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Elucidation of Genetic Relationships in the Genus *Cajanus* Using Random Amplified Polymorphic DNA Marker Analysis

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Abstract

In this present investigation, a total of 27 RAPD primers were used to elucidate the genetic relationships between cultivated *Cajanus cajan* cultivars and 10 allied species of primary, secondary and tertiary gene pool, including *C. cajanifolius, C. scarabaeoides, C. platycarpus, C. albicans, C. volubilis, C. sericeus, C. acutifolius, C. lineatus, C. lanceolatus* and *C. reticulates*. All primers showed polymorphism at species level and produced 215 unequivocal polymorphic bands, with an average of 7.96 bands per primer. These polymorphic primers exhibited variation with regard to average band informativeness, resolving power, and showed high polymorphism information content value. No single primer was able to distinguish between all the two cultivars of *C. cajan* and ten allied species of *Cajanus*, but several species specific amplified fragments were observed. The pair wise Jaccard's similarity coefficient values revealed high level of inter-specific genetic variation in the genus *Cajanus*. Cluster analysis exhibited the grouping of two *C. cajan* accessions with *C. cajanifolius* in one cluster, while except *C. platycarpus*, all the nine wild *Cajanus* species belonging to the secondary and tertiary gene pool form another cluster. The present analysis more or less agreed with the sectional classification of the genus *Cajanus,* and it has been hypothesized that cultivated pigeonpea has evolved through multi-genomic interaction through *C. cajanifolius*, and it has experienced minor genomic reorganization during its divergence. Again, identification of species specific amplification pattern substantiated the utility of RAPD markers on selection of suitable species to transfer the desirable trait into cultivated *C. cajan,* through marker aided breeding for its genetic augmentation, and also for the effective management of genetic resources of *C. cajan*.

Keywords: *Cajanus*; Phylogenetic analysis; RAPD

Abbreviations: RFLP: Restriction Fragment Length Polymorphism; RAPD: Random Amplified Polymorphic DNA; AFLP: Amplified Fragment Length Polymorphism; SSR: Simple Sequence Repeat; PIC: Polymorphism Information Content; I_b: Band informativeness; AvI_b: Average Band informativeness; Rp: Resolving power; UPGMA: Unweighted Pair group Method with Arithmetic mean

Introduction

Pigeonpea, botanically known as *Cajanus cajan* (Linn.) Millsp, is an important grain legume crop of the semi-arid tropics, with somatic chromosome complement 2n=2x=22. This legume crop refers as *SKIPPER* of pulse world. It is widely cultivated in Asia and Africa, in addition to some parts of Australia and Latin America [1]. The major centre of world production is undoubtedly India, where pigeonpea is the second most important pulse crop next to chickpea. Among the countries growing this crop, India has the largest area (3.53 million hectares) under pigeonpea cultivation, and also contributes a major share (2.51 million tons), about 75% of world production, with an average yield of 0.78 tons/hectare [2]. Being a monotypic crop with poor cultivation practice in marginal and submarginal land and heterozygous genome structure, the genetic improvement depends upon the alien gene transfer from its secondary and tertiary gene pool [1,3,4], and to selection and perpetuation of useful kind of gene action through conventional recombination breeding [5]. Hence, there is a need for the assessment of genetic relationship between *C. cajan* and its wild allies, the donor source for desired agro-economic traits. Studies on genetic origin of pigeonpea are still unsettled and reports were available in favour of both monophyletic and polyphyletic origin. Studies on morphology [3,4,6], cytology and crossability [7,8], isozymes [9] and nuclear RFLPs [10], suggested a monophyletic origin from *C. cajanifolius*. On the other hand, studies on seed protein profiling [11-13] and nuclear DNA amounts [14], suggested a polyphyletic origin of the *C. cajan.*

In the last couple of decades, the DNA based markers are potentially used for elucidation of phylogenetic relationships across the taxa, in addition to its exploitation in marker aided breeding, molecular tagging and mapping of genes [15]. Among the DNA markers, RAPD based marker system was simple, and are equally reliable for genetic studies because RAPDs are indefinite in number, ubiquitously distributed throughout the genome, and capable of high level polymorphism. RAPD markers have also been widely used in the grain legumes for the depiction of genetic relationships among cultivars, among wild allies, or between cultivars and wild allies [16-25]. In *Cajanus* species, RAPD markers have been employed for genetic fingerprinting, genetic diversity assessment and gene tagging [26-28]. We report here on the utilization of RAPD markers to elucidate and validate the genetic relationships between the cultivated *C. cajan* and ten allied species of *Cajanus,* being the potential donor source of genes during inter-specific breeding programmes.

