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Comparative Analysis of Genetic Diversity among Cultivated Pigeonpea (*Cajanus cajan* (L) Millsp.) and Its Wild Relatives (*C. albicans* and *C. lineatus*) Using Randomly Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) Fingerprinting

Kusum Yadav[1*], Sanjay Kumar Yadav[1], Anurag Yadav[2], Veda Prakash Pandey[1], Upendra Nath Dwivedi[1]

[1]Department of Biochemistry, University of Lucknow, Lucknow, India
[2]Department of Microbiology, Sardarkrushinagar Dantiwada Agricultural University, Palanpur, India
Email: [*]anukusum@gmail.com

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Abstract

Genetic relationships of 16 cultivars of pigeonpea (*Cajanus cajan* (L) Millsp.) and its two wild relatives (*C. albicans* and *C. lineatus*) from different parts of the India were analysed using 22 random amplified polymorphic DNAs (RAPDs) primers and 10 inter simple sequence repeats (ISSRs) primers. Twenty two RAPD primers yielded 151 polymorphic markers (71.2%) with an average of 6.8 polymorphic band/primer. Cluster analysis based on these 151 RAPD markers revealed relatively low level (0.434 - 0.714) of genetic diversity among cultivars and high level of diversity between cultivars and wild relatives. Ten ISSR primers produced 100 bands across 16 cultivars and its wild relatives out of which 93 (93%) were polymorphic with an average of 9.3 polymorphic band/primer. Cluster analysis based on these 93 ISSR markers also revealed relatively higher level (0.328 - 0.827) of genetic diversity among cultivars as compared to RAPD markers. The polymorphic markers obtained by both RAPD and ISSR primers were pooled and the genetic diversity analysis based on these 244 markers was analysed. Jaccard's similarity coefficient obtained by pooled data revealed very narrow range (0.477 - 0.720) among cultivated and high range between cultivated

[*]Corresponding author.

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and wild species *C. albicans* (0.240 - 0.331) and *C. lineatus* (0.163 - 0.193). In the UPGMA based dendrogram the 16 cultivars were grouped into three distinct clusters. Cluster I contained two genotypes, cluster II had six and cluster III had eight genotypes. Principal components analysis (PCA) also resulted in similar pattern as that of UPGMA based analysis. The first three PCs contributed 56.26%, 5.71% and 4.97% of variation, respectively, with cumulative variation of the first three PCs was 66.96%. Both the markers and the combined data revealed similar pattern with narrow diversity among cultivars and higher diversity between cultivars and wild one, but the genetic diversity range obtained by ISSR markers was relatively higher as compared to RAPD and pooled data. Furthermore, both the markers also correlated the clustering of stress resistant genotypes together. Cultivar Pusa-2002 possessed more diversity with other genotypes in ISSR dendrogram.

Keywords

Genetic Diversity, RAPD, ISSR, Pigeonpea

1. Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important crop in semi-arid tropical and subtropical farming systems, providing high quality vegetable protein (20% - 22%), animal feed, and firewood. Pigeonpea is a tall, woody, perennial legume with centre of diversity in India [1]. It is only cultivated food crop of the cajaninae sub-tribe and popularly known as red gram (Arhar or Tur). It has diploid genome (2n = 22) and estimated at about 0.853 pg [2]. In terms of global grain legume production, it is sixth after *Phaseolus* beans, peas, chickpeas, broad beans, and lentils. The nitrogen-fixing ability of pigeonpea makes it an important component in sustainable cropping systems, and farmers recognize and appreciate the ability of pigeonpea to "replenish" the soil when planted after a cereal crop [3].

The productivity of pigeonpea in the last five decades has remained low at about 700 kg/ha (http://faostat.fao.org/site/339/default.aspx). Pigeonpea is an important pulse crop of India but as compared to other grain legumes it has received relatively little research attention. Moreover, genetic improvement in production and productivity of pulse crops has been very slow owing to several constraints. Therefore, many pigeonpea breeding programmes are focused on improving the genetic potential both to increase yield and to provide protection against abiotic and biotic stresses. In order to enhance genetic potential, there must be a comprehensive understanding of the amount and pattern of genetic variation that exists within and between the available cultivated and wild accessions.

A large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner [4]. This limits the use of morphological characters and isozymes as these markers had the environmental influences also. Molecular genetic markers have developed into a powerful tool to analyze genetic relationships and genetic diversity. DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers [5]. Assessment of genetic variability in crops has been done using various molecular markers viz., RAPD [6]-[8], AFLP [9]-[11], RFLP [11] [12], SSR [11] [13], PCR-RFLP [14]. RAPDs are generated by PCR amplification using single, short, synthetic, random oligonucleotide as a primer that acts as both forward as well as reverse primer. An RAPD primer is able to hybridise to several hundred sites in the target DNA; exponential amplification can occur only when the primer anneals at two sites, on the opposite strands of the DNA, within about 2 kb of each other. The conditions are normally chosen so that the number of fragments generated is 1 - 20; thus a relatively small number of primers can generate a very large number of fragments, usually from different regions of the genome. As a consequence, multiple loci may be examined very quickly, and genomic DNA from individuals can be screened for detecting variations in sequences. RAPD markers have been used for the identification of cultivars and for assessing the genetic diversity among cultivars of several crops like bean [15], cowpea [16], pea [17], mungbean [4] [18], and *Vigna angularis* [19].

ISSR marker is based on primers having microsatellite sequences, and detects variation in the size of inter-

satellite regions; therefore, a prior knowledge of the template DNA sequence is not required [20]. This technique uses a single primer that may consist of solely a microsatellite sequence or a microsatellite sequence plus a short (usually 2 nucleotides long) arbitrary sequence either at the 3'- or the 5'-end of the primer (anchored microsatellite). In all these cases, amplification will occur only of such regions that are flanked by the SSR sequence used as primer; it is essential that the flanking SSR sequences are in reverse orientations. ISSR markers have been successfully used to estimate the extent of genetic diversity at inter and intra specific level in a wide range of crop species, which include rice [21], wheat [22], *Vigna* [23], blackgram [24], barley [25].

