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Genetic analysis of wild and cultivated germplasm of pigeonpea using random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) markers

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The reliability of the quantification of genetic diversity using only one type of marker has been questioned as compared to the combined use of different markers. To compare the efficiency of the use of single versus multiple markers, the genetic diversity was quantified among 12 diverse pigeonpea germplasm comprised of eight wild and four cultivated using both random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers, and how well these two types of markers discriminated the diverse pigeonpea germplasm was evaluated. The pigeonpea germplasm including eight wild species and four cultivated varieties was subjected to 40 RAPD and 40 microsatellite primers. The level of polymorphism as revealed by RAPD primers produced a total of 517 DNA fragments and all were found to be polymorphic that is, 100% and in SSR analysis 101 fragments were produced that too showed 100% polymorphism. The high similarity index value revealed by RAPD was 0.931 between GT-100 and ICPL-87 whereas through SSR, it was 1.00 between GTH-1 and GT-100 as well as *Rhyncosia rothi* and *Rhyncosia minima*. The least similarity index value revealed by RAPD (*R. rothi and* GTH-1) and SSR (*Rhyncosia bracteata* and ICPL-87) were 0.07 and 0.133, respectively. Using RAPD marker, the calculated arithmetic mean heterozygosity and the marker index were 0.90 and 22.47, respectively. The *R. bracteata* and ICPL-87 were found distinct from rest of other cultivars by showing only 13% similarity. Average PIC value shown by RAPD and SSR primers were found to be 0.90 and 0.18, respectively.

Key words: Pigeonpea, random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) markers.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a grain legume of the *Cajaninae* sub-tribe of the economically important leguminous tribe *Phaseoleae* (Young et al., 2003). The genus *Cajanus* comprises 32 species most of which are found in India (18) and in Australia (13), although one is native to West Africa. Pigeon pea is the only cultivated food

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Abbreviations: RAPD, Random amplified polymorphic DNA; **STR,** simple tandem repeats; **SSR,** simple sequence repeats; **CTAB,** cetyl trimethyl ammonium bromide; **PCR,** polymerase chain reaction; **PIC,** polymorphic information content; **MI,** maker index.

crop of the *Cajaninae* sub-tribe and has a diploid genome comprising 11 pairs of chromosomes (2n = 22) (Greilhuber and Obermayer, 1998). Wild relatives have now been reported to possess many agronomically important traits such as resistance to pests and diseases (Reddy et al., 1996). *Rhynchosia bracteata* Benth possesses resistance to pod fly damage (Sharma et al., 2003), which would be useful in cultivated pigeonpea for breeding. Genetic diversity has an important role in plant breeding programme. To test genetic resources for their productivity, quality parameters and stress tolerance, field trials are usually time consuming, therefore, molecular markers and DNA technology are used to assess diversity in the gene pool to identify genes of interest and to develop a set of markers for screening progeny (Karp et al., 1996).

Random amplified polymorphic DNA (RAPD) markers have been used for numerous applications in plant molecular genetics research despite having disadvantages of poor reproducibility and not generally being associated with gene regions (Welsh et al., 1990; Williams et al., 1990). RAPD, being a multi locus marker (Karp et al., 1997) with the simplest and fastest detection technology, have been successfully employed for determination of intraspecies genetic diversity in several grain legumes. These include *Vigna unguiculata* (Ba et al., 2004), *Vigna radiate* (Souframanien et al., 2004), *Lens* sp. (Sharma et al., 1995; Ahmad et al., 1996), *Phaseolus* sp. (Beebe et al., 2000; Chiorato et al., 2007), *Glycine* sp. (Jeffrey et al., 1998; Barroso et al., 2003), *Cicer* sp. (Ahmad, 1999), *Pisum* sp. (Cheghamirza et al., 2002; Taran et al., 2005) and *C. cajan* (Kotresh et al., 2006; Ratnaparkhe et al., 1995; Choudhury et al., 2008).

Although known by many names and acronyms, including simple tandem repeats (STR), microsatellites and simple sequence repeats (SSR), SSR have received considerable attention and are probably the current marker system of choice for marker based genetic analysis and marker assisted plant breeding (Akkaya et al., 1992). Only 20 SSRs have been previously reported in pigeonpea, of which only half were polymorphic in cultivated pigeonpea germplasm (Burns et al., 2001). In contrast, more than 1000 microsatellites have been mapped in soybean [*Glycine max* (L.)] (Song et al., 2004), and several hundred are available in chickpea (*Cicer arietinum* L.; Lichtenzveig et al., 2005), common bean (*Phaseolus vulgaris* L.; Blair et al., 2003) and groundnut (*Arachis hypogaea* L.; Ferguson et al., 2004).

SSR are generally among the most reliable and highly reproducible among molecular markers. Indeed SSR are now widely recognized as the foundation for many framework linkage maps. SSR have played a critical role even in merging disparate linkage maps, since they define specific locations in the genome unambiguously (Bell and Ecker, 1994; Akkaya et al., 1995). Hence, considering the potential of the DNA marker based genetic diversity analysis, the present study was aimed to analyze the genetic diversity among the diverse germplasm of pigeonpea as well as to evaluate the relative usefulness of RAPD and SSR markers for diversity analysis.