Materials and Methods

Plant materials

Seeds of two cultivars of pigeonpea(*C. cajan* (L.) Millsp.), BDN-2 and DSLR-17 and 10 wild species (Table 1) were obtained from

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Section	Species	Accession No	Geographical Origin
Cajanus	*C. cajan*	BDN 2	India
	C. cajan	DSLR 17	India
	C. cajanifolius	ICPW 031	India
Atylia	*C. lineatus*	ICPW 041	India
	C. sericeus	ICPW 159	India
Fruticosa	*C. acutifolius*	ICPW 001	Australia
	C. lanceolatus	ICPW 038	Australia
	C. reticulates	ICPW 075	Australia
Cantharospermum	*C. albicans*	ICPW 017	India
	C. scarabaeoides	ICPW 094	Sri Lanka
Volubilis	*C. volubilis*	ICPW 169	India
Rynchosoides	*C. platycarpus*	ICPW 066	India

Table 1: Genotypes of different species of *Cajanus* used in the present study along with their sectional classification [3].

ICRISAT, Patancheru, Andhra Pradesh. The species are maintained in the experimental garden of School of Life Sciences, Jyoti Vihar, Odisha.

DNA extraction and purification

Fresh and young leaf samples of equal quantity (~ 1.2g) were collected for isolation of genomic DNA. Genomic DNA was isolated by using SDS method [29], with few modifications [30]. DNA was dissolved in 10 mM Tris/1 mM EDTA ($T_{10}E_1$) buffer. DNA concentration and purity was measured by using UV-Vis spectrophotometer (UV 1601, Shimadzu, Japan), with $T_{10}E_1$ buffer (pH 8.0). For further confirmation, the quantification of DNA was accomplished by analyzing the purified DNA on 0.8% agarose gel, along with diluted uncut λ DNA as standard. DNA from two cultivars of *C. cajan* and 10 wild species were equilibrated to concentration of 10 ng/µl, using $T_{10}E_1$ buffer.

RAPD marker generation

For RAPD analysis, PCR amplification of 20 ng of genomic DNA, obtained from two cultivars of *C. cajan* and 10 wild species, were carried out using 27 standard decamer oligonucleotide primers (OPA and OPB series; Operon Tech., Alameda, CA, USA), individually [31]. Each amplification reaction mix of 25 µl contained the 30 ng template DNA, 2.5 µl of 10X assay buffer (100 mM Tris.Cl, pH 8.3; 0.5 M KCl; 0.1% gelatin), 2 mM $MgCl_2$, 200 µM each of the dNTPs, 20 ng primers, 1.0 U Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The amplification was carried out in a thermal cycler (Veriti-96, Applied Biosystem, USA), programmed for initial denaturation of 5 min at 94°C; 45 cycles of 2 min denaturation at 94°C, 1 min annealing at 37°C and 2 min elongation at 72°C, and final elongation step of 5 min at 72°C. The PCR products were separated on 1.4% agarose gel for electrophoresis in TAE (40 mM Tris acetate; 2 mM EDTA) buffer at 50 V for 4 h, stained with ethidium bromide and visualized by UV transilluminator (M-15, UVP, Upland, CA 91786, USA). The size of the amplified fragments were determined using 250 bp ladder (B. Genei, Merck Bioscience, India), as molecular weight marker and TOTAL LAB SOLUTIONS-V 2003.02 software. The presence and absence of amplified fragments in each case was scored as '1' and '0', respectively.

