present study, to a large extent, are in agreement with the sectional classification of the genus *Cajanus*.

The closer affinity between pigeonpea and its wild relative *C. cajanifolius* has been established through the study of morphological traits (Mallikarjuna *et al*., 2011), analysis of esterase isozymes (Krishna and Reddy, 1982) and SDS-PAGE (Panigrahi *et al*., 2007). In the case of SDS-PAGE, the banding patterns revealed *C. cajanifolius* to be the closest to *C. cajan*, with *C. platycarpus* as an outgroup species justifying its status as a tertiary gene pool species (van der Maesen 1986). The close affinity between *C. cajan* and *C. cajanifolius* has also been observed through RFLP (Nadimpalli *et al*., 1994) and SSR analyses (Mallikarjuna *et al*., 2011). There is further evidence from cytology that *C. cajanifolius* is the progenitor species of *C. cajan* as the two have a similar karyotype, and the hybrids between the two species show normal meiosis with high pollen fertility and high seed set (Pundir and Singh, 1985a).

In the present study, *C. platycarpus*, belonging to the sect. Rhynchosioides formed an isolated cluster with its two accessions. Sivaramakrishnan *et al*. (2002), while assessing the genetic diversity in 12 species of *Cajanus*

including other accessions of pigeonpea and species of *Rhynchosia* using RFLP made similar inference on status of *C. platycarpus.* The distinctness of *C. platycarpus* in this study also corroborates well with the earlier reports on the interrelationships of *C. platycarpus* and other wild relatives of *Cajanus* (Krishna & Reddy, 1982 and Pundir & Singh, 1985a).

The dendrogram constructed using UPGMA method based on molecular data in the present analysis revealed the grouping of the two species namely, *Cajanus scarabaeoides* and *Cajanus albicans*, which correspond to the sect. Cantharospermum of the genus *Cajanus* proposed by van der Maesen (1986). Similar conclusion was drawn by Krishna & Reddy (1982) based on their study of esterase isozymes among *Cajanus cajan* and 6 species of *Atylosia* (now *Cajanus*), who detected 3 common bands justifying the close relationship between the two species. Karyotypes of *C. albicans* and *C. scarabaeoides* were very similar and none of the two species had a chromosome pair with *r*-index >2.0, which is reflected in very similar symmetry indices (Ohri & Singh, 2002). The findings of the present work are in accordance with the relationship established by the above studies.

The results of the present study led to placement of 3 accessions of *C. lineatus* and 2 accessions of *C. sericeus* in a tight sub-cluster and 1 accession of each of *C. reticulatus, C. albicans, C. platycarpus* and *C. mollis* in another sub-cluster; both the sub-cluster share a common node at 57% level of similarity. Three of the above species namely, *C. lineatus*, C. sericeus and *C. reticulatus* belong to the sect. Atylia. According to Ohri & Singh (2002), the karyotypes of *C. lineatus* and *C. sericeus* belonging to sect. Atylia were similar in respect of maximum *r*-index and the ratio of longest and shortest chromosomes in their respective complements. Using RAPD marker, Ratnaparkhe *et al*. (1995) found a similar affinity between *C. lineatus* and *C. sericeus* but contrary to the sectional arrangement of the genus, *C. albicans* belonging to the sect. Cantharospermum formed a cluster with the above two taxa. The grouping of an accession of *C. albicans* with members of the sect. Atylia in the current piece of work corroborates the findings of Ratnaparkhe *et al*. (1995) and Nadimpalli *et al*. (1992). Though closeness between Australian *C. reticulatus* and Indian *C. platycarpus* has been reported in one of the studies (Parani *et al*., 2000) based on ribosomal DNA variation, inclusion of an accession of *C. mollis* in the cluster is difficult to explain.

The genetic relatedness between the two species of the sect. Volubilis namely, *C. crassus* and *C. mollis* was very close as could be established from the present study. In a number of molecular phylogenetic studies, the relationship between *C. volubilis* and *C. mollis* have been derived (Jha & Ohri, 1996; Sivaramakrishnan *et al*., 2002) but no reference was found with regard to the genetic similarity between *C. mollis* and *C. crassus*. Upadhyaya *et al*. (2012) assessed 18 species of the genus *Cajanus* including *C. mollis and C. crassus* for 27 morpho-agronomic traits and found them in two different clusters. However, there were similarities in respect of climbing habit, larger sized leaves, higher seed protein contents etc. among these two species.