MATERIALS AND METHODS

A total of 12 germplasm including eight wild *viz*.,*Cajanus scarabaeoides*, *Cajanus platycarpus, Cajanus cajanifolius*, *Rhyncosia rufescence, Rhyncosia minima, R. bracteata, Rhyncosia canna* and *Rhyncosia rothi* and four cultivated namely GTH-1, GT-100, ICPL-87 and GT-1 were procured from the Centre for Excellence for Research on Pulses, S.D Agricultural University, S.K. Nagar (Gujarat) India. First, 25-30 seeds of each cultivar were treated with 0.1% carbendazim (fungicide), washed thoroughly with autoclaved distilled water and sown in earthen pots containing sand: soil : farmyard manure (1:1:1).

Isolation of genomic DNA

For genomic DNA isolation, about 500 mg of fresh leaf material from 4 to 5 week old seedlings of each of the 12 cultivars were ground to fine powder in liquid nitrogen and DNA was isolated following the cetyl trimethyl ammonium bromide (CTAB) method as described by Rogers et al. (1980) with some modification. The quality and concentration of the extracted DNA was estimated by spectrophotometer and the samples were diluted to a final concentration of 30 ngμl^{-1}.

RAPD and SSR based marker analysis

The 40 primers each of RAPD (10-mer primers) and SSR procured from Bangalore Genie, Bangalore, India, were screened for the genomic DNA extracted for polymorphism survey. However, finally eight random primers *viz*., OPA-07, OPA-09, OPA-10, OPA-11, OPA-12, OPA-14, OPA-16 and OPA-19 and 5 SSR primers namely CCB1, CCtta001, CCat001, CCtta003 and CCtta005 were found to be polymorphic and hence, taken for further analysis (Table 1). Polymerase chain reaction (PCR) reactions were carried out in a 25 µl reaction volume containing 1 unit of Taq DNA polymerase, 30 ng of tempelate DNA, 0.2 µM of primer, 2 µl of each of dATP, dCTP, dGTP and dTTP, 1x PCR reaction buffer. Amplifications were performed in a DNA thermocycler (Eppendorf, Hamburg, Germany), programmed for initial denaturation for 3 min at 94°C, followed by 40 consecutive cycles of 30 s at 94°C, 1 min at 35°C and 72°C for 1 min and final extension at 72°C for 10 min. The annealing temperatures of the cycling parameter were readjusted for each microsatellite primers according to their calculated Tm based on the sequence composition: Tm =4°(G+C) + 2° (A+T)-3°C. The amplified products were subjected to electrophoresis in 1.2 % agarose gel in TAE [tris acetate ethylene diamine tetra-acetic acid (EDTA)] buffer running at 80 v for 2.5 h. The gels were stained using ethedium bromide and documented using Alpha Imager-1200^{TM} (Alpha Innotech, San Leandro, USA). Duplicated independent DNA preparations for each sample were done and only major bands consistently amplified were scored.

Data scoring and statistical analysis

Data analysis was carried out only for those primers that gave scorable patterns. Data were scored for computational analysis on the basis of the presence or absence of the PCR products. If a product was present in a genotype, it was designated as 1 and, 0 if absent. The data were maintained in the spreadsheet format for

Table 1. List of polymorphic RAPD and SSR primers along with their nucleotide sequence.

S/N	RAPD primer			SSR primer		
	Primer	Primer sequence	GC content %	Primer	Primer sequence	GC content %
1	OPA-07	5'GAAACGGGTG3'	60	CCB1	F: AAGGGTTGTATCTCCGCGTG	55
					R: GCAAAGCAGCAATCATTTCG	45
2	OPA-09	5'GGGTAACGCC3'	70	CCtta001	F: TTCTGGATCCCTTTCATTTTTC	36
					R: TGACACCCTTCTACCCCATAA	47
3	OPA-10	5'GTGATCGCAG3'	60	CCat001	F: CTTCCCCCAACTAAGATCCA	50
					R: GTTCGTTCTCTTTAATTGACTTGC	37
4	OPA-11	5'CAATCGCCGT3'	60	CCtta003	F: CCAAGAAAAGGTGCTCCAAGT	47
					R: TTGCTTCTTTTCTCGCTTGC	45
5	OPA-12	5'TCGGCGATAG3'	60	CCtta005	F: TCTTCCATTGCATGGTGTT	42
					R: GCATGATATGAGATGATGACGA	40
6	OPA-14	5'TCTGTGCTGG3'	60			
7	OPA-16	5'AGCCAGCGAA3'	60			
8	OPA-19	5'CAAACGTCGG3'	60			

further analysis. The data were entered into binary matrix and subsequently analyzed using NTSYSpc version 2.02*i* (Rohlf, 1994). Coefficients of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by agglomerative technique using the unweighted pair group method with arithmetic mean (UPGMA) method by SAHN clustering function of NTSYS-pc. Relationships between the pigeonpea germplasm were graphically represented in the form of dendrograms. The cophenetic correlation analysis was carried out using the COPH function of NTSYS-pc. In this method, the dendrogram and similarity matrix were correlated to find the goodness-of-fit of the dendrogram constructed based on the similarity coefficients. The correspondence between the SSR and RAPD based on similarity coefficient matrices was tested based on cophenetic correlation analysis and Mantel matrix correspondence test. The Mantel matrix correspondence test was carried out using the MXCOMP function in the NTSYSpc version2.02*i*. The expected heterozygosity (Hn), arithmetic mean heterozygosity (Hav) and marker index (MI) were calculated using the methodology of Nei (1973) and Powell et al. (1996). The expected heterozygosity, Hn for a molecular marker was calculated as, $Hn = 1-\sum Pi2$, where pi is the allele frequency of the i^{th} allele. The arithmetic mean heterozygosity, Hav was calculated for each marker class as, $Hav = \sum Hn/n$, where n is the number of markers or loci analyzed. Marker index (MI) is calculated as, MI = E(Hav)p, where E is the effective multiplex ratio ($E = n\beta$, where β is the fraction of polymorphic markers or loci).