Statistical analysis

The information content of RAPD marker system was calculated for each marker and locus using the polymorphism information content {PIC=1-Σ $f^2_{(1-n)}$, where 'f' is the frequency of allele (1-n) } [32], band informativeness {I_b=1- (2×[0.5-p]), where, 'p' is the proportion of the total genotypes having the band}, average band informativeness {Av I_b=Σ I_b/n, where, I_b–Band informativeness and 'n' is the number of markers loci analysed}, and resolving power {Rp=Σ I_b} of the primer [33]. The RAPD marker data was arranged in a binary matrix, with rows corresponding the RAPD banding pattern, and column to the taxa in question (OTUs). The scores were '1' for presence and '0' for absence of a RAPD band. NTSYS-PC 2.02e was used to estimate the similarity matrices among the taxa using Jaccard's coefficient indices. The similarity matrix thus generated was used to construct a dendrogram by Unweighted Pair Group Method with Arithmetic mean (UPGMA), using Sequential, Agglomerative, Hierarchical and Nested (SAHN) method [34], and the confidence limits were measured by squared elucidation distance interval. Again, the Principal Coordinate Analysis (PCoA), based on Jaccard's similarity coefficient was done using eigen vector of NTSYS-PC 2.02e.

Results

Generation of RAPD markers and genotypic characterization

For this purpose, two cultivars of *C. cajan* and ten different species of the genus *Cajanus* were considered. A total of 27 decamer primers (Table 2) were used for RAPD analysis. Amplification of all 27 primers generated 215 unequivocal scorable bands, which are polymorphic at species level (Figures 1a and b). The size of amplification products ranges from 140 bp to 4,030 bp. The maximum of 13 loci were amplified with primer OPA 15, whereas only one loci was observed with primer OPB 04. These 27 polymorphic primers exhibited variation, with regard to average band informativeness (*AvIb*) and resolving power (*Rp*). The primer OPA 16 showed highest *AvIb* (0.675), while OPB 03 and OPB 08 showed lowest *AvIb* of 0.142. The primer OPA 16 showed highest *Rp* (7.426) and the primer OPA 10 showed lowest *Rp* (0.432) values. All the 27 primers exhibited high PIC values. But among them, OPB 03 and OPB 08 showed high PIC (0.994) and OPA 16 showed low PIC (0.806) values. Although all 27 primers were found to be polymorphic, no single primer was able to differentiate the 11 species of *Cajanus*. But,

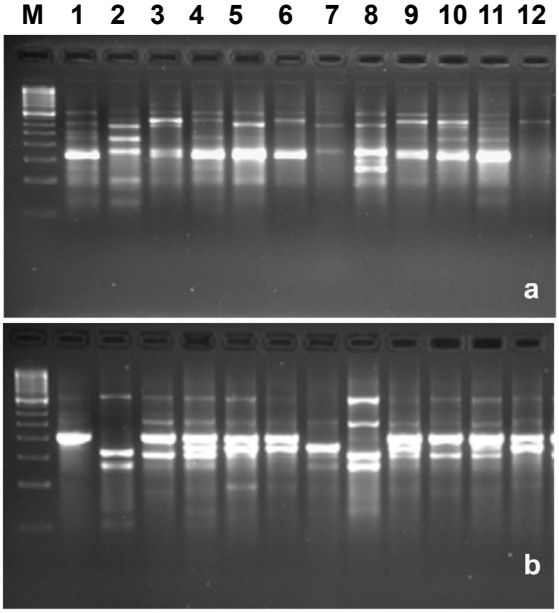

Figure 1: RAPD banding pattern obtained with two cultivars of *C. cajan* and 10 wild species of *Cajanus,* using primer OPA 09 (a) and OPB 06 (b). Lane 'M' represents molecular weight marker (250 bp ladder, B.Genei, Merck Bioscience, India). Lane '1 & 2' represents the cultivars *C. cajan* (DSLR 17 and BDN 2), and Lane '3 to 12' represents the wild allied species *C. cajanifolius, C. scarabaeoides, C. sericeus, C. albicans , C. lineatus, C. volubilis, C. acutifolius, C. platycarpus, C. lanceolatus* and *C. reticulates,* respectively.