4.2 Genetic diversity analysis in pigeon pea (Cajanus cajan) accessions

In the present study, 2 local accessions of *Cajanus cajan* (Pigeonpea) collected from Kandhamal and Nayagarh districts of Odisa (India) and 9 accessions procured from ICRISAT were analysed to derive the genetic relationship among them using RAPD and ISSR markers. Intra-specific genetic diversity analysis of pigeon p*e*a with the use of a total of 36 RAPD and ISSR primers revealed distinct segregation of genotypes of Indian and African origin. Among the accessions from Indian states, those from Madhya Pradesh, Andhra Pradesh and Odisha formed separate clusters justifying the proximity of the geographical area of their occurrence and cultivation. Two local landraces collected from Kandhamal and Nayagarh of Odisha (locally known as "Kandula") were genetically very close to each other and to the accession from adjoining state of Madhya Pradesh. Songok *et al*. (2010) found similar distinction of pigeon pea genotypes from India and East Africa. While analysing phylogenetic relationships of *Cajanus* and allied genera using AFLP markers, Nadimpalli *et al*. (1992) also observed grouping of accessions of individual species from different countries.

The dendrogram constructed based on UPGMA method using data obtained from RAPD and ISSR markers, revealed the clear segregation of pigeonpea genotypes of African origin. The accession Tanzania (ICP No. 12825) and Malawi (ICP No. 13434) got separated from rest of the Indian genotypes (except 1 from Kenya) sharing a similarity of 63%. Songok *et al.* (2010) also found clear distinction of pigeonpea genotypes from India and East Africa. They apprehended that after domestication, pigeonpea is believed to have been taken from India, the country of its origin, to Malaysia, then to East Africa and then to Egypt through the Nile valley around 2000 BC (Songok *et al*., 2010; van der Maesen, 1990; Smartt, 1990). Though a self-pollinated crop, out-crossing (40-70%) does occur through insect pollination and over the years, substantial genetic variability among these geographically isolated populations of India and Africa might have been taken place.

However, Wasike *et al*. (2005) using AFLP studied the genetic variability and relatedness between Asian and African pigeonpea cultivars found no major clustering patterns according to country of origin. Though there was a close genetic relationships between them, East African pigeonpeas are less genetically diverse than Indian cultivars. It was opined that the Indian cultivars could be used as a source of germplasm for future improvement of East African pigeonpea. The non-clustering of the accession from Kenya (ICP No. 9150) with African genotypes may be due to the reason explained above or it might have been a recent introduction from an Asiatic country. In a similar type of work, Boehringer *et al*. (1991) used allozymes to detect polymorphism between Indian and Zambian genotypes of pigeonpea.

Among the collections from Indian states, which came in a separate cluster, the genotypes from Madhya Pradesh (ICP No.7182 & 7613), Andhra Pradesh (ICP No.9880, 11975 and 12746) and Odisha (Ca-c1 and Ca-c2/1) formed separate clusters justifying their geographical area of occurrence and cultivation. Two local cultivars collected from Kandhamal and Nayagarh Districts of Odisha and locally known as "Kandula" were very close with about 88% similarity between them and along with an accession from adjoining state of Madhya Pradesh (ICP No. 7035), they occupied a distinct sub-cluster in the dendrogram. Kandula, the local variety of pigeonpea has a different taste and the 'dal' prepared out of it is somewhat pasty in nature. The relatively close relationship of pigeonpea accessions from Odisha and Andhra Pradesh indicates gene flow among populations of these adjoining coastal states and perhaps adaptation to prevailing environmental conditions and cultural practices. Thus, a clear distinction of genotypes from different states/ geographical locations could be observed in the present investigation as has been established by Songok *et al*. (2010), who analysed cultivars/ accessions from Indian states of Odisha, Madhya Pradesh, Andhra Pradesh, Tamilnadu, Maharasthra and Gujrat along with those from Africa.

In the face of decreasing production of pulses in several parts of the world including India, there is an increasing need to broaden the genetic base and introduce traits for various biotic stresses and desirable traits. There is a renewed interest to exploit more wild relatives and such efforts would have a considerable impact on broadening the genetic base of variation of different leguminous crops and introduction of useful biotic, abiotic and agronomic traits. The possibility of exploiting wild relatives from the various gene pools of cultivated crops especially of grain legumes, has opened up new vistas for enhancing the genetic variability and the findings of the present work will help in the process of evaluation of genetic diversity and selection of species, accessions and landraces of legumes for utilization in breeding and crop improvement programmes in India or elsewhere.

References

Baker, J. G. (1878). Leguminosae. In: Hooker, J. D. (ed.) Flora of British India, Part-V. L. Reeve & Co, London, pp. 56-306.