RESULT

Genetic relatedness was studied among 12 germplasm of Pigeonpea using RAPD and SSR markers, so that genetically distinct germplasm showing different responses towards *Helicoverpa armigera* can be utilized in breeding for *H. armigera* for generating mapping populations to be used to identify genes or quantitative trait loci. The banding pattern thus obtained by both RAPD and SSR primers clearly distinguished cultivars into different clusters showing sufficient diversity.

Polymorphism survey using RAPD and SSR markers

In pooled RAPD analysis, all eight arbitrary oligonucleotide primers finally selected out of total 40, generated a total of 517 scorable bands with 150 loci. Among them all, 150 loci were found polymorphic, showing 100% polymorphism (Table 2). The size of the amplified products ranged from 65 to 3607 bp. A representative RAPD profile obtained by primer OPA-10 is shown in Figure 1.

Out of a total of 22 loci (289-1511bp), all 22 were polymorphic (100%). In case of SSR analysis, CC based five microsatellite (SSR) primers out of total 40, were used to analyze the genetic diversity among 12 pigeonpea germplasm (Table 3). The SSR analysis with five microsatellite primers produced a total of 12 alleles. Among these five primers, maximum amplified allele size of 250 bp was generated by CCtta-005 and minimum amplified allele size of 71 bp by CCtta001 marker. A maximum of three alleles were recorded for primers CCtta-003 and CCtta-005 (Figure 2), while primers CCB-1, CCat-001 and CCtta-001 produced two alleles each, which were the lowest in the present investigation.

Cluster analysis

The polymorphic information content (PIC) values, a reflection of allele diversity and frequency among the germplasm, were uniformly higher for all the RAPD loci tested. The PIC value ranged from 0.78 (OPA-12) to 0.94 (OPA-19) with a mean of 0.98. The similarity coefficients based on RAPD ranged from 0.070 to 0.931 with an average value of 0.501. The least similarity index value revealed by RAPD was 0.07 between *R. rothi and* GTH-1 whereas arithmetic mean heterozygosity and the marker

Table 2. Summary of genetic diversity study using RAPD analysis.

S/N	Locus name	Number of band	Total loci	Polymorphic loci	Percentage polymorphism	PIC value	Molecular weight range (bp)
1	OPA-07	63	21	21	100	0.92	147-1778
2	OPA-09	74	23	23	100	0.93	111-3204
3	OPA-10	84	22	22	100	0.93	289-1511
4	OPA-11	82	23	23	100	0.93	112-2435
5	OPA-12	28	8	8	100	0.78	65-1850
6	OPA-14	60	20	20	100	0.91	73-3607
7	OPA-16	37	11	11	100	0.86	212-1891
8	OPA-19	89	22	22	100	0.94	173-3230
	Total	517	150	150	100	7.2	-