The objectives of the present investigation were to study and compare genetic diversity patterns among 16 pigeonpea genotypes and its wild relatives (*C. albicans and C. lineatus*), using RAPD and ISSR markers, and to evaluate the degree of polymorphism generated by each technique as a pre-requisite for their applicability to breeding programme in pigeonpea.

2. Materials and Methods

2.1. Plant Materials

The plant material comprised of 18 accessions of pigeonpea including 16 cultivars of *C. cajan* and 2 accessions of its wild progenitor (*C. albicans* and *C. lineatus*) (**Table 1**). All the above material was procured from core collection maintained at Indian Institute of Pulses Research, Kanpur, India.

2.2. Genomic DNA Isolation

Pigeonpea seeds were surface sterilized with 0.2% $HgCl_2$ and washed 3 - 4 times and 3grown in pots in sunlight for three weeks. DNA was isolated from young leaves using HiPurA™ Plant genomic DNA Miniprep Purification spin kit (Himedia Laroratories Pvt. Ltd). Quality and quantity of isolated DNA was checked by spectrophotometry as well as by 0.8% agarose gel electrophoresis. The DNA yield obtained was in the range of 1.0 µg to 3.0 µg.

2.3. RAPD Amplification

A total of 30 decamer primers (GC content 60% - 90%) were used for RAPD analysis. Out of 30 primers 19 (OPA-19, OPB-14, OPB-17, OPB-19, OPC-05, OPH02, OPH-03, OPH-05, OPH-10, OPH-11, OPH-12, OPM-07, OPP-07, OPP-09, OPAQ-05 OPAQ-18, OPAQ-19, OPAZ-05 and OPAZ-18) belong to Operon series (Operon Technologies USA) and 11 (P-23;GTAGGCGTCG, P-24;GGCTCGTACC, P-25;GACCCCGGCA, P-26; CAGGGGACGA, P-27;CGCCACGTTC, P-28;GCCTCCTACC, P-29;GGCGTCGGGG, P-30;CAGGGCCGCT, P-31;CTCTCCGCCA, P-32;CTCGGCTGGA and P-33;AGGCCCGATG) were selected randomly. The PCR amplification protocol is described in our published paper [26].

2.4. ISSR Amplification

ISSR-PCR was performed by means of 15 primers out of which 11 were 3'-anchored [GAGAGAGAGAGAGAGAT, GAGAGAGAGAGAGAGAC, GAGAGAGAGAGAGAGAA, ACACACACACACACACCT, AGAGAGAGAGAGAGAGCT, GTGTGTGTGTGTGTGTA, GTGTGTGTGTGTGTGTCT, CACCACCACGC, CTCTCTCTCTCTCTCTGC, GAGAGAGAGAGACC, AGAGAGAGAGAGAGAGTG] and four were non-anchored [GACAGACAGACAGACA, GACTGACTGACTGACT, GTGTGTGTGTGTGTGTGT, ACTGACTGACTGACTG] primers. Each amplification reaction contained 10 mM Tris-HCl, pH 9.0, 2 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 200 µM of each dNTP, 4 µM of primer, 0.6 Units of Taq DNA polymerase (Bangalore Genei, Bangalore, India) and 30 ng of genomic DNA in total volume of 25 µl. The PCR amplification was carried out for 40 cycles in a thermal cycler (PTC-200, Bio-Rad USA). The reaction had initial denaturation step at 94˚C for 5 min, followed by 40 cycles of 94˚C for 1 min, annealing temperature for 1 min, 72˚C for 2 min. The final extension step was at 72˚C for 5 min. Amplified products were separated on 1.8% agarose gels having 0.5 $\mu g \cdot ml^{-1}$ of the ethidium bromide at 50 V for 3 h. The gels were observed under a UV light source in a gel documentation system (BIOVIS Gel, Expert Vision Labs Pvt. Ltd, India).

2.5. Data Analysis for RAPD and ISSR

Clearly resolved, unambiguous polymorphic band were scored visually for their presence or absence with each

Table 1. List of genotypes of pigeon pea (*Cajanus cajan* (L) Millsp.) and its wild relatives used for genetic diversity analysis.

S. No.	Variety	State	Pedigree	Place of origin	Year of release	Maturity duration (days)	100 seed wt. (g)	Special features
1.	ICPL87 Pragati	M.P, Gujarat, M.S	T21 × JA277	ICRISAT Patancheru	1986	116 - 126	8.6	Determinate, brown seeded, spreading, resistant to wilt
2.	AK-101	Maharashtra	Selection from germplasm	Akola (Maharashtra)	-	145 - 160	7.9	Tolerant to wilt, semi-spreading, Indeterminate,
3.	Vamban 1	Tamil Nadu	(Prabhat × HY3A) × (T21 × 102)	TNAU, Vamban	1993	95 - 100	7.5	Determinate, Suitable for Peanut intercropping
4.	Jawahar (JKM7)	M.P., Gujarat, Maharashtra	ICP8863 × LRG30	Jknvv, Khargon	1996	173 - 180	9.3	Tolerant to wilt and Phytophthora blight
5.	ICPL85063 (Laxmi)	A.P.	BDN1 × (T21 × JA275)	RAS, Lam	2000	160 - 200	9.9	semi-spreading, suitable for rabi planting also
6.	Azad	UP, Bihar	Bahar × NP (Wr) 15	CSAUA & T, Kanpur	1997	153 - 210	10.1	Indeterminate, resistant to sterility mosaic semi-spreading
7.	Pusa 2002	New Delhi	P945 × Pusa 78	IARI, New Delhi	2002	130	7.5	Early maturing, indeterminate
8.	BDN- 2	Maharashtra	Sel. From local bori11-132-A-1	ARS, Badnapur	1978	150 - 160	8.6	Indeterminate, Tolerant to wilt
9.	GT 101	Gujarat	BWR 24 × Pusa Sweta	S.D. Agri. Univ. S.K. Nagar	2002	133 - 185	11.0	Indeterminate, semi-spreading
10.	Malviya Vikalp (MA-3)	M.P., Gujarat, Maharashtra	Sel. From land races no MA-2	BHU, Varansi	1999	178 - 262	8.0	Spreading, Constricted pod, resistant to pod fly
11.	C11	M.P, Gujarat, M.S	Sel. from sanga Reddy (A.P.)	A.P.	1982	195 - 200	9.0	Profuse branching, brown seeded, Tolerant to wilt
12.	Manak	Punjab, Haryana, North Raj, West U.P.	T21 × UPAS120	HAU, Hisar	1985	120 - 130	6.5	Indeterminate, semi-spreading, small seeded
13.	Paras (H82-1)	Haryana	EE76 × UPAS120	HAU, Hisar	1998	133 - 145	7.8	Indeterminate, tolerant to wilt
14.	Birsa Arhar	Bihar	Local sel. Land races Ranchi	BAU, Kanke (Bihar)	1992	180 - 200	-	Resistant to wilt under field condition
15.	Pusa 84	Punjab, Haryana, North Raj, West U.P	Pusa Ageti × T21	IARI, New Delhi	1985	140 - 150	7.5	Semi-spreading, determinate, semi tall, brown seeded
16.	T15x15	Gujarat	Sel. From land races	GAU, Gujarat	1985	200 - 210	9.0	Indeterminate, White seeded, suitable for vegetable purpose also
17.	*Cajanus albicans* (wild)		NKR 185	A.P.				
18.	*Cajanus lineatus* (wild)		JM 3366	Tamil Nadu				