Sl. #	Primer	Primer sequence	No. of Loci amplified	No. of polymorphic loci	%Polymorphism	Range (bp)	PIC	Average band Informativeness (Av Ib)	Resolving power (Rp)
1	OPA 01	5'-CAGGCCCTTC-3'	09	09	100%	300-2855	0.975	0.285	2.564
2	OPA 02	5'-TGCCGAGCTC-3'	06	06	100%	450-1485	0.813	0.647	3.882
3	OPA 03	5'-AGTCAGCCAC-3'	11	11	100%	300-1585	0.919	0.454	5.002
4	OPA 04	5'-AATCGGGCTG-3'	07	07	100%	343-1700	0.833	0.658	4.606
5	OPA 05	5'-AGGGGTCTTG-3'	08	08	100%	290-1450	0.954	0.268	2.140
6	OPA 06	5'-GGTCCCTGAC-3'	10	10	100%	140-1490	0.937	0.372	3.722
7	OPA 07	5'-GGTCCCTGAC-3'	12	12	100%	510-1930	0.913	0.443	5.314
8	OPA 08	5'-GTGACGTAGG-3'	11	11	100%	100-1645	0.950	0.389	4.276
9	OPA 09	5'-GGGTAACGCC-3'	08	08	100%	480-1770	0.892	0.429	3.434
10	OPA 10	5'-GTGATCGCAG-3'	03	03	100%	280-640	0.948	0.144	0.432
11	OPA 12	5′ TCGGCGATAG 3′	7	7	100%	395-2023	0.872	0.448	3.142
12	OPA 13	5′ CAGCACCCAC 3′	12	12	100%	395-2250	0.868	0.440	5.284
13	OPA 14	5′ TCTGTGCTGG 3′	3	3	100%	565-1280	0.906	0.476	1.428
14	OPA 15	5′ TTCCGAACCC 3′	13	13	100%	865-3305	0.812	0.55	7.15
15	OPA 16	5′ TTCCGAACCC 3′	11	11	100%	1090-3255	0.806	0.675	7.426
16	OPA 18	5′ AGGTGACCGT 3′	9	9	100%	1225-3635	0.822	0.666	5.996
17	OPA 19	5′ CAAACGTCGG 3′	10	10	100%	1200-4030	0.874	0.428	4.282
18	OPA 20	5′ GTTGCGATCC 3′	10	10	100%	1295-3335	0.880	0.628	6.282
19	OPB 01	5′ GTTTCGCTCC 3′	7	7	100%	1097-3830	0.920	0.469	3.284
20	OPB 02	5′ TGATCCCTGG 3′	12	12	100%	593-3700	0.910	0.5	6
21	OPB 03	5′ CATCCCCCTG 3′	8	8	100%	650-3670	0.994	0.142	1.136
22	OPB 04	5′ GGACTGGAGT 3′	1	1	100%	647	0.919	0.572	0.572
23	OPB 06	5′ TGCTCTGCCC 3′	7	7	100%	530-3485	0.963	0.326	2.282
24	OPB 08	5′ GTCCACACGG 3′	5	5	100%	1237-2047	0.994	0.142	0.71
25	OPB 09	5′ TGGGGGACTC 3′	3	3	100%	1403-2863	0.989	0.19	0.57
26	OPB 10	5′ CTGCTGGGAC 3′	3	3	100%	1860-3020	0.872	0.571	1.714
27	OPB 15	5′ GGAGGGTGTT 3′	9	9	100%	1240-3920	0.974	0.285	2.568

Table 2: Details of RAPD primers (including polymorphic informativeness) used for analysis of genome diversity of *C. cajan species*.

Sl. #	Species with Acc No. or cultivar	Primer	No. of Amplified fragments	Marker (s)
1	*C. cajan* DSLR 17 & *C. cajan* BDN 2	OPA 02, OPA 05, OPA 08	03	OPA 02_{725}, OPA 05_{290}, OPA 08_{1450}
2	*C. cajanifolius* ICPW 031	OPA 05, OPA 09, OPB 06	03	OPA 05_{1020}, OPA 09_{1160}, OPB 06_{530}
3	*C. scarabaeoides* ICPW 094	OPA 03, OPA 04	02	OPA 03_{700}, OPA 04_{955}
4	*C. sericeus* ICPW 159	OPA 03, OPA 13, OPA 15	03	OPA 03_{1183}, OPA 13_{1500}, OPA 15_{865}
5	*C. albicans* ICPW 017	OPA 05, OPA 06, OPA 08, OPA 18, OPA 19	05	OPA $05_{1450,}$ OPA $06_{1490,}$ OPA 08_{860}, OPA 18_{1665}, OPA 19_{1725}
6	*C. lineatus* ICPW 041	OPB 01,OPB 06	02	OPB 01_{1710}, OPB 06_{3530}
7	*C. acutifolius* ICPW 001	OPA 03, OPA 05, OPA 07, OPA 18	04	OPA 03_{1585}, OPA 05_{690}, OPA 07_{1315}, OPA 18_{1525}
8	*C. platycarpus* ICPW 066	OPA 07, OPA 16, OPB 02	03	OPA $07_{1420,}$ OPA 16_{1090}, OPB 02_{3055}
9	*C. lanceolatus* ICPW 038	OPA 10	02	OPA $10_{640,\ 555}$