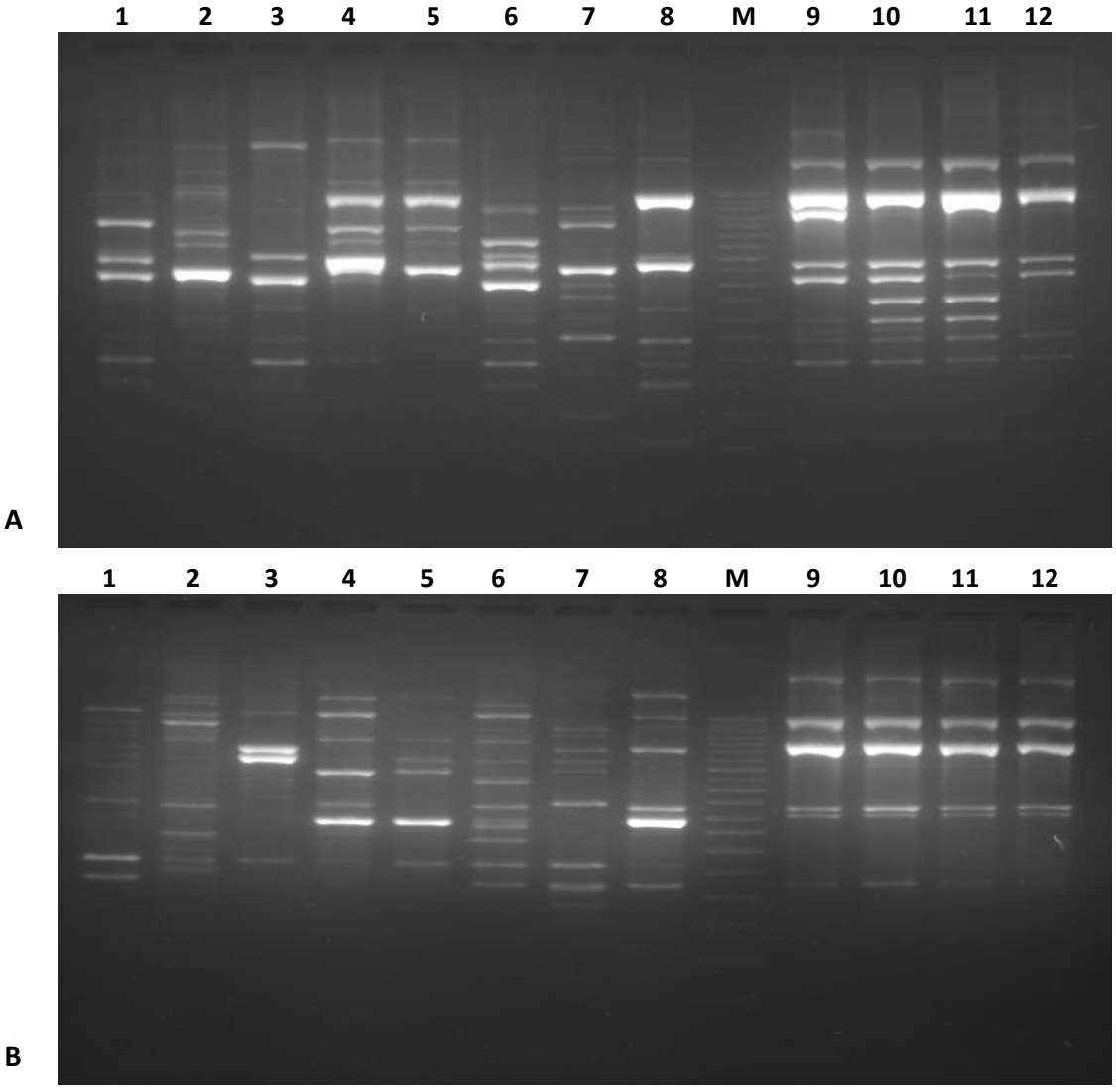

Figure 1. RAPD profiles of the eight wild and four cultivated germplasms. Lanes 1 to 8 represent wild germplasm; M, ladder and 9 to 12 cultivated germplasms; A, OPA -10; B, OPA-11; C, OPA-14; D, OPA-19. 1, *Cajanus scarabaeoides*; 2, *Rhyncosia bracteata*; 3,*Cajanus cajanifolius*; 4, *Cajanus platycarpus*; 5, *Rhyncosia rothi*; 6, *Rhyncosia canna*; 7, *Rhyncosia minima*; 8, *Rhyncosia rufescence*; M-50 bp ladder, 9-GTH-1, 10-GT-100, 11-ICPL-87, 12-GT-1.

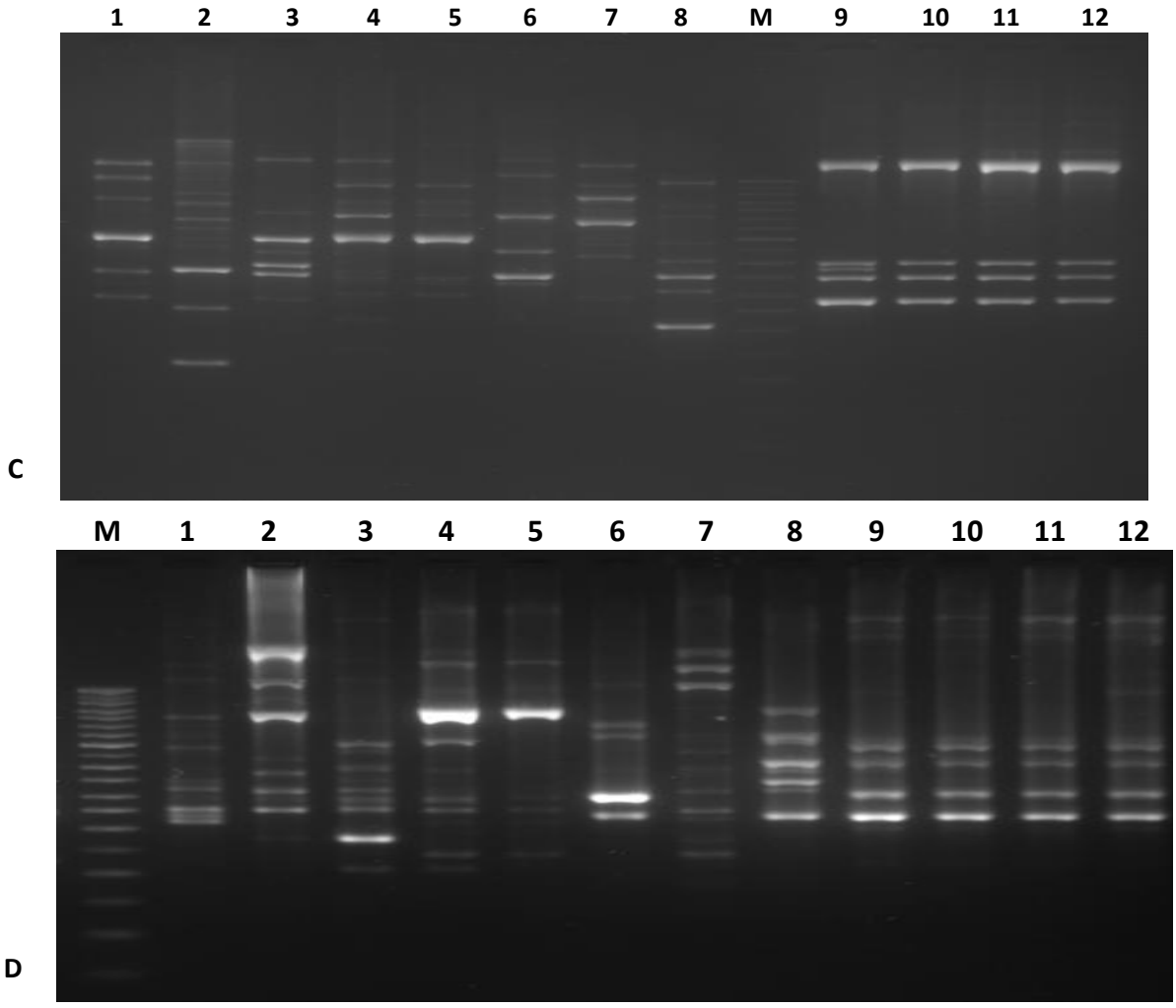

Figure 1. Contd.