RAPD and ISSR primer. The scores were obtained in the form of a matrix with "1" and "0", which indicate the presence and absence of bands in each variety respectively. The individual RAPD and ISSR profiles as well as pooled data (RAPD+ISSR) were analysed for genetic diversity analysis. Jaccard's similarity coefficient [27] was estimated from these binary data using FreeTree [28] software. The resulting similarity matrix was used for UPGMA based dendrogram construction. Support for clusters obtained was evaluated by bootstrap analysis with FreeTree and Tree View [29] software through generating two thousand samples by re-sampling. PCA was also

done to check the results of UPGMA based clustering using EIGEN module of NTSYSpc [30].

3. Results

3.1. Polymorphism Detected by RAPD Markers

In RAPD analysis, our earlier results [26] based on 30 primers tested were included in the analysis. Out of these, 22 primers yielded a total of 212 fragments (average 9.6 bands/primer), of which 151 amplicons (71.2%) were polymorphic, the number of polymorphic bands per primer ranged from 2 to 17, the average being 6.8 (**Table 2**). Primers OPB17, OPB-19 OPM-07, OPAZ-05, OPAZ-18, P-24, P-26, P-27, P-29, and P-30 were the most informative primers as 75% or more of the amplicons were polymorphic. These ten highly polymorphic primers produced a total of 83.1% polymorphism. Amplification pattern obtained by primer P-27 is given in **Figure 1**. A total of nine intense, distinct, species-specific markers were amplified by primers OPP-09, P-23, P-24, P-25, P-27, P-28, P-29, P-30 in all the 16 cultivars of pigeonpea but absent in both the wild species.

3.2. Polymorphism Detected by ISSR Markers

ISSR primers produced different numbers of DNA fragments, depending on their simple sequence repeat motifs. Out of the 15 primers tested, 10 produced reproducible and polymorphic patterns. The 10 primers yielded a total of 100 fragments (average 10 bands/primer), of which 93 amplicons (93%) were polymorphic, the number of polymorphic bands per primer ranged from 5 to 15, the average being 10 (**Table 2**). All the 10 Primers [GAGAGAGAGAGAGAGAC, GAGAGAGAGAGAGAGAA, GACAGACAGACAGACA, CACCACCACGC, GTGTGTGTGTGTGTGTGT, AGAGAGAGAGAGAGAGCT, ACTGACTGACTGACTG, AGAGAGAGA-GAGAGAGTG, GAGAGAGAGAGACC, ACACACACACACACACCT] were the most informative primers as 71% or more of the amplicons were polymorphic. Amplification pattern generated by primer GAGAGA-GAGAGAGAGAC is given in **Figure 2**.

Table 2. Comparison of PCR based DNA marker systems in pigeonpea.

Marker system	Number of primer	Polymorphism (%)	Average number bands per primer	Average number of polymorphic bands per primer
RAPD	22	71.2	9.6	6.8
ISSR	10	93	10	9.3
RAPD + ISSR	32	76.2	9.7	7.6

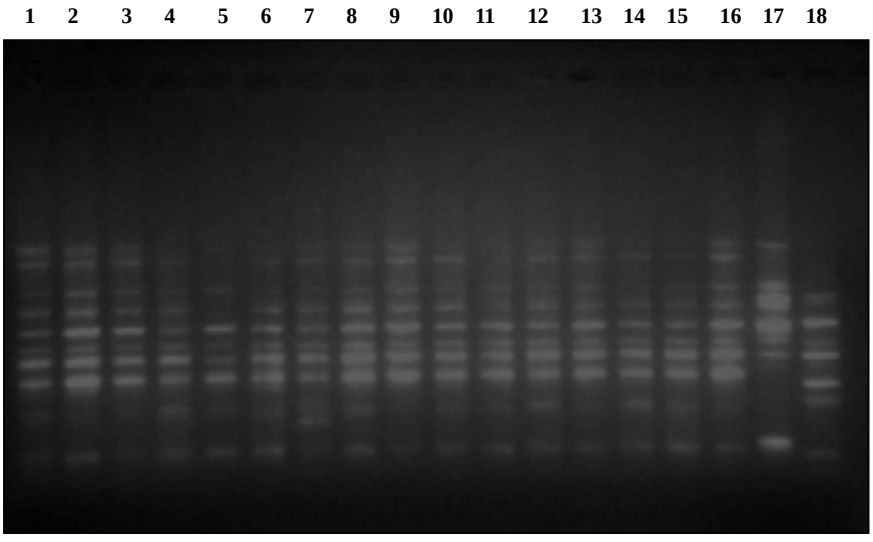

Figure 1. RAPD profile of pigeonpea genotypes, using the random primer P-27. Lanes 1 - 18 are pigeonpea genotypes as listed in **Table 1**.