Table 3: Primer response for the generation of species specific RAPD markers among the 11 *Cajanus* species.

amplification by different primers was informative for the identification of two cultivars of *C. cajan* and eight species of *Cajanus* (Table 3).

Genetic relationships in the genus *Cajanus*

The similarity matrix indices were estimated among 11 species of *Cajanus* using 215 RAPD markers, to quantify the level of polymorphism for inter-specific studies. The pair wise Jaccard's similarity indices values ranged from 0.416 to 0.954 (Table 4), which evidenced large amount of genetic variation exist between the species of *Cajanus* at the genome level. Among all the allied species, *C. cajanifolius* is pretty close to *C. cajan* genotypes, with similarity indices (0.796 and 0.833). The Jaccard's similarity matrix data was utilized to construct a dendrogram. Dendrogram analysis exhibited the clustering of *C. cajan* accessions with *C. cajanifolius* (Section-Cajanus) in one cluster, while rest of the wild *Cajanus* species except *C. platycarpus*, belonging to the tertiary

	C. cajan BDN-2	*C. cajan DSLR-17*	*C. cajanifolius*	*C. scarabaeoides*	*C. sericeus*	*C. albicans*	*C. lineatus*	*C. volubilis*	*C. acutifolius*	*C. platycarpus*	*C . lanceolatus*	*C . reticulates*
C. cajan BDN-2	1.000											
C. cajan DSLR-17	0.954	1.000										
C. cajanifolius	0.796	0.833	1.000									
C. scarabaeoides	0.667	0.700	0.740	1.000								
C. sericeus	0.569	0.569	0.661	0.731	1.000							
C. albicans	0.640	0.640	0.714	0.761	0.740	1.000						
C. lineatus	0.653	0.654	0.725	0.848	0.784	0.782	1.000					
C. volubilis	0.653	0.620	0.660	0.739	0.623	0.791	0.684	1.000				
C. acutifolius	0.607	0.640	0.714	0.761	0.673	0.773	0.674	0.674	1.000			
C. platycarpuss	0.416	0.416	0.458	0.523	0.460	0.561	0.478	0.657	0.561	1.000		
C. lanceolates	0.634	0.667	0.775	0.867	0.764	0.884	0.808	0.778	0.841	0.558	1.000	
C. reticulates	0.640	0.673	0.750	0.884	0.740	0.857	0.864	0.750	0.773	0.524	0.928	1.000

Table 4: Jaccard's similarity indices based on 1-0 binary matrix of RAPD marker data generated for two *C. cajan* cultivars and 10 wild species in the genus *Cajanus.*