Table 3. Summary of genetic diversity study usingSSR analysis.

S/N	Locus name	Number of bands amplified	Molecular weight range (bp)	Difference in bp	Total number of alleles	PIC value
1	CCB-1	8	110-206	96	2	0.25
2	CCat-001	18	101-222	121	2	0.25
3	CCtta001	12	71-119	48	2	0.076
4	CCtta-003	15	87-187	100	3	0.14
5	CCtta-005	14	156-250	94	3	0.19

index were 0.90 and 22.47, respectively (Table 2).

Clustering pattern of dendogram generated by using the pooled molecular data of eight RAPD loci indicated that two clusters namely A and B are formed at a similarity coefficient of 0.14 (Table 4). Cluster A was again divided into two sub clusters A1 and A2. First, A1 sub cluster formed two sub clusters A1a and A1b, in which cluster A1a included *C. scarabaeoides*, *C. cajanifolius*, *R. rufescence* and *R. canna*. In this cluster, the *C. scarabaeoides* and *Cajanus cajanifolius* showed 37% similarities with the other wild germplasm of the cluster A1a. The second minor sub cluster A1b included all cultivars viz., GTH-1, GT-100, ICPL-87 and GT-1. In this cluster, GT-100 and ICPL-87 showed 93% similarities

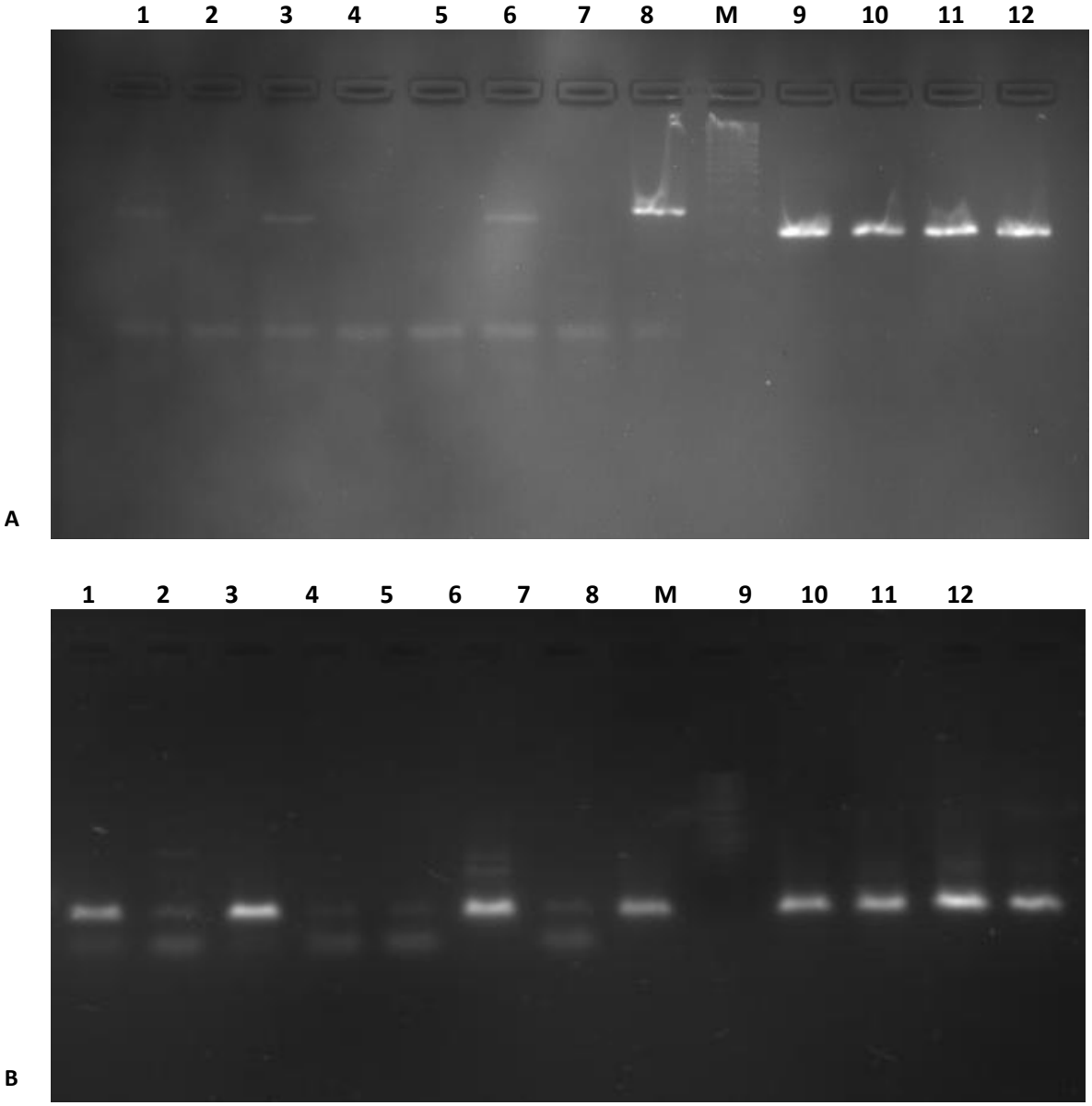

Figure 2. SSR profile of the eight wild and four cultivated germplasms. Lanes 1 to 8 represent wild germplasm; M, ladder and 9 to 12 cultivated germplasms. A, CCtta-003; B, CCtta005. 1, *Cajanus scarabaeoides*; 2, *Rhyncosia bracteata*; 3, *Cajanus cajanifolius*; 4, *Cajanus platycarpus*; 5, *Rhyncosia rothi*; 6, *Rhyncosia canna*; 7, *Rhyncosia minima*; 8, *Rhyncosia rufescence*; M-50 bp ladder, 9-GTH-1, 10-GT-100, 11-ICPL-87, 12-GT-1.

with the other cultivars. Sub cluster A2 included *R. minima* only. The second major cluster B formed two sub clusters in which first sub cluster B1 included *R. bracteata*. Second sub Cluster B2 contained *C. platycarpus* and *R. rothi*, which was found to be more closely related and showed 59% similarity (Figure 3).