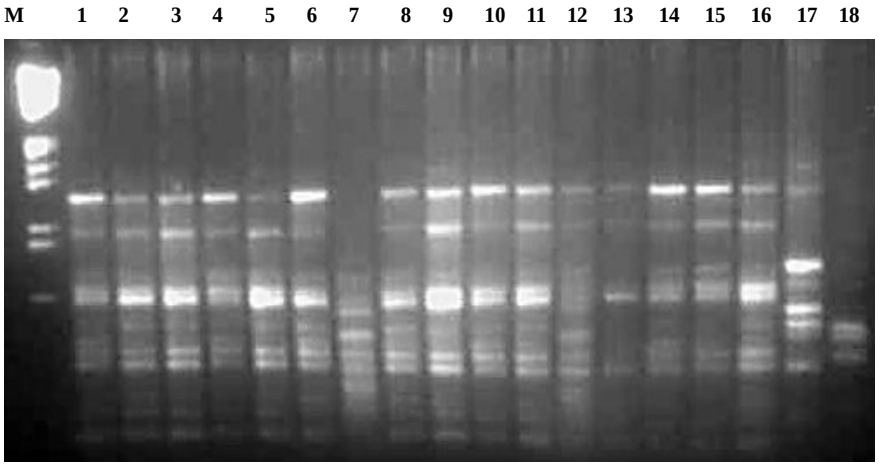

Figure 2. ISSR profile of pigeonpea genotypes using the ISSR primer GAGAGA-GAGAGAGAGAC. Lane M is λDNA EcoRI/ HindIII double-digest marker. Lanes 1 - 18 are pigeonpea genotypes as listed in **Table 1**.

3.3. Genetic Diversity Based on RAPD and ISSR Markers

The genetic diversity among pigeonpea cultivars and wild relatives was analyzed by UPGMA method. In RAPD analysis, 151 polymorphic markers amplified were analyzed for genetic diversity. Jaccard's similarity coefficients among *C. cajan* cultivars ranged from 0.434 - 0.714 [26] Lowest similarity was obtained between GT-101 and Azad (0.434) followed by that between Birsa arhar and Vamban-1 (0.451). Highest similarity was obtained between Pusa-2002 and Manak (0.714) followed by that between ICPL-87 and Azad (0.703). In the dendrogram 16 genotypes were grouped into two distinct clusters; cluster I and cluster II with seven and nine genotypes, respectively, whereas the two wild species did not group into clusters (**Figure 3**).

Genetic diversity analysis based on 93 ISSR markers yielded Jaccard's similarity coefficients range from 0.328 - 0.827 among *C. cajan* cultivars (Table not shown). Lowest similarity was obtained between Pusa-2002 and ICPL-85063 (0.328) followed by that between Pusa-2002 and ICPL-87 (0.379). Highest similarity was obtained between GT-101 and Vamban-1 (0.827) followed by that between GT-101 and BDN-2 (0.813). A dendrogram based on UPGMA analysis with ISSR data has revealed three main clusters *viz*., cluster I, II and III with two, three and ten genotypes, respectively (**Figure 4**). Cluster III was further divided into three sub-clusters (IIIA, IIIB and IIIC). The two wild species and one cultivar Pusa-2002 did not group into clusters.

The RAPD and ISSR data were combined for UPGMA cluster analysis. The 244 markers (151 RAPD + 93 ISSR) revealed varying degrees of genetic relatedness among wild and cultivated genotypes. Jaccard's similarity coefficients among *C. cajan* cultivars revealed very narrow range from 0.477 - 0.720 and high range between *C. cajan* cultivars and its wild relatives *C. albicans* (0.240 - 0.331) and *C. lineatus* (0.163 - 0.193) (**Table 3**). Lowest similarity was obtained between Pusa-2002 and ICPL-85063 (0.477) followed by that between Pusa-2002 and Vamban-1 (0.487) and between Pusa-84 and ICPL-85063 (0.487). Highest similarity was obtained between Vamban-1 and AK-101 (0.720) followed by that between Vamban-1 and GT-101 (0.705). The UPGMA dendrogram obtained from the cluster analysis of RAPD+ISSR data gave three distinct clusters *viz*., cluster I, II and III with two (Pusa-2002 and Manak), six (Vamban-1, AK-101, GT-101, Paras, T-15-15 and ICPL-85063) and eight (Pusa-84, Birsa-Arhar, JKM-7, Azad, MA-3, BDN-2, C-11 and ICPL-87) genotypes, respectively, whereas the two wild species did not group into clusters (**Figure 5**). Principal Components Analysis (PCA) of the combined RAPD and ISSR revealed that the PC1, PC2, and PC3 accounted for 56.26%, 5.71%, and 4.97% of the variation, respectively. Together, the first three PCs accounted for 66.99% of the total variation. Two dimensional (**Figure 6**) and three dimensional (**Figure 7**) plots were prepared by using the first two and first three PCs, respectively. In both 2-D and 3-D plots the wild species *C. lineatus* and *C. albicans* occupied distant positions from the *C. cajan*. The wild relatives occupied lower left and *C. cajan* genotypes clustered to the upper right corner of the biplot. The pattern of genetic divergence obtained by PCA is in close agreement with the results of UPGMA based cluster analysis. In both the methods wild species formed out groups whereas all the 16 cultivated genotypes showed narrower range of diversity.