Boehringer, A., Lebot. V. and Aradhya, M. (1991) Isozyme variation in twenty-one perennial pigeonpea genotypes. Int. Pigeonpea Newslett. 14: 6-7.

De, D. N. (1974). Pigeonpea. In: Hutchinson, J. (ed.) Evolutionary Studies on World Crops. Cambridge Press, London, UK, pp. 79-87.

Deodikar, G. B. and Thakar, C. V. (1956). Cytotaxonomic evidence for the affinity between *Cajanus indicus* Spreng. and certain erect species of *Atylosia* W. and A. Proc. Indian Acad. Sci. 43: 37–45.

Doyle, J. J. and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Dutta, S., Kumawat, G., Singh, B. P., Gupta, D. K., Singh, S., Dogra, V., Gaikwad, K., Sharma, T. R., Raje, R. S., Bandhopadhya, T. K., Datta, S., Singh, M. N., Fakrudin, B., Kulwal, P., Wanjari, K. B., Varshney, R. K., Cook, D. R., Singh, N. K. (2011). Development of genic-SSR markers by deep transcriptome sequencing in pigeonpea [*Cajanus cajan* (L.) Millspaugh]. BMC Plant Biol. 11: (doi: 10.1186/1471-2229-11-17).

Ganapathy, K. N., Gnanesh, B. N., Byre Gowda, M., Venkatesha, S. C., Gomashe, Sunil S. and Channamallikarjuna, V. (2011). AFLP analysis in pigeonpea [*Cajanus cajan* (L.) Millsp.] revealed close relationship of cultivated genotypes with some of its wild relatives Genet. Resour. Crop Evol. 58: 837–847.

Grear, J. W. (1978). A revision of the New World species of *Rhynchosia* (Leguminosae-Faboideae). Mem. New York Bot. Gard. 31 (1): 1-168.

Haines, H. H. (1919). Some new species of plants from Behar and Orissa. J. Asiatic Soc. Beng. (NS) 15: 309-317.

Jha, S. S. and Ohri, D. (1996). Phylogenetic relationships of pigeonpea [*Cajanus cajan* (L.) Millsp.] and its wild relatives based on seed protein profiles. Genet. Resour. Crop Evol. 43: 275–281.

Krishna, T. G. and Reddy, L. J. (1982). Species affinities between *Cajanus cajan* and some *Atylosia* species based on esterase isoenzymes. Euphytica 31: 709-713.

Kumar, L. S. S., Thombre, M. V. and D'Cruz, R. (1958). Cytological studies of an intergeneric hybrid of [*Cajanus cajan* (L.) Millsp.] and *Atylosia lineata* Wt. & Arn. Proc. Indian Acad.Sci. Section B. 47 (4): 252-262.

Lackey, J. A. (1978). Leaflet anatomy of Phaseoleae (Leguminosae: Papilionoideae) and its relation to taxonomy. Bot. Gaz. 139 (4): 436-444.

Mallikarjuna, N., Saxena, K. B. and Jadhav, D. R. (2011). *Cajanus*. In: Cole, C. (ed.) Wild Crop Relatives: Genomic and Breeding Resources of Legume Crops and Forages. Springer, pp. 21-33.

Nadimpalli, R. J., Jarret, R. L., Pathak, S.C. and Kochert, G. (1992). Phylogenetic relationships of the pigeonpea [*Cajanus cajan* (L.) Millsp.] based on nuclear restriction fragment length polymorphisms. Genome 36: 216-223.

Odeny, D. A., Jayashree, B., Gebhardt, C., and Crouch, J. (2009). New microsatellite markers for pigeonpea [*Cajanus cajan* (L.) Millsp.]. BMC Res. Notes (doi: 10.1186/ 1756-0500-2-35).

Ohri, D. and Singh, S. P. (2002). Karyotypic and genome size variation in pigeonpea [*Cajanus cajan* (L.) Millsp.] and some of its relatives. Genet. Resour. Crop Evol. 49: 1–10.

Panguluri, S. K., Janaiah, K., Govil, J. N., Kumar, P. A. and Sharma, P. C. (2006). AFLP fingerprinting in pigeonpea [*Cajanus cajan* (L.) Millsp.] and its wild relatives. Genet. Resour. Crop Evol. 53 (3): 523-531.

Panigrahi, J., Kumar, D. R., Mishra, M., Mishra, R. P. and Jena, P. (2007). Genomic relationships among 11 species in the genus *Cajanus* as revealed by seed protein (albumin and globulin) polymorphisms. Plant Biotechnol. Rep. 1: 109–116.