In case of SSR analysis, the highest PIC value was recorded for CCB-1 (0.25), CCat-001 (0.25) and the lowest for CCtta001 (0.076) (Table 3). The highest similarity index value of 1.00 was found between *R. rothi* and *R. minima* as well as between GTH-1 and GT-100, while the least similarity index value of 0.13 was between *R. bracteata* and ICPL-87 (Table 5). The arithmetic mean heterozygosity and the marker index were 0.18 and 33.66, respectively.

Clustering pattern of dendogram generated by using the pooled molecular data of five SSR primers indicated that two clusters namely A and B are formed at a similarity coefficient of 0.18. Cluster A was again divided into two sub clusters A1 and A2. First A1 sub cluster formed two minor sub clusters A1a and A1b. Cluster A1a was further subdivided into A1a(1) and A1a(2) in which first minor sub cluster A1a(1) included *C. scarabaeoides*, *R. rufescence* and *C. cajanifolius*. This further indicated that

Table 4. Similarity matrix for jaccard's coefficient for 12 wild and cultivated pigeonpea germplasm based on RAPD analysis.

	C. scarabaeoides	*R. bracteata*	*C. cajanifolius*	*C. platycarpus*	*R. rothi*	*R. canna*	*R. mini*	*R. rufescence*	GTH-1	GT- 100	ICPL- 87	GTH-1
C. scarabaeoides	1.000											
R. bracteata	0.140	1.000										
C. cajanifolius	0.372	0.125	1.000									
C. platycarpus	0.188	0.137	0.213	1.000								
R. rothi	0.152	0.166	0.149	0.591	1.000							
R. canna	0.328	0.189	0.305	0.172	0.140	1.000						
R. minima	0.208	0.153	0.200	0.151	0.166	0.160	1.000					
R. rufescence	0.359	0.116	0.315	0.197	0.136	0.298	0.170	1.000				
GTH-1	0.269	0.112	0.210	0.125	0.070	0.177	0.186	0.283	1.000			
GT- 100	0.274	0.113	0.239	0.141	0.086	0.179	0.157	0.305	0.851	1.000		
ICPL- 87	0.258	0.110	0.242	0.142	0.088	0.181	0.175	0.291	0.829	0.931	1.000	
GT- 1	0.271	0.120	0.235	0.148	0.092	0.180	0.180	0.280	0.840	0.860	0.920	1.000