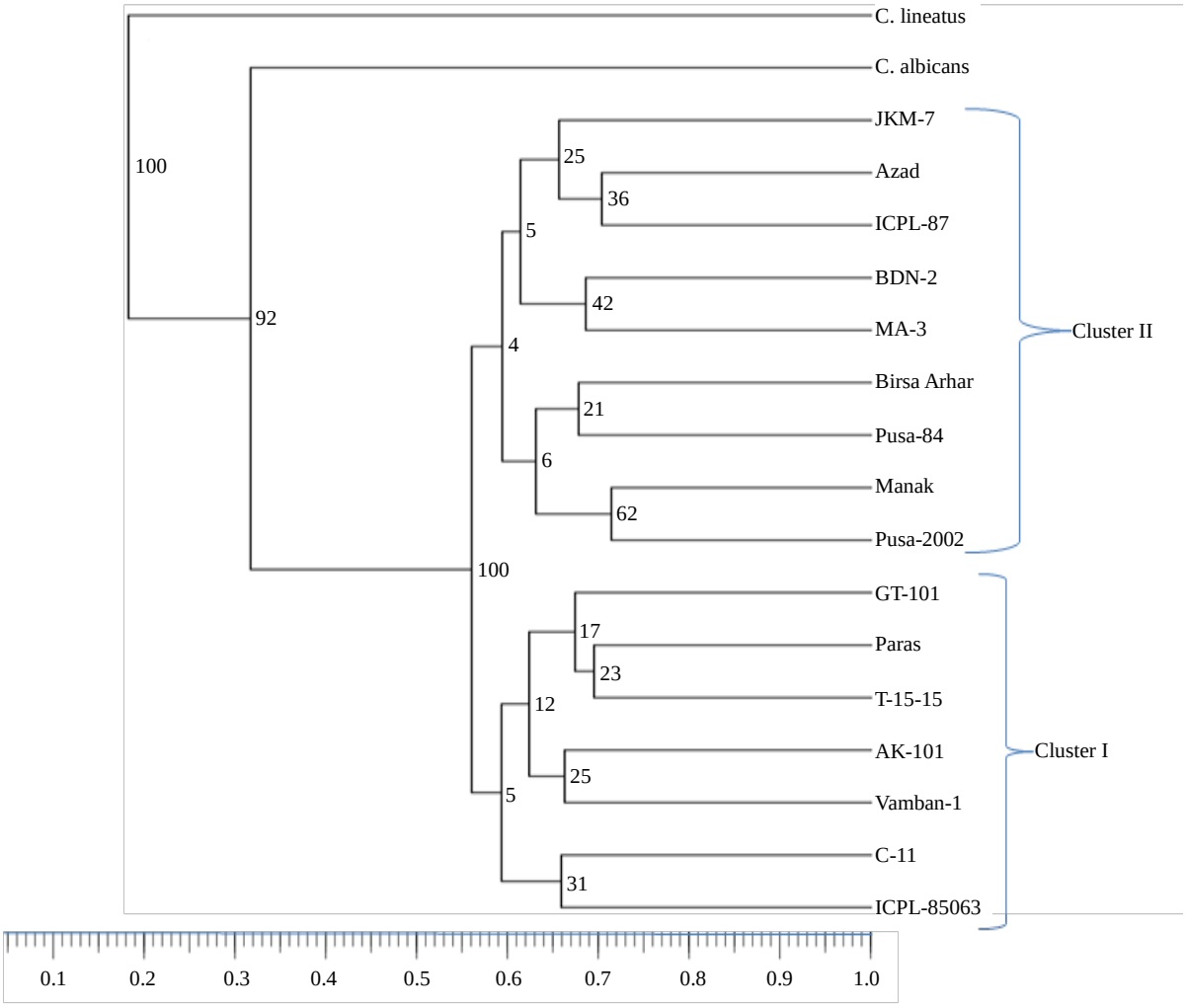

Figure 3. Dendrogram of pigeonpea along with wild relative showing genetic similarity based on RAPD markers.

4. Discussion

4.1. Polymorphism and Marker Efficiency

In the present investigation, two multilocus marker systems viz., RAPD and ISSR were evaluated for their efficiency in revealing the genetic diversity among the pigeonpea genotypes and its wild relatives studied. RAPD markers produced 71.2% polymorphic band with an average 9.63 band per primer. ISSR markers amplified 93% polymorphic band with an average 10 band per primer. Clearly the ISSR markers are more efficient than the RAPD assay, as they revealed more polymorphism than that of RAPD markers. Similar results were obtained in several other studies involving pigeonpea [31] [32], groundnut [33] and wheat [22]. Many authors reported the higher reproducibility of ISSR-PCR is because of use of longer primers as compared to RAPD [34]-[36].

Jaccard's similarity coefficients among 16 *C. cajan* cultivars used ranged between 0.434 - 0.714 (RAPD), 0.328 - 0.827 (ISSR) and 0.477 - 0.720 (RAPD + ISSR). ISSR marker showed relatively higher range of similarity coefficients as compared to RAPD and RAPD + ISSR data in *C. cajan* cultivars. Similar results were also found in chickpea [37] and *Vigna* [24]. A possible explanation for the difference in resolution of RAPDs and ISSRs is that the two-marker techniques target different portions of the genome. The ability to resolve genetic variation among different genotype may be more directly related to the number of polymorphisms detected with each marker technique rather than a function of which technique is employed.