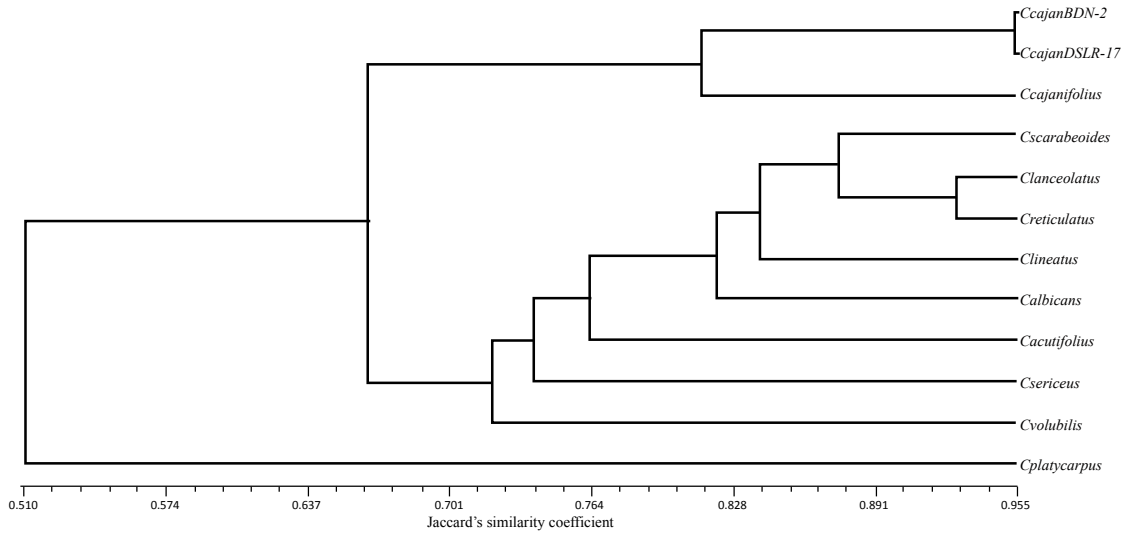

Figure 2: Dendrogram showing the clustering of two cultivars of *C. cajan* and 10 allied species within the genus *Cajanus,* based on the Jaccard's similarity coefficients using RAPD banding pattern.

gene pool, formed another cluster respectively (Figure 2). However, two species (*C. lanceolatus* and *C. reticulates*), belonging to section *Fruticosa* were clubbed together with similarity indices 0.928. Similarly, species belonging to *Atylia* (*C. lineatus* and *C. sericeus*), *Cantharospermum* (*C. albicans* and *C. scarabaeoides*) and *Fruticosa* (*C. acutifolius, C. lanceolatus* and *C. reticulates*) also form close clusters. *C. sericeus* (sec. *Atylia*) shows greater similarity with *C. volubilis* (sec. *Volubilis*) than with *C. lineatus* of the same section. *C. platycarpus* (sec. *Rhynchosoides*) is found to be out grouped. These clustering patterns were further supported by two and three dimensional principal coordinate analysis (Figures 3a and b).

Discussion

C. cajan is the only domesticated species in the subtribe *Cajaninae* (genus-*Cajanus*), and is predominantly self-pollinated. So, its genetic augmentation has been restricted to selection and hybridization within cultivars. With gradual realization that wild relatives of *C. cajan* are potential sources for its genetic improvement, attempts were made for wide hybridization, to introduce some desirable traits into *C. cajan*. However, this requires the knowledge of species affinities and the phylogenetic relationships *inter se*. RAPD based DNA markers has been extensively utilized to deduce the genetic relatedness of plant cultivars and plant populations, as well as the inter- and intra-specific genetic relationships between plant species [15-25]. This is due to the simplicity of this technique, as only very small amounts of DNA are required, and information on template DNA sequence is not needed [31].

In the present investigation, the RAPD marker used not only for the elucidation of genetic relationship in the genus *Cajanus,* but also for the identification of species specific RAPD markers in the genus *Cajanus*, at least for the 11 species used. All the responded 27 primers were found