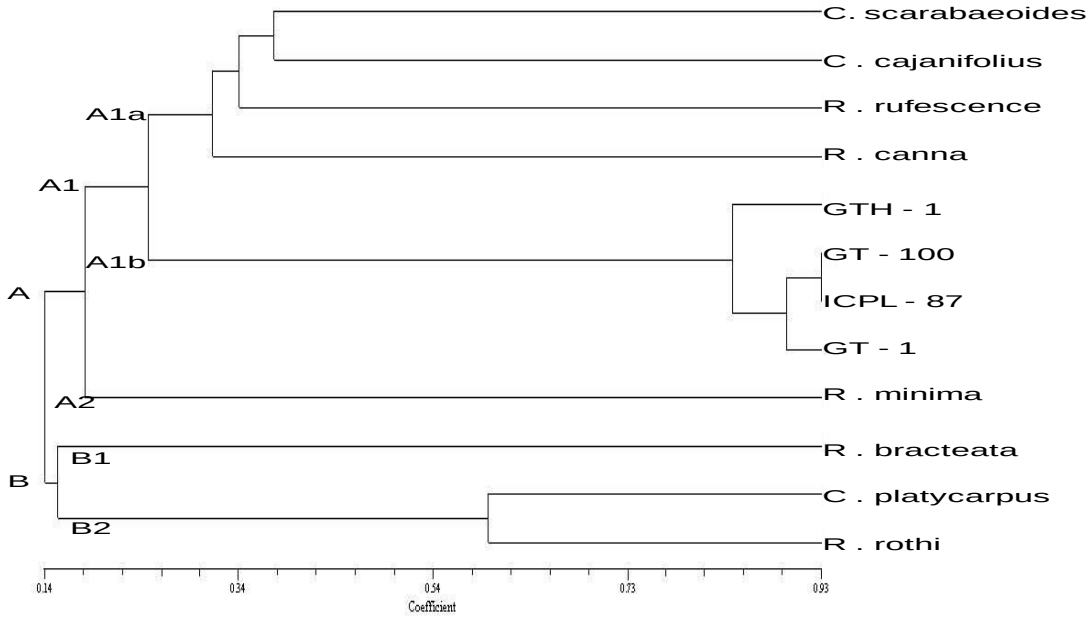

Figure 3. Dendogram showing clustering of 12 pigeonpea germplasm constructed using UPGMA based on Jaccard's coefficient obtained from RAPD analysis.

Table 5. Similarity matrix for Jaccard's coefficient of SSR for 12 pigeonpea germplasm.

	C. scarabaeoides	*R. bracteata*	*C. cajanifolius*	*C. platycarpus*	*R. rothi*	*R. canna*	*R. minima*	*R. rufescence*	GTH-1	GT- 100	ICPL- 87	GT- 1
C. scarabaeoides	1.000											
R. bracteata	0.181	1.000										
C. cajanifolius	0.888	0.200	1.000									
C. platycarpus	0.666	0.250	0.750	1.000								
R. rothi	0.333	0.166	0.375	0.500	1.000							
R. canna	0.818	0.250	0.727	0.545	0.272	1.000						
R. minima	0.333	0.166	0.375	0.500	1.000	0.272	1.000					
R. rufescence	0.900	0.166	0.800	0.600	0.300	0.750	0.300	1.000				
GTH-1	0.615	0.142	0.538	0.500	0.250	0.533	0.250	0.692	1.000			
GT- 100	0.615	0.142	0.538	0.500	0.250	0.533	0.250	0.692	1.000	1.000		
ICPL- 87	0.571	0.133	0.500	0.461	0.230	0.600	0.230	0.642	0.923	0.923	1.000	
GT- 1	0.727	0.166	0.636	0.600	0.300	0.615	0.300	0.818	0.833	0.833	0.769	

two wild germplasms *C. scarabaeoides* and *R. rufescence* are more closely related and showed 90% similarity, while second minor sub cluster A1a(2) contained *R. canna* and *C. platycarpus* include sub cluster A1b. Second sub cluster A2 contained all cultivated germplasm *viz*., GTH-1, GT-100, ICPL-87 and GT-1. The cluster A2 contained GTH-1 and GT-100 which were highly close related and showed 100% similarity, while GT-100 and ICPL-87 showed 92% similarity. Second major Sub cluster B included *R. rothi* and *R. minima* which were grouped in one cluster and showed 100% similarity and the remaining *R. bracteata* did not form clustering (Figure 4).

DISCUSSION

The wild relatives of pigeonpea have considerable diversity. These may possess useful genes controlling economically important traits. These cultivars may serve as germplasm source of new genes in pigeonpea for *H. armigera* resistance. In the present investigation, RAPD primers were used to study the genetic diversity of 12 pigeonpea germplasm. Out of 40, eight primers showed 100% polymorphic bands. Similar results were reported by Choudhury et al. (2008) where they found the level of polymorphism ranging from 9.1 to 100%. Lohithaswa et al. (2003) showed 63.46% polymorphism in their study to assess genetic diversity among 11 pigeonpea cultivars with RAPD markers. Malviya et al. (2010) observed the genetic diversity of 17 cultivars of pigeonpea using 17 RAPD primers. Nine out of 17 primers depicted more than 80% polymorphism.

In RAPD markers study, Jaccard similarity coefficient ranged from 0.07 to 0.93. Lakhanpaul et al. (2000) subjected 32 Indian cultivars of green gram to RAPD analysis using 21 decamer primers. Jaccard similarity coefficient values ranged from 0.65 to 0.92. *C. scarabaeoides* and *C. cajanifolius* showed highest similarities (37%) in other wild germplasm and GT-100 and ICPL-87 showed highest similarities (93%) in local cultivars. The present study revealed high level of polymorphism among wild pigeonpea germplasm as compared to cultivated germplasm. Ratnaparkhe et al. (1995) also reported low level of genetic diversity among cultivated pigeonpea as compared to the wild relatives.

In the present study, the arithmetic mean heterozygosity and the marker index in RAPD analysis were 0.90 and 22.47, respectively. Choudhury et al. (2008) found the arithmetic mean heterozygosity to be 0.48, whereas the value of the maker index (MI) was 5.027 in pigeonpea using RAPD analysis. As microsatellite or SSR markers are highly polymorphic, reproducible, co-dominant in nature and distributed throughout the genome, they have become the ideal marker system for genetic analysis and breeding application. In the present study, out of 40, five SSR markers were polymorphic in 12 pigeonpea germplasm.