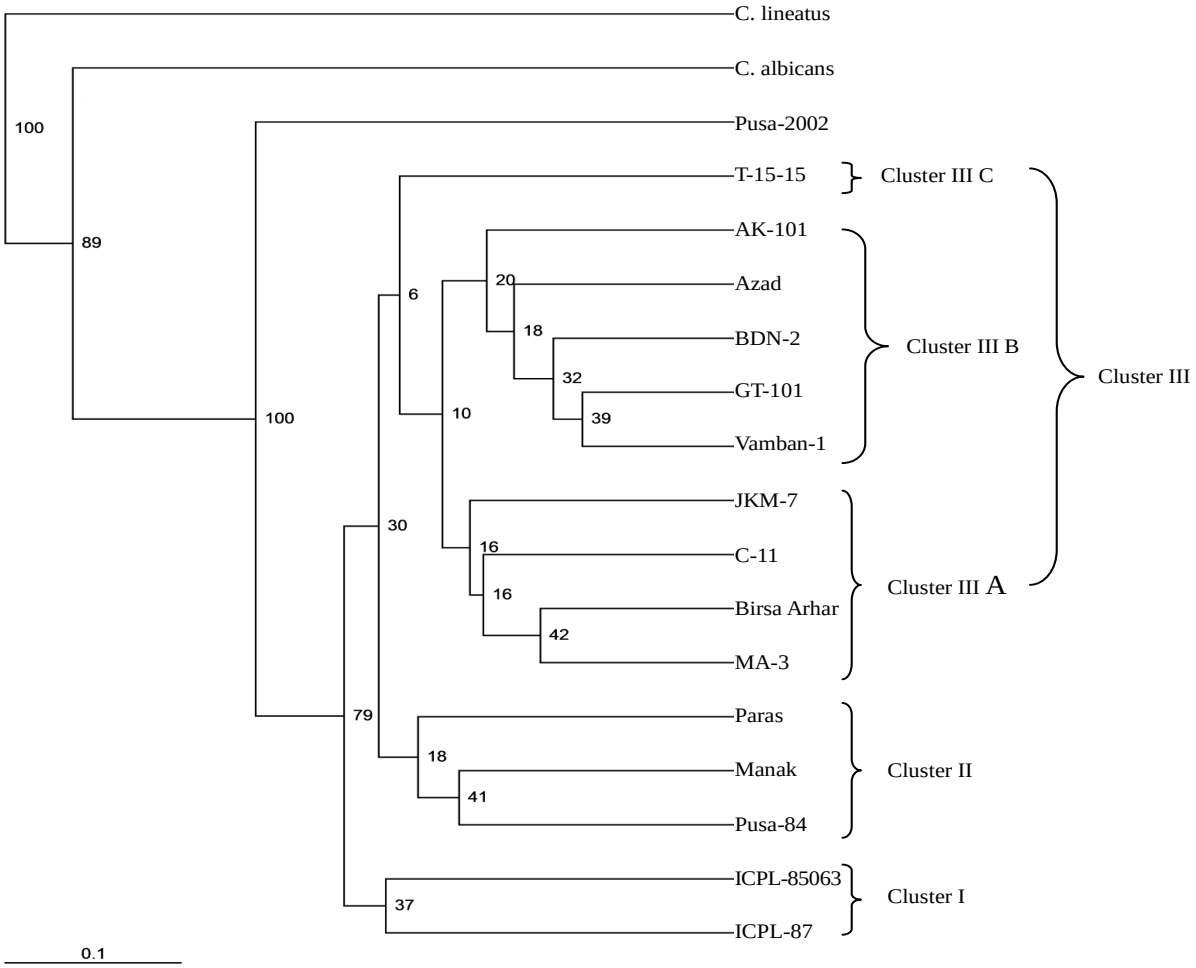

Figure 4. Dendrogram of pigeonpea along with wild relative showing genetic similarity based on ISSR markers.

4.2. Genetic Diversity

Cluster analyses revealed three main clusters viz., cluster I, II and III in RAPD + ISSR dendrogram, which has similar pattern to that of ISSR dendrogram whereas, the dendrogram based on RAPD showed some variation in the clustering of genotypes. Sixteen genotypes were grouped in three clusters in ISSR and ISSR + RAPD data; whereas the dendrogram based on RAPD data showed two main clusters. Clustering of genotypes within groups was not similar when RAPD and ISSR derived dendrogram were compared, whereas the pattern of clustering of the genotypes remained more or less the same in ISSR and combined data of RAPD + ISSR. In all the three dendrograms, both the wild species *C. lineatus* and *C. albicans* formed out groups as they did not cluster with any of the pigeonpea cultivars. Pusa-2002 showed more diversity from other cultivars in ISSR dendrogram; this pattern was not found in RAPD and RAPD + ISSR dendrograms. On comparing these results it can be concluded that ISSR marker has more differentiating power than RAPD. In ISSR and RAPD + ISSR dendrograms, we obtained the almost same sample distributions while dendrogram from RAPD is little different. The differences in clustering pattern of genotypes using RAPD and ISSR markers may be the different number of PCR products analysed, this confirms the importance of the number of loci and their coverage of the overall genome for producing reliable estimates of genetic relationships among cultivars [38].

The dendrogram based on RAPD + ISSR markers clearly separated various biotic and abiotic stress tolerant genotypes together (**Figure 5**). All the members, except Pusa-84, of cluster III are tolerant to certain biotic and abiotic stresses *viz*., Birsa arhar, JKM-7, BDN-2, C-11, ICPL-87 are all tolerant to wilt while JKM-7 Azad and MA-3 are *Phytophthora* blight sterility mosaic and pod fly resistant, respectively. However, two wilt resistant genotype viz., Paras and AK-101 could not group in cluster III instead it occupied place in cluster II. More or

Table 3. Jaccard's similarity coefficient of the 16 pigeonpea cultivars and two wild species based on RAPD + ISSR markers.

	ICPL-87	AK-101	Vamban-1	JKM-7	ICPL-85063	Azad	Pusa-2002	BDN-2	GT-101	MA-3	C-11	Manak	Paras	Birsa Arhar	Pusa-84	T-15-15	*C. albicans*	*C. lineatus*
ICPL-87	1																	
AK-101	0.614	1																
Vamban-1	0.527	0.72	1															
JKM-7	0.596	0.562	0.585															
ICPL-85063	0.559	0.65	0.626	0.496	1													
Azad	0.659	0.6	0.6	0.682	0.568	1												
Pusa-2002	0.533	0.506	0.487	0.569	0.477	0.522	1											
BDN-2	0.56	0.613	0.657	0.632	0.582	0.678	0.598	1										
GT-101	0.535	0.693	0.705	0.536	0.64	0.553	0.515	0.69	1									
MA-3	0.635	0.612	0.623	0.656	0.549	0.678	0.525	0.701	0.647	1								
C-11	0.607	0.64	0.606	0.664	0.631	0.626	0.539	0.639	0.6	0.697	1							
Manak	0.52	0.564	0.523	0.532	0.513	0.53	0.638	0.555	0.57	0.553	0.557	1						
Paras	0.639	0.686	0.626	0.54	0.572	0.568	0.546	0.604	0.696	0.591	0.608	0.621	1					
Birsa Arhar	0.634	0.565	0.513	0.629	0.503	0.666	0.594	0.609	0.541	0.678	0.637	0.615	0.635	1				
Pusa-84	0.588	0.557	0.516	0.558	0.487	0.543	0.553	0.6	0.573	0.643	0.615	0.617	0.635	0.691	1			
T-15-15	0.528	0.63	0.607	0.529	0.531	0.506	0.496	0.574	0.655	0.54	0.577	0.578	0.669	0.602	0.651	1		
C. albicans	0.252	0.298	0.306	0.24	0.289	0.258	0.268	0.314	0.331	0.272	0.256	0.305	0.311	0.288	0.302	0.331	1	
C. lineatus	0.186	0.171	0.184	0.163	0.167	0.18	0.17	0.17	0.182	0.181	0.179	0.182	0.167	0.185	0.187	0.187	0.193	1

Name of genotypes 1 to 18 is given in **Table 1**.