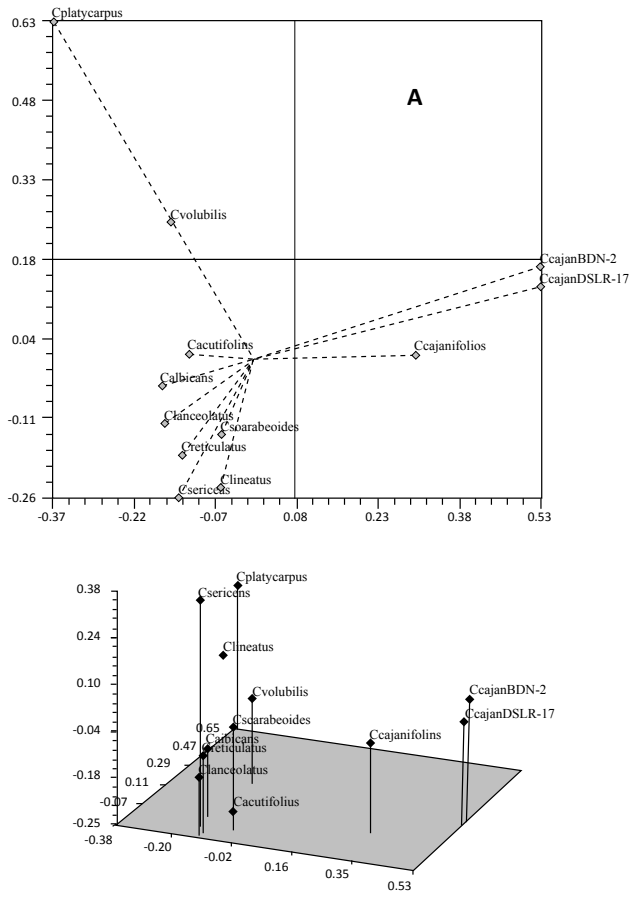

Figure 3: Principal coordinate analysis (2-D View (a) and 3-D View (b)) of two cultivars of *C. cajan* and 10 allied species within the genus *Cajanus,* based on the Jaccard's similarity coefficients using RAPD banding pattern.

to be polymorphic at species level and generated 215 unequivocal scorable polymorphic bands. The size of amplification products ranges from 140 bp to 4,030 bp. Maximum 13 loci were amplified with one primer OPA 15, whereas minimum one amplicon was observed with the primer OPB 04. The *PIC*, *AvIb* and *Rp* values were varied from one primer to another. The RAPD markers OPA 02_{725}, OPA 05_{290}, OPA 08_{1450}, OPA 05_{1020}, OPA 09_{1160}, OPB 06_{530},OPA 03_{700}, OPA 04_{955}, OPA 03_{1183}, OPA 13_{1500}, OPA 15_{865}, OPA $05_{1450,}$ OPA $06_{1490,}$ OPA 08_{860}, OPA 18_{1665}, OPA 19_{1725}, OPB 01_{1710}, OPB 06_{3530},OPA 03_{1585}, OPA 05_{690}, OPA 07_{1315}, OPA 18_{1525}, OPA $07_{1420,}$ OPA 16_{1090}, OPB 02_{3055} and OPA $10_{640,555}$ were unique to different species of *Cajanus,* while OPA 02_{725}, OPA 05_{290} and OPA 08_{1450} were unique to the *C. cajan* cultivars used in the present study. Several RAPD markers have detected for the identification of pigeonpea cultivars, as well as the allied species of *Cajanus* [25]. However, the primer set, cultivar set and allied species are different in both studies. Recently, species specific RAPD and ISSR markers have detected to differentiate *C. cajan* and *C. scarabaeoides* [35], and SSR markers to differentiate *C. cajan* and *C. cajanifolius* [36]. No other information was available on the identification of pigeonpea cultivars and wild species at DNA level. As a result, pigeonpea breeding relies heavily on a phenotypic selection method. Secondly, pigeonpea is one of the exceptions among grain legumes, in that it has tendency towards out crossing. As result of frequent outcrossing, existing standard cultivars have become heterogeneous for several important agronomic characters, such as pest and disease resistance. For instance, the cultivar '*Bahar*' lost its resistance to sterility mosaic disease, perhaps as a result of out crossing with susceptible cultivars [37]. The maintenance of germplasm in pigeonpea is very tedious, and problems of contamination have been enormous. The identification of DNA markers for specific traits in pigeonpea will, therefore, be helpful in assessing the purity and stability of the genotypes entering into the breeding programme. Similarly, the species could clearly be distinguished, with as few as one selected primer, or with polymorphic amplicons. These species specific markers may also be utilized to track the introgressive wide hybridization programme for the genetic augmentation in pigeonpea.