The PIC value was found to be ranging from 0.076

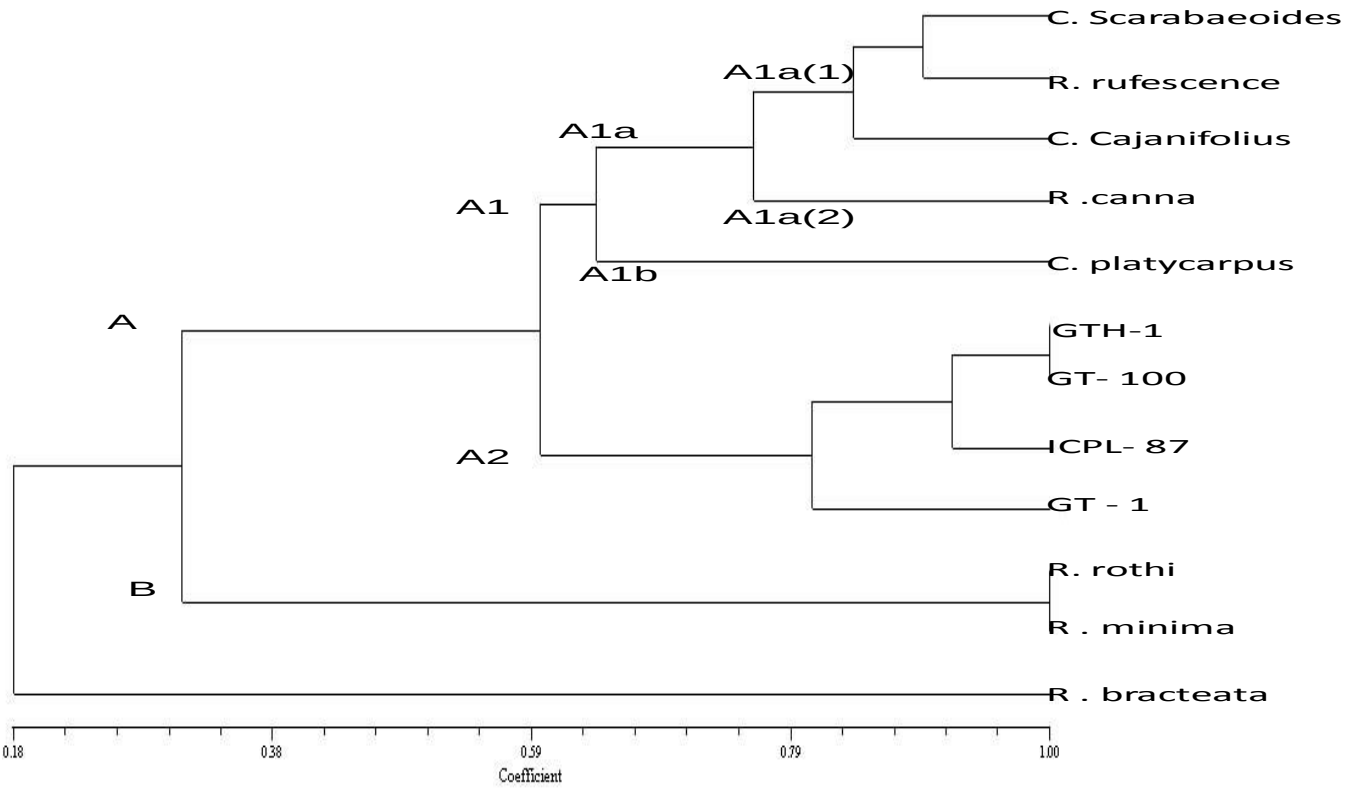

Figure 4. Dendogram showing clustering of 12 wild and cultivated pigeonpea germplasm constructed using UPGMA based on Jaccard's coefficient obtained from SSR analysis.

to 0.25. Odeny et al. (2007) reported that 20 SSR primers were polymorphic in 15 cultivated and nine wild pigeonpea relatives. Nearly, all amplifying SSR primers detected polymorphism amongst the 24 diverse accessions. The PIC value was ranged from 0.17 to 0.80. In the results obtained by Sexena et al. (2010), 13 SSR primers were polymorphic amongst 32 cultivated and eight wild pigeonpea genotype representing six *Cajanus* species. The polymorphic information content for these markers ranged from 0.05 to 0.55.

In SSR pooled data, five polymorphic SSR primers were used for diversity analysis of 12 pigeonpea germplasm. This indicated that the cultivated GTH-1 and GT-100 are closely related and showed 100% similarity. Odeny et al. (2007) used 19 polymorphic SSR primers for diversity analysis of 24 *Cajanus* genotypes. They found that, cultivated ICP 7543 and ICP 14144 revealed the highest genetic similarity (98%). The high polymorphic results indicate a wide genetic base in pigeonpea accessions and genetic diversity may be due to their characteristics, wide distribution, amplification protocol used/selection of suitable primers.

The present analysis using pigeonpea germplasm showed that both techniques may provide consistent data and can thereby be used to study genetic diversity in pigeonpea, showing concordant values of genetic diversity. This led us to generate an opinion that RAPD markers can be considered as effective as SSR markers if we succeeded to achieve the following stringency: 1, high purity of DNA; 2, selection of RAPD primes aimed at identifying DNA segment that are well separated and reproducible; 3, optimization of reagent concentrations that are critical in the amplification process, providing reliable and replicable results, and 4, identification of more strongly stained DNA segments, selecting properly different amplification programs.

Therefore, the option to analyze pigeonpea genetic diversity using RAPD markers kept track and guided genetic breeding programs can be considered an adequate strategy. On practical grounds, the OPA19, OPA10 and OPA11 primers for instance which produced the greatest number of bands and which showed the greatest potential to discriminate polymorphic DNA segments, can be recommended for future analysis of the pigeonpea genome using RAPD markers.

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