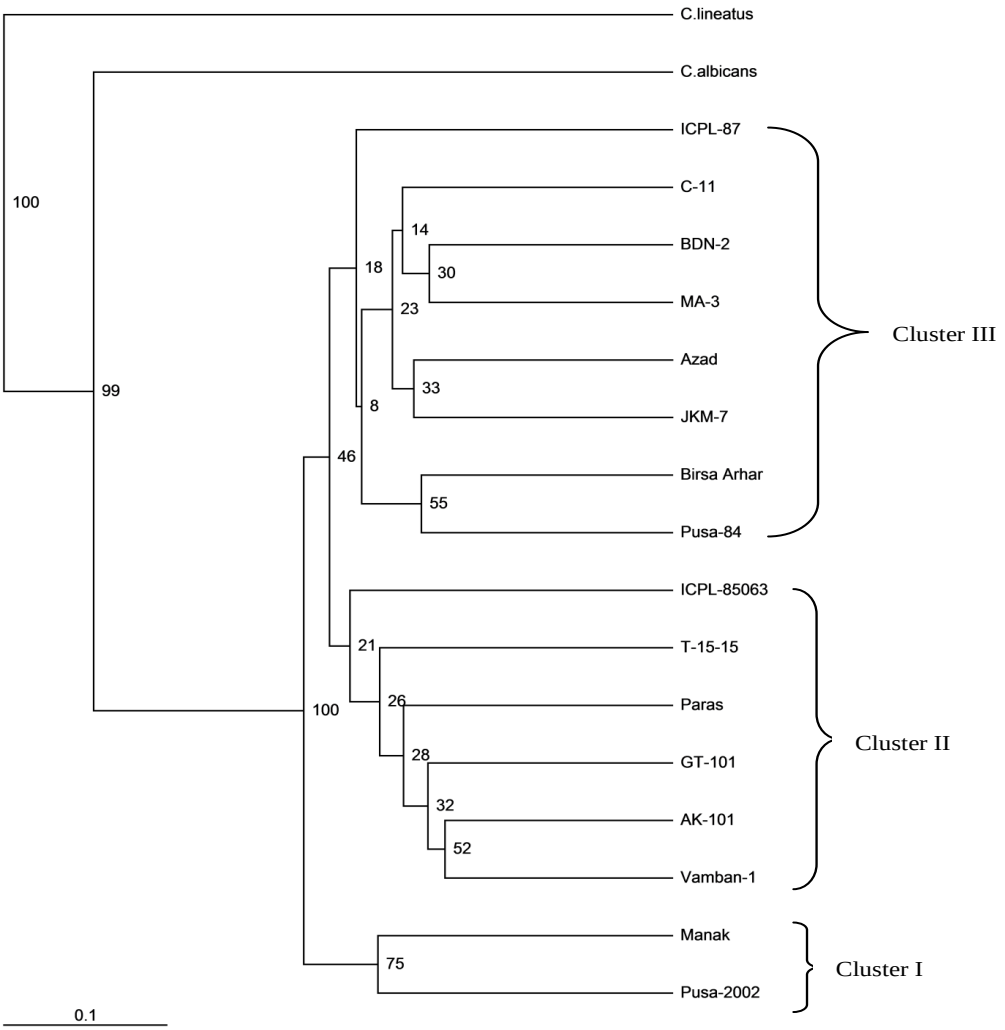

Figure 5. Dendrogram of pigeonpea along with wild relative showing genetic similarity based on RAPD + ISSR markers.

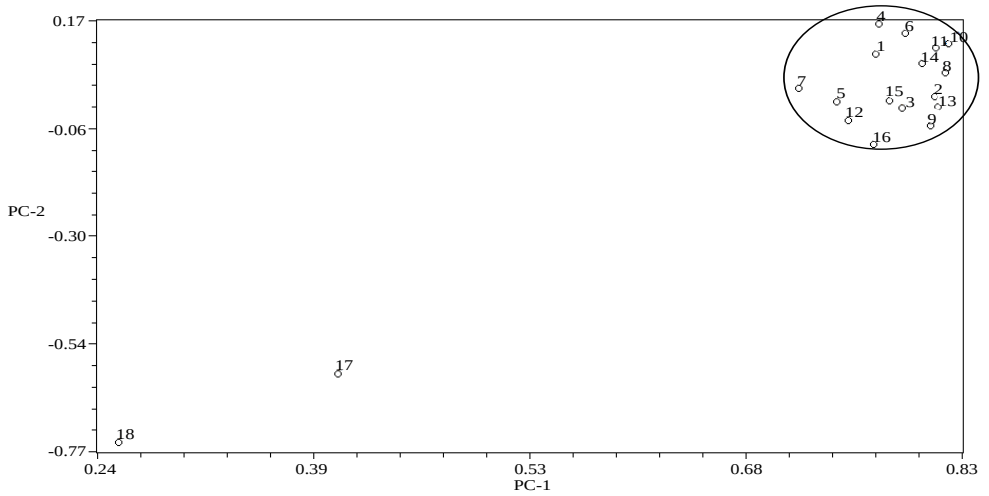

Figure 6. Two dimensional plot of principal components, PC-1 and PC-2 based on RAPD + ISSR markers. Name of the genotypes 1 to 18 is given in **Table 1**.