Parani, M., Lakshmi, M., Senthil Kumar, P. and Parida, A. (2000). Ribosome DNA variation and phylogenetic relationships among [*Cajanus cajan* (L.) Millsp.] and its wild relatives. Curr. Sci. 78 (10): 1235–1238.

Pundir, R. P. S. and Singh, R. B. (1985a). Cytogenetics of F1 hybrids between *Cajanus* and *Atylosia* species and its phylogenetic implications. Theor. Appl. Genet. 71: 216–220.

Pundir, R. P. S. and Singh, R. B. (1985b). Biosystematic relationships among *Cajanus, Atylosia and Rhynchosia* species. Theor. Appl. Genet. 69: 531-534.

Pundir, R. P. S. and Singh, R. B. (1985 c). Crossability relationships among *Cajanus*, *Atylosia* and *Rhynchosia* species and detection of crossing barriers. Euphytica 34: 303-308.

Ratnaparkhe, I. B., Gupta, V. S., Venmurthy, M. R. and Ranjekar, P. K. (1995). Genetic fingerprinting of pigeonpea [*Cajanus cajan* (L.) Millsp.] and its wild relatives using RAPD markers. Theor. Appl. Genet. 91: 893-898.

Reddy, L. J., and De, D. N. (1983). Cytomorphological study in *Cajanus cajan* X *Atylosia lineata.* Indian J. Genet. 43: 96-103.

Reddy, L. J. and Faris, D. G. (1981). A cytoplasmic genetic male-sterile line in pigeonpea. Int. Pigeonpea Newslett. 1: 16–17.

Reddy, L. J. (1981a). Pachytene analyses in *Cajanus cajan* x *Atylosia lineata* and their hybrid. Cytologia 46: 397-412.

Reddy, L. J. (1981b). Pachytene analyses in *Atylosia scarabaeoides* and *Cajanus cajan* X *A. sericea* hybrid. Cytologia 46: 576-577.

Rohlf, F. J. (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software: Setauket, New York.

Saxena, K. B. and Sharma, D. (1990). Pigeonpea. In: Nene, Y. L., Hall, S. D. & Sheila, V. K. (ed.) The Pigeonpea. CAB International, UK, pp. 137-157.

Sivaramakrishnan, S., Kannan, S. and Reddy, L. J. (2002). Diversity in selected wild and cultivated species of pigeonpea using RFLP of mtDNA. Euphytica 125: 21–28.

Smartt, J. (1990). Grain Legumes: Evolution and Genetic Resources. Cambridge University Press, Cambridge, pp. 278-293.

Songok, S., Ferguson, M., Muigai, A. W. and Silim, S. (2010). Genetic diversity in pigeonpea [*Cajanus cajan* (L.) Millsp.] landraces as revealed by simple sequence repeat markers. African J. Biotechnol. 9 (22): 3231-3241.

Stirton, C. H. (1981). Psoraleceae. In: Polhill, R. M. and Raven, P. H. (ed.) Advances in Legume Systematics, Part 2. Royal Botanic Gardens, Kew, UK, pp. 337–343.

Upadhyaya, H. D., Kashiwagi, J., Varshney, R. K., Gaur, P. M., Saxena, K. B., Krishnamurthy, L., Gowda, C. L. L., Pundir, R. P. S. *et al*. (2012). Phenotyping chickpeas and pigeonpeas for adaptation to drought. Frontiers Plant Physiol. 3 (179): 1-10.

Van der Maesen, L. J. G. (1980). India is the native home of the pigeonpea. Miscellaneous papers 19, Landbouwhoge school, Wageningen, pp. 257-262.

Van der Maesen, L. J. G. (1986). *Cajanus* DC. and *Atylosia* Wt. & Arn. (Leguminosae). Agricultural University Wageningen Papers 85 (4): 1–225, Wageningen, Netherlands.

Van der Maesen, L. J. G. (1985). Revision of the genus *Pueraria* DC with some notes on *Teyleria* Backer (Leguminosae), Agricultural University Wageningen Papers 85 (1): 1–132, Wageningen, Netherlands.

Van der Maesen, L. J. G. (1990). Pigeonpea: Origin, history, evolution and taxonomy. In: Nene, Y. L., Halls, D. and Sheila, V. K. (ed.) The Pigeonpea. CAB International, UK, pp. 15–46.

Wasike. S., Okori. P. and Rubaihayo. P. R. (2005) Genetic variability and relatedness of the Asian and African pigeonpea as revealed by AFLP. African J. Biotechnol. 4 (11): 1228-1233.