The phylogenetic analysis in the genus *Cajanus* is equivocal. Studies based on morphology [3-4,6], cytology and crossability [7], isozymes [9], nuclear RFLPs [10] and r-DNA analysis [38], suggest a monophyletic origin from *C. cajanifolius*. On the other hand, the seed storage protein profiles [11-13] and nuclear DNA amounts [14] suggest a polyphyletic origin of the cultigens. In the present study, eleven species representing the genus *Cajanus* (including cultigen *C. cajan*) showed high genetic variation, ranging from 0.416 to 0.954. The low similarity indices might be due to non-response of four species, *C. volubilis, C. platycarpus, C. reticulates* and *C. lanceolatus* to five primers for amplification. However, earlier studies reported the similarity indices value of 0.22 to 0.801 among eight wild species of *Cajanus,* including *C. acutifolius*, *C. albicans*, *C. goensis*, *C. grandifolius*, *C. sericeus*, *C. volubilis*, *C. lineatus*, *C. reticulates*, and five species of three genera *Dunbaria*, *Rhynchosia* and *Flemingia* [25]. This incongruence in the result might be due to the different set of primers, different species, and different genotypes taken into consideration. However, the variation between two cultivated accessions is much less than their differences with *C. cajanifolius* pattern. The allied species showed varying degrees of similarity with the cultigens, indicating their relative genetic closeness to *C. cajan*. This substantiates the gene introgression from allied species (which is still effective) has also taken place during the course of evolution of pigeonpea in the genus *Cajanus* [13]. Further diversification of cultivated species might be due to structural alterations of the genome and environment specific expression of house keeping genes.

On the basis of RAPD banding pattern and Jaccards similarity indices, a dendrogram was constructed (SAHN), which exhibited the clustering of *C. cajan* accessions with *C. cajanifolius* (Section-Cajanus) in one cluster, while species belonging to the section *Cantharospermum (C. albicans* and *C. scarabaeoides), Atylia* (*C. lineatus* and *C. sericeus*), *Fructicosa* (*C. acutifolius*, *C. lanceolatus*, *C. reticulates*), and *Volubilis* (*C. volubilis*) form separate cluster. The species *C. platycarpus* belongs to section *Rhynchosoides,* out grouped by maintaining equidistant from species of both clusters. The clustering pattern was also supported by two dimensional and three dimensional principal coordinate analyses. RAPD banding pattern indicated *C. reticulates* and *C. lanceolatus* are close to each other than to *C. acutifolius* and, *C. acutifolius,* showed equal sharing of RAPD bands with the species of the section *Fructicosa, Atylia* and *Cajanus,* including *C. cajan*. Likewise, a study based on esterase zymogram [9], also showed a close relationship between *C. albicans* and *C. scarabaeoides* and close homology between *C. cajan* and *C. cajanifolius*, while the pattern of *C. platycarpus* was found to be distinctly different from other species. However, the clustering of *C. lineatus* and *C. sericeus* (belongs to section Atylia) into a single cluster was also reported earlier [25]. This variation might be due to different set of primers used in both studies. The overall cluster pattern based on RAPD banding pattern partially supports the sectional classification of the genus [3]. These results also agreed with nuclear RFLPs based parsimony analysis [10], and seed albumin and globulin profiling [13]. Again in the present study, *C. platycarpus* is out grouped and justified its

status in the tertiary gene pool [3]. However, the results of present study were at variance with crossability relationships and DNA content studies [8,14], and it might be due to the genetic divergence during the course of evolution under selection pressure. The evidences obtained from different studies till now, including the present study, unequivocally support *C. cajanifolius* to be the closest relative of cultivated pigeonpea [3,4,6,7,9-11,13]. RAPD banding pattern shared several homology not only with *C. cajanifolius,* but also with several allied species, including *C. scarabaeoides, C. lineatus, C. albicans, C. volubilis* and *C. sericeus.* These results based RAPD pattern, similarity indices and clustering pattern substantiated that *C. cajan* and *C. cajanifolius* are a product of multigenomic interaction, at least involving *C. scarabaeoides,* and both had experienced minor genomic reorganization during its divergence, due to existing gene flow within the genus.

Again this study presented the suitability of RAPD marker as potential tool on elucidation of genetic relationship and species differentiation. Future studies on genetic association and validation of identified species specific RAPD markers led to a platform for transfer of the desirable agronomic traits into cultigen *C. cajan* for its genetic augmentation, at least by involving two species *C. cajanifolius* and *C. scarabaeoides*.

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