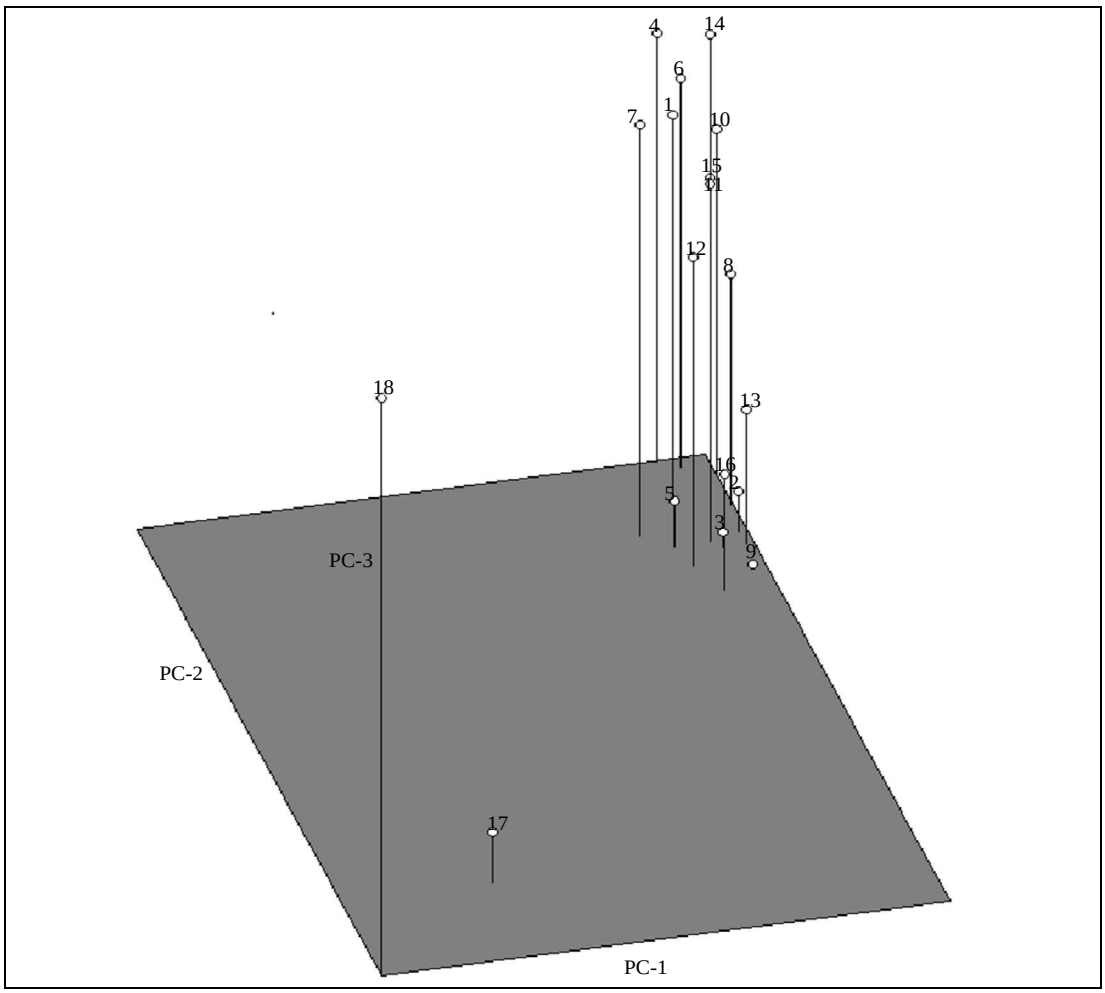

Figure 7. Three dimensional plot of principal components PC-1, PC-2 and PC-3 based on RAPD + ISSR markers. Name of the genotypes 1 to 18 is given in **Table 1**.

less similar pattern was observed in ISSR and RAPD dendrograms. In RAPD dendrogram the cultivar AK101 together with other resistant cultivar C-11 clustered with non resistant genotypes in cluster I. Whereas in ISSR based dendrogram AK101 grouped with tolerant genotypes of cluster III. The cultivar ICPL-87 which is resistant to wilt grouped in cluster I in ISSR dendrogram while in RAPD and RAPD + ISSR dendrogram it is sharing same group with resistance genotypes. The possible reason for clustering of stress resistant genotypes together could be the binding and amplification of RAPD and ISSR primers near or within loci responsible for resistance and all resistant genes may be sharing some common regions to which these primers anneal.

Genetic variation among elite genotypes could be useful to select parents to be crossed for generating appropriate populations intended for both genome mapping and breeding purposes. In this study in both ISSR and RAPD+ISSR dendrogram Pusa-2002 and ICPL-85063 are the most diverse cultivars having similarity coefficient of 0.328 and 0.477, respectively. These two genotypes also possesses some contrasting quantitative traits viz., Pusa-2002 is early maturing (130 days), indeterminate and relatively low yielding (100 seed wt. 7.5 g) while ICPL-85063 is late maturing (160 - 200 days), semi spreading and relatively high yielding (100 seed wt. 9.9 g), however the genetic diversity possessed by these cultivars is still not enough to be applied in breeding programmes.

In this study we obtained higher diversity between the wild species and between wild and cultivated genotypes than among the cultivated which is similar to earlier study in pigeonpea [39]. Very narrow genetic diversity in pigeonpea cultivars was reported also by many other researchers [8] [10] [13]. Such narrow genetic base can put a serious impediment to breeding progress in pigeonpea [39]. The reason for narrow genetic diversity in

pigeonpea and in other pulses is because of use of highly similar genotypes as parents in crossing programmes for the development of new cultivars which leads to narrowing down the genetic base of cultivated germplasm of pulses. Thirty five (41%) and 16 (34%) of the released cultivars in chickpea and in pigeonpea, respectively, were developed in India involving one or two genotypes as one of the ancestors in their pedigree [40]. This range of genetic diversity has been found to be narrow among both the cultivated and wild relatives of pigeon pea and lentil as compared to other pulses [41].

5. Conclusion

The present work can be summarized that all the genotypes could be distinguished by RAPD and ISSR markers, respectively. However, the relationship among the accessions revealed by RAPD analysis was somewhat different from that revealed by ISSR. RAPD markers revealed relatively narrower genetic diversity among pigeonpea as compared to ISSR markers and the range of genetic variability was further narrowed when RAPD markers were pooled with ISSR markers. Furthermore, both the markers also correlated the clustering of stress resistant genotypes together. Finally Pusa-2002 possessed more diversity with other genotypes in ISSR dendrogram. It can be concluded that genetic diversity obtained among cultivars is narrow so there is an urgent need to incorporate diverse parents in breeding programmes of pigeonpea and generate more DNA markers which can be useful in molecular breeding programmes of this crop